

## EFFECT OF EXTRACTION METHOD ON SOME PROPERTIES OF KARKADE PETALS EXTRACT

Alian A.<sup>(1)</sup>; Y. Reyad<sup>(1)</sup>; M. Rabeih<sup>(2)</sup> and Doaa Mahmoud<sup>(2)</sup>.

<sup>(1)</sup> Food Science and Technology Department, Faculty of Agriculture, Cairo University.

<sup>(2)</sup> Food Technology Research Institute, Agriculture Research Center, Ministry Of Agriculture.

### ABSTRACT

Effect of extraction methods of karkade using water or water with 2% citric acid on some chemical and physical properties has been carried out. Extraction methods were carried out for 15min up to 24 hrs at 5 to 100 C. T.S.S. content was found to be increased by increasing the period of extraction as well as by increasing the temperature, hence it was 13-36 during extraction with water and 20-55 by adding 2% citric acid during extraction. The change in refractive index was quite slight and ranged between 1.3345 and 1.3394. pH values for karkade extracted with water were 3.75-3.62, while they were 3.71-3.48 by adding 2% citric acid to extraction water.

**Keywords:** karkade petals, *Hibiscus sabdariffa*, T.S.S, refractive index

### INTRODUCTION

Erect, mostly branched, annual; stem to 3.5m tall, flowers large, red to yellow with dark center and capsules 5cm long and 5.3cm wide, as described by Duke (1983).

Native to Old World Tropics, probably in the East Indies karkade now cultivated throughout the tropics. It contains citric acid and salts, serving as a diuretic. Karkade is used in jams, jellies, sauces, wines, syrup, gelatin, refreshing beverages, and dried roselle is used as tea, Tender leaves and stalks are eaten as salad and as a pot-herb and are used for seasoning curries. Seeds have been used as an aphrodisiac coffee substitute (Watt and Breyer-Brandwijk, 1962).

Roselle is used as remedy for antiseptic, aphrodisiac, astringent, cholagogue, digestive, diuretic, refrigerant, resolvent, sedative, stomachic, and tonic. Roselle is a folk remedy for abscesses, cancer, cough, fever, hangover, heart ailments, hypertension, neurosis, scurvy, and strangury. The drink made by placing, the calyx in water, is said to be a folk remedy for cancer (Perry, 1980).

### MATERIALS AND METHODS

#### Materials:

##### Source of karkade petals:

Two samples of both dark and light roselle calyces purchased from local market were used in this investigation.

#### Methods:

##### 1- Preparation of karkade extract.

Fifty grams from each light and dark karkade were soaked in 500 ml water or in acidic water with 2% citric acid as described by Du *et al.*, (1975) at 5, 25, 50, 75 and 100°C for different periods from 15 min. up to 24 hrs. The produced extracts were kept in refrigerator till used.

## **2- Chemical and physical determinations.**

The total soluble solids (T.S.S) content was measured according to the method outlined in A.O.A.C.(2005)

Refractive index of the extract samples under investigation was measured by the method described in the A.O.A.C.(2005).

The pH values of the roselle calyces extract were measured using the procedure of Jacobs (1951).

The color parameter was determined by Hunter equipment according to the procedure described in Tsai and Ou's (1996).

## **RESULTS AND DISCUSSION**

Two methods were carried out for preparation of karkade extract from both light and dark cultivars. Soaking in distilled water or water plus 2% citric acid at different temperatures (5, 25, 50, 75 and 100 C<sup>0</sup>) for several periods (1/4 : 24 hrs) and the following analyses have been carried out for evaluation of the prepared karkade extract.

Total soluble solids (T.S.S) amount is one of the indices that are used for controlling the period of soaking. Total soluble solids of karkade petals extract of both light and dark cultivars, as affected by the methods of extraction, are presented in Table (1). The obtained data showed the correlation between extended time of soaking and the percentage of T.S.S. As soaking time extended, the T.S.S. percentage increased in both water or acidic water (2% citric acid).

Also, as soaking temperature increased, the T.S.S. percentage increased. Moreover, it was observed that using soaking at 50<sup>0</sup>C for 1/4, 1/2, 1, 2 and 4 hours, the T.S.S. percentage was clearly increased, especially in the light one. It could be noticed also that T.S.S. percentage was almost the same by soaking at 50 and 100 C<sup>0</sup>, which indicates that soaking up to 50<sup>0</sup>C is quite suitable for preparation of karkade extract.

However, it could be noticed that there was an increase in T.S.S. percentage by using an acidic water (2% citric acid). It was clear that soaking at 50<sup>0</sup>C for 1/4 hr produced only 26% T.S.S. in water, while soaking in an acidic water produced 33% T.S.S., which indicates the importance of using an acid water to release more quantities of soluble substances. Moreover, soaking at high temperature should eliminate the deleterious effect of microorganisms during the preparation of karkade extract.

El-Bedewy, (1973) determined the total soluble solids of dried roselle calyces and found that there was a slight difference as that reported in Table (1), hence the extraction method was different.

The refractive index is one of the physical properties, that is easily determined, it could give an idea about the degree of extractability by the different methods. Refractive index of karkade petals extract of both light and dark cultivars as affected by methods of extraction is presented in Table (2).

From the obtained results, it could be noticed that as soaking temperature increased, the refractive index increased, especially in case of dark cultivar.

**Table (1): Total soluble solids of karkade petals extract of both light and dark cultivars as affected by extraction method calculated on dry weight basis.**

Soaking temperature (°C)	Soaking period (hr)	Extraction method			
		Light petals		Dark petals	
		Water	Acidic Water (2% citric acid)	Water	Acidic Water (2% citric acid)
5	1	13	20	12	23
	2	15	22	16	26
	4	19	25	18	29
	8	22	29	19	31
	16	24	32	23	34
	24	26	35	27	36
25	1	19	25	18	26
	2	22	36	24	35
	4	25	39	28	37
	8	27	43	32	42
	16	32	46	35	47
	24	35	48	37	49
50	¼	26	33	25	32
	½	29	37	27	39
	1	33	42	29	41
	2	34	45	34	45
75	¼	22	33	21	34
	½	27	34	25	36
	1	35	38	29	44
	2	39	45	35	46
100	¼	26	39	25	42
	½	29	46	32	52
	1	35	53	36	55

Refractive index of light karkade extracted was found to be increased by increasing soaking period or increasing soaking temperature. The same trend was observed with the dark cultivar.

Meanwhile, concerning the soaking media, it could be noticed that the refractive index showed higher readings by using acidic water, than those obtained by using water.

The obtained results are quite closed to those obtained by El-Bedawy, (1973).

pH value is considered to be one of the main factors that limit the acceptability of a given product by consumers. Materials could be impregnated to a certain level by addition of sugars to modify the acid/sugar ratio in the final product. In this view the determination of acidity of karkade petals was conducted to find out the required amount of sugar to be added through the preparation and consumption of the karkade extract, (El-Bedawi, 1973).

Table (2): Refractive index of karkade petals extract of both light and dark cultivars as affected by extraction method.

Soaking temperature (°C)	Soaking period (hr)	Extraction method			
		Light petals		Dark petals	
		Water	Acidic Water (2% citric acid)	Water	Acidic Water (2% citric acid)
5	1	1.3345	1.3364	1.3351	1.3369
	2	1.3354	1.3375	1.3359	1.3373
	4	1.3358	1.3376	1.3365	1.3377
	8	1.3360	1.3376	1.3367	1.3379
	16	1.3362	1.3376	1.3369	1.3381
	24	1.3365	1.3379	1.3370	1.3385
25	1	1.3349	1.3366	1.3350	1.3367
	2	1.3353	1.3372	1.3353	1.3371
	4	1.3358	1.3382	1.3357	1.3378
	8	1.3360	1.3385	1.3365	1.3380
	16	1.3362	1.3388	1.3367	1.3385
	24	1.3364	1.3393	1.3369	1.3388
50	¼	1.3350	1.3368	1.3357	1.3366
	½	1.3361	1.3377	1.3368	1.3373
	1	1.3366	1.3382	1.3370	1.3381
	2	1.3370	1.3390	1.3373	1.3383
	4	1.3373	1.3397	1.3378	1.3394
	75	¼	1.3354	1.3366	1.3356
½	1.3359	1.3374	1.3364	1.3370	
1	1.3362	1.3374	1.3366	1.3381	
2	1.3368	1.3385	1.3369	1.3386	
100	¼	1.3365	1.3369	1.3364	1.3368
	½	1.3367	1.3382	1.3368	1.3387
	1	1.3374	1.3390	1.3373	1.3394

The obtained results of pH of karkade petals extract of both light and dark cultivars as affected by method of extraction are presented in Table (3). From the results, it could be noticed that there was a variation in pH values in karkade extract depending on soaking methods, e.g. soaking temperature, soaking period, and soaking media. As soaking temperature increased from 5 to 25°C, pH value of the extract increased when water was used as soaking medium. However, using water + 2% citric acid, pH of the extract was decreased. On the other hand, as soaking temperature increased to 50, 75 and 100°C, pH value of the extract was decreased. The pH value of light karkade extract that was prepared through boiling (100°C) in water showed a gradual decrease from 3.69 to 3.63, when period of boiling was 1/4 and 1 hour, respectively. The same trend was observed in case of the dark extract as that of light cultivar.

Such results are confirmed by those obtained by Diab,(1968) and Beristain *et al.*,(1994) .

Roselle petals are considered to be one of the excellent sources of anthocyanin. To follow, the changes in the color of karkade extract during the preparation, it was aimed to determine the L, a and b through the different steps of preparation.

**Table (3): pH of karkade petals extract of both light and dark cultivars as affected by extraction method.**

Soaking temperature (°C)	Soaking period (hr)	Extraction method			
		Light petals		Dark petals	
		Water	Acidic Water (2% citric acid)	Water	Acidic Water (2% citric acid)
5	1	3.75	3.71	3.78	3.71
	2	3.73	3.67	3.79	3.71
	4	3.72	3.66	3.79	3.69
	8	3.72	3.65	3.78	3.68
	16	3.71	3.63	3.78	3.66
	24	3.69	3.62	3.68	3.66
25	1	3.79	3.70	3.81	3.78
	2	3.76	3.67	3.78	3.76
	4	3.75	3.65	3.76	3.73
	8	3.74	3.62	3.71	3.56
	16	3.69	3.62	3.69	3.49
	24	3.67	3.62	3.68	3.48
50	¼	3.78	3.77	3.80	3.66
	½	3.76	3.67	3.79	3.64
	1	3.76	3.67	3.75	3.56
	2	3.75	3.63	3.75	3.53
	4	3.64	3.56	3.76	3.48
	75	3.77	3.75	3.77	3.66
75	¼	3.75	3.72	3.75	3.62
	½	3.65	3.64	3.65	3.57
	1	3.63	3.61	3.63	3.53
	2	3.63	3.61	3.63	3.53
100	¼	3.69	3.64	3.64	3.55
	½	3.64	3.63	3.63	3.52
	1	3.63	3.61	3.62	3.48

Color of karkade petals extract was measured according to the principle of the tristimulus color measurement (L\*,a\*,b\* color system).

The effect of different steps of preparation on Hunter index values i.e. L\*: lightness, a\*: redness and b\*: yellowness were studied for karkade extract prepared from two different cultivars and under several conditions. The results in Table (4) indicate that lightness (L-value) of light karkade extract recorded ~ 30. This means that all extracts were less lightness. The effect of soaking temperature on lightness indicated that, as soaking temperature increased, L\* values decreased. For instance, L-value of light karkade extract in water at 5°C for one hour recorded 33.14, while recorded 31.51, 30.85, 30.03 and 29.96 at 25, 50, 75 and 100°C for one hour, respectively.

Results indicate also, that color parameters of light karkade were affected by soaking medium (water or acidic water 2% citric acid). Adding 2% citric acid to water caused a decrease in lightness values (L value) at all different temperatures and periods. This means that adding citric acid caused a less lightness for the extract than using water only. On the other side, redness (a-values) of light karkade with 2% citric acid was higher than that extracted with water only. The same trend was observed with b-values. Redness values of light karkade under different conditions ranged from 1.00 to 4.97. The effect of soaking period was found to be more noticeable in related to a-values.

The results in Table (4) indicate also that as soaking period increased from hour to 24 hours, the a-value increased. For instance, at 5°C for one hour, the a-value recorded 1.00, while at 8 hours recorded 3.00, and at 24 hours it recorded 4.35. The same trend was observed with the effect of soaking temperature, as soaking temperature increased, a-values increased.

Meanwhile, results of Table (5) showed the color parameters of dark karkade extract as affected by methods of extraction.

**Table (4): Color parameter of light karkade petals extract as affected by extraction method.**

Soaking temperature (C) <sup>o</sup>	Soaking period (hr)	Extraction method									
		Water					Acidic Water (2% citric acid)				
		L	a	b	h	c	L	a	b	h	c
5	1	33.14	1.00	-1.28	-1.28	1.62	30.99	2.21	-2.50	-1.18	3.28
	2	32.74	1.87	-1.28	-0.68	2.27	30.69	2.21	-2.32	-1.09	3.14
	4	32.18	2.09	-0.95	-0.45	2.29	30.58	2.78	-1.61	-0.58	3.21
	8	31.74	3.00	-0.88	-0.29	3.13	30.23	3.75	-1.38	-0.37	3.99
	16	30.16	3.99	-0.58	-0.15	4.03	30.16	3.89	-1.07	-0.28	4.03
	24	30.66	4.35	-0.71	-0.01	4.35	29.41	4.86	-1.03	-0.21	4.97
25	1	31.51	1.82	-2.14	-1.18	2.81	31.05	3.06	-1.42	-0.46	3.37
	2	31.46	1.84	-1.28	-0.69	2.24	31.12	3.66	-1.30	-0.36	3.88
	4	31.06	2.27	-1.17	-0.52	2.55	30.76	3.73	-1.20	-0.32	3.92
	8	30.52	2.51	-0.62	-0.25	2.59	30.37	3.84	-1.20	-0.31	4.02
	16	30.35	3.36	-0.56	-0.17	3.41	29.43	4.22	-0.07	-0.01	4.22
	24	28.84	4.36	-0.28	-0.07	4.37	28.59	4.39	-0.05	-0.01	4.39
50	¼	31.31	1.64	-1.93	-1.18	2.53	30.40	2.02	-1.33	-0.66	2.42
	½	31.06	1.87	-1.23	-0.66	2.24	29.94	3.45	-0.94	-0.27	3.58
	1	30.85	3.24	-1.01	-0.32	3.39	29.66	3.60	-0.64	-0.18	3.66
	2	30.42	3.62	-0.96	-0.27	3.75	28.90	4.39	-0.61	-0.14	4.43
	4	29.61	4.18	-0.52	-0.13	4.21	28.39	4.69	-0.55	-0.12	4.72
75	¼	30.90	3.49	-1.79	-0.52	3.92	30.88	2.72	-1.69	-0.62	3.20
	½	30.08	3.87	-1.52	-0.39	4.16	30.14	2.94	-1.55	-0.53	3.33
	1	30.03	3.96	-1.51	-0.38	4.24	28.11	4.11	-1.02	-0.25	4.24
	2	28.59	4.97	-0.53	-0.12	4.99	27.34	4.11	-0.69	-0.17	4.18
100	¼	31.66	4.18	-1.72	-0.41	4.52	31.06	3.60	-1.75	-0.49	4.00
	½	31.59	3.98	-0.86	-0.22	4.07	31.04	3.55	-1.26	-0.35	3.77
	1	29.96	3.84	-0.58	-0.15	3.88	30.81	3.42	-1.21	-0.35	3.64

The results in Table (5) indicate that lightness (L-values) of dark karkade extract ranged from 27.18 to 31.60. This means that all extracts were less lightness. The effect of soaking temperature on lightness values indicated that, as soaking temperature increased, L-values decreased. For instance, L-value of dark karkade extract in water at 5°C for one hour recorded 31.31, while decreased to 29.98 at 100°C for the same period. Redness values of dark karkade under different conditions ranged from 1.59 to 4.61. The effect of soaking period was more pronounced in related to a-values (redness). The results in Table (5) show also that, as soaking period increased (from 1 hour to 24 hours) the a-values increased. It was reported that at 5°C for one hour, the a-value increased and recorded 2.27, while after

24 hours it was 4.61. The same trend was noticed with the effect of soaking temperature, hence its increasing raised a-values.

Regarding b-values of dark karkade petals extract with water, results in Table (5) indicate that b-value ranged from -0.09 at 100°C for one hour to -2.03 at 5°C for same period. Also, as soaking period increased, b-values decreased. Moreover, using soaking temperature from 5 to 100°C, b-values decreased from -2.03 to -0.09.

Additionally, results indicated that color parameters of dark karkade were affected by soaking medium (water or acidic water with 2% citric acid). Adding 2% citric acid to water caused a decrease in L-values at all both different used temperatures and periods, this means that citric acid caused the extracts to be less lightness than using water only. On contrary, redness (a-values) of dark karkade extract prepared with 2% citric acid was higher than that of using water only. The same trend was observed with b-values.

The extract of calyces of (*Hibiscus sabdariffa* L.) was found to be the most well-known colorants that contain anthocyanins. Several investigators studied such coloring materials, and the first report carried out was by Yamamoto *et al.*, (1932). Later on structure and content, of main coloring constituents of commercial roselle color was identified by Sato *et al.*, (1991).

Table (5): Color parameter of dark karkade petals extract as affected by extraction method.

Soaking temperature (°C)	Soaking period (hr)	Extraction method									
		Water					Acidic Water (2% citric acid)				
		L	a	b	h	c	L	a	b	h	c
5	1	31.13	2.27	-2.03	-0.89	3.05	31.23	2.09	-1.65	-0.79	2.66
	2	30.77	2.64	-1.90	-0.72	3.25	30.37	2.15	-1.51	-0.70	2.63
	4	30.10	2.87	-1.86	-0.65	3.42	29.04	2.34	-1.42	-0.61	2.74
	8	29.61	2.99	-1.60	-0.54	3.39	28.87	2.92	-1.30	-0.45	3.19
	16	28.72	3.47	-1.07	-0.31	3.63	28.85	3.14	-0.89	-0.28	3.26
	24	28.04	4.61	-0.88	-0.19	4.69	28.25	4.73	-0.52	-0.12	4.76
25	1	31.60	1.93	-1.96	-1.02	2.75	31.91	1.02	-1.95	-1.91	2.20
	2	30.94	1.94	-1.85	-0.95	2.68	31.74	2.04	-1.94	-0.95	2.82
	4	29.29	2.13	-1.75	-0.82	2.76	31.61	2.24	-1.47	-0.66	2.68
	8	29.08	2.26	-1.50	-0.66	2.71	29.96	3.21	-1.38	-0.43	3.49
	16	28.66	2.43	-1.46	-0.60	2.83	28.52	4.31	-0.95	-0.22	4.41
	24	27.90	3.42	-1.18	-0.35	3.62	28.32	4.99	-0.81	-0.16	5.06
50	¼	30.96	1.98	-1.52	-0.77	2.49	29.57	2.32	-1.12	-0.48	2.28
	½	30.15	2.00	-1.48	-0.74	2.49	29.24	2.59	-1.55	-0.59	3.02
	1	29.47	2.18	-1.00	-0.46	2.39	29.20	2.65	-1.82	-0.67	3.21
	2	29.27	3.01	-0.30	-0.09	3.02	28.73	3.59	-1.88	-0.52	4.05
	4	27.18	3.82	-0.26	-0.07	3.83	28.62	4.05	-0.96	-0.24	4.16
75	¼	30.60	1.59	-1.49	-0.94	2.18	30.41	1.87	-1.78	-0.95	2.58
	½	30.07	2.59	-1.45	-0.56	2.97	29.97	1.90	-1.77	-0.93	2.59
	1	29.85	2.79	-1.43	-0.51	3.14	28.79	2.51	-1.71	-0.68	3.04
	2	28.92	2.80	-1.16	-0.41	3.03	28.49	3.15	-1.43	-0.45	3.46
100	¼	30.92	3.19	-1.30	-0.41	3.44	30.72	2.91	-1.64	-0.56	3.34
	½	30.60	3.17	-1.14	-0.36	3.37	28.72	3.34	-1.61	-0.48	3.71
	1	29.98	2.69	-0.09	-0.03	2.69	27.43	3.38	-1.58	-0.47	3.73

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تأثير طريقة الاستخلاص على بعض خواص مستخلص الكركدية

أحمد عليان<sup>(1)</sup>، يوسف رياض<sup>(1)</sup>، محمد ربيع<sup>(2)</sup> و دعاء محمود<sup>(2)</sup>

<sup>(1)</sup> قسم الصناعات الغذائية - كلية الزراعة - جامعة القاهرة

<sup>(2)</sup> معهد بحوث تكنولوجيا الأغذية - مركز البحوث الزراعية - وزارة الزراعة

تم دراسة تأثير طريقة الاستخلاص على بعض الخواص الكيميائية والفيزيائية حيث تم الاستخلاص بالماء والماء المضاف إليه 2% حمض الستريك وذلك على درجات حرارة من 5 إلى 100°م ولقترات من 1/4 إلى 24 ساعة. حيث وجد أن كمية المولد الصلبة الذاتية ارتفعت بزيادة مدة الاستخلاص وكذا بارتفاع درجة الحرارة حيث تراوحت بين 14 إلى 36 في حالة الاستخلاص بالماء في حين ارتفعت في وجود الحمض من 25 إلى 55.

أما بخصوص معامل الانكسار، فكان التغيير بسيطاً حيث تراوح بين 1,3345 إلى 1,3394 وكذلك تأثرت درجة الـ pH حيث تراوحت بين 3,75 إلى 3,62 في حالة الاستخلاص بالماء و 3,71 إلى 3,48 في حالة الاستخلاص بالماء المضاف إليه حمض الستريك 2%.

وبتقدير اللون ظهر انخفاض قيمة L مع ارتفاع قيمة كل من a,b وذلك بزيادة مدة الاستخلاص وارتفاع درجة الحرارة.