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Effect of Engineering Treatments on Extraction of Roselle Juice (Karkdah)

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ABSTRACT

Roselle juice are a good source of anthocyanin with several potential applications in the food, pharmaceutical and cosmetic industries a far as anthocyanin extract is concerned. The search for optimal operating conditions to maximize the efficiency of the process in necessary. In this work increasing the adsorption kinetics and isotherms was studied to determine the parameters to be optimized to recover the anthocyanins from aqueous extracts of the roselle. The results revealed that, the best combination of independent variables, in order to obtain the highest of anthocyanins (1250 mg/ 100g d.s.), Vitamic c (145 mg/ 100 g d.s.) and total phenols (4163 mg / 100 g d.s.) were obtained by extracting using acidified water with 2% citric acid for 210 min as retention time at 25 degrees ˚C, using the highest solid – to – solvent ratio (1/25)
The deepest red - purple (Hue = 14.04 & purity (C) 51.39) colored solution was observed under this condition. The best DPPH scavenging activity expressed as EC_{50} was obtained (40.50 u/ml).Therefore, results of this study can be used to determine application of roselle anthocyanins in a variety of food products as food colorants such as confectionery products, gelatin desserts, snaks, cake, pudding, ice cream and beverages .

Key words: Roselle; anthocyanins; vitamin C; total phenols; antioxidant activity

Introduction

Hibiscus sabdariffa L. (roselle) is a medicinal plant grown in Africa, South East Asia, Central America in Mexico, it is known as Jamaica flowers, Sorrel and karkdah (in Egypt), belongs to the Malvaceae family. This species in native to West Africa, described as an annual, bushy plant with a height of up to 2.5 m; its flowers consist of a purple – red calyx and corolla. Some varieties with light red calyxes. Varieties with dark red calyxes contains five to seven times higher anthocyanin content than varieties with light red calyxes (Salinas – Moreno *et al.,* 2012).

Roselle is cultivated in tropical and subtropical regions e.g. Sudan, China, Thialand, Egypt, Mexico, and West India (Dominguez *et al.,* 2008).

The calyxes, which dry naturally in sun ligh and air contain organic acids (tartaric, citric, malic, and hibiscic), glucoside compounds and contains a rich of bioactive compounds such as phtosterols and polyphenols, some of them with antioxidant properties such as anthocyanins like delphinidin -3- glucoside which are responsible for the red color (Mady *et al.,* 2012). Roselle also contains vitamins; ascorbic acid, thiamine, riboflavin and niacin and minerals (Ca, P and Fe).

The most common use of Roselle calyxes is for the extraction of soluble solids to obtain an aromatic infusion to use as a flavoring for sauces, jellies, marmalades and soft drinks or to use as a colorant for foods(Sandra and Jose, 2014) which roselle appear to be good and promising sources of water soluble natural red colorants.

In the medical – health area, the infusion is used for its diuretic, astringent, and digestive properties and also to treat different type of cancer, to reduce blood pressure, to deliver renal stones, and to treat high cholesterol (Sandra and Jose, 2014)

Cahlikova *et al.,* (1015) explained that the active constituents of the extracts of Roselle, which widely used in folk medicine to combat many illnesses and have been shown on several occasions to be anthocyanins and they ascertained through UHPLC – ESI MS/MS that delphinidin - 3 – sambubioside is the major one but the predominant one is cyanidin – 3 – sambubioside.

Also Roselle extracts are reported to have an antimicrobial effect on different pathogenic and food spoilage micro-organisms due to its metabolities of phenolic compounds such as anthocyanins. They are associated with the prevention of illnesses generated by oxidative stress (Heba *et al*., 2014).

Antioxidants are strong scavengers of free radicals, which are unstable chemical species that react rapidly with other chemical species in a biological system.

Reactive species can attack stable molecules in a healthy organism and produce illness. Antioxidants may neutralize the oxidative effect of free radicals (Faudale *et al*., 2008) .

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Misnawi *et al*., (2014) proved that the roselle extract can be used a substitute for synthetic antioxidant to prolong the shelf life and reusability of bulk frying oil. They showed that the addition of Roselle extract could retard the formation of free fatty acids to maximum 0.1226 mg KOH/g oil (4% addition) and lowered hydroperoxide formation as low as 7.47 Meq O_2/Kg oil after treated with 20 times of successive frying , and showed that the addition of 0.04% of Roselle extract could prolong the usage of oil up to 6 times in the open frying treatment. On the other hand, only 0.01 % of the Roselle extract was needed to prolong the oil usage up to 20 times in deep frying practice.

Anthocyanin is relatively unstable and because of their high reactivity it may be easily degraded and form colorless or undesirable brown – colored compounds during extraction processing and storage (Durst and wrolstad, 2001) Indeed, temperature, pH, light oxygen, metals, organic acids, sugars, ascorbic acid, enzymes, sulfer dioxide, co – pigmentation and interactions with food components may affect both the structure and stability of anthocyanins (Zuhaili *et al*., 2012).

Today several studies were done to improve the antioxidant activities of the aqueous extract and prevent the degradation of anthocyanins, therefore, researchers studied the average particle size of calyxes, the extraction temperature, the type of solvent, and the solid –solvent ratio which have a significant impact on the extraction (Domingnez *et al*. 2008; Cisse *et al*., 2012) but there were little studied reported about the influence of these variables on the efficiency of the batch extraction process and optimize the parameters for obtaining an extract with both a high extraction yield and high concentration of anthocyanins with strong antioxidant activity.

It will be important to investigate the optimal extraction condition efficiencies for subsequent bioactivity experiments.

For instant, it has been found that the use of temperatures higher than 70 ˚C for prolonged periods may cause significant degradation of anthocyanins (Cisse *et al*. 2012). Also it has been found that acid may cause partial hydrolysis of the acyl moieties in acylated anthocyanins, especially in anthocyanins acylated with dicarboxylic acid such as malonic acid (Cisse *et al*., 2009).

The extraction kinetics involves multiple steps, however, the main part of the operation is limited by diffusion because the natural structure of plants opposes a resistance to any penetration by a liquid, thus the process is very slow. The rate of mass transfer during solid – liquid extraction might be increased by reducing the particle size, increasing the effective diffusion coefficient due to the increase in extraction medium temperature or change in the pH medium, and in the structure of material (Ben Amor and Allaf, 2009).

The antioxidant activity of roselle extract is pH dependent (pH 2 to 7), the activity decreases as pH increase. However, at a constant pH, only a relatively small decrease in antioxidant activity and total phenolic content is observed (Suliman *et al*., 2011).

Azza *et al*. (2011) found that time & temperature of extract processing have a great influence on anthocyanin and ascorbic acid stability and revealed that traditional high – temperature processing methods may cause color degradation and reduce its fresh flavours, therefore, it is essential to maintain a uniformity of color and ascorbic acid stability over the production run and avoid colour changes brought about by chemical reaction during processing.

Mady *et al*. (2012) showed that the solid to solvent ratio and the particle size had a strong effect on both extraction velocity and anthocyanin extraction yield but not enhance the anthocyanin content and they recommended with a compromise must be found between the anthocyanin extraction yield and the anthocyanin content.

The aim in this work, is to study the effect of temperature, contact time, and solvent – to – solid ratio on the aqueous anthocyanins extraction and evaluate the influence of all these variables on the efficiency of the batch extraction process and on obtaining a quality juice.

At the same time, optimize the parameters for obtaining a Roselle extract with both a high extraction yield and high concentration of anthocyanin.

Two aspects of the desorption of anthocyanin were evaluated:

1- The speed or release of the anthocyanins (desorption kinetics),
2- The extent of the anthocyanin desorption depending on the adsorption contact time.

Material and methods

Batch extraction:

To prepare the different solid – to – solvent ratio, sun dried flowers of roselle were purchased from a local market in Giza. Egypt. The flowers were from the Sudan region. One batch was used for all extractions.

Dried flowers & calyxes were manual grinding using a domestic blender (Ken wood chef Classic, model KM 330), particles were sieved through sieve of 100 um meshs and the powder collected for extraction using distilled water acidified with 2% citric acid (as a solvent). The solid – to solvent ratio used in $KgKg^{-1}$ were 1/5 (50g /250g distilled water, 1/15 (37.5 g/250g distilled water) and 1/25 (10g / 250g distilled water). The temperature values used were 25 ˚C and 90 ˚C, whereas the extraction time used were 30, 90 and 210 min.

Amber bottles of 250 ml were used as batch extractors and were maintained in a thermostatic water bath with slight orbital stirring (Memmert, Schwabach, Germany) at the extraction temperature. Fitted to a filled sealed bottle, was used as a control to measure the sample temperature during experiments. At specific time intervals, the bottles were taken from the batch extractor, immediately cooled by submersion in an ice bath then filtered with a 0.45 um filter before analysis. All trials and measurements were done in triplicate.
Eighteen combination samples differ in solid – to – solvent ratio, time, and temperature of extraction were obtained to reach the optimum conditions. The extraction yields of anthocyanin is defined regarding the initial concentration of anthocyanin in the solid.

Chemicals:

All chemicals used were at least analytical grade. 2, 2- diphenyl picrylhydrazyl (DPPH), gallic acid, and citric acid were obtained from Sigma chemicals Co. (St. Louis, USA) . Anhydrous sodium carbonate (Na_2Co_3), Folin-Ciocalteu phenol reagent, hydrochloric acid (HCL), potassium chloride (PCL), sodium acetate buffer and methanol alcohol were obtained from Merch (Darmstadt, Germany).

Methods

Determination of pH, total soluble solids content and titratable acidity:

The pH of the samples was measured with a glass – electrode digital pH meter (Cyberscan 500, Singapore). The total soluble solids content was determined using a digital refractometer (Atago, Tokyo, Japan) with a scale of o - 45˚ Brix, Total titratable acidity was determined by titrating 100 ml of the samples with 0.1 M NaoH until pH 8.1 and the results were expressed as percent malic acid. All determinations assessed according to AOAC (2000).

Determination of ascorbic acid:

The HPLC method for analysis of ascorbic acid (chromatography system) was modified from Wimalasiri and wills (2010) was used. Briefly. 4 ml of sample was mixed with 4 ml methanol and 10 ml distilled water, filterated through 0.45 um membrane filter (Whatman International Ltd, Maidstone, Uk). The first 3 ml of the filtrate was discarded and the next 1 ml was collected for analysis. 20 ul of the sample was injecting into the HPLC system, Column effluents were monitored at 254 nm, the UV absorbance maximum for ascorbic acid. The results were expressed in mg per 100 g dry weight.

Determination of total phenols:

Total phenols were determined by the Folin – Ciocalteu method of Chew *et al*. (2009). Briefly, 1.5 ml Folin – Ciocalteu reagent (10% v/v) was mixed with 1.2 ml 7.5% (w/v) Na_2Co_3 solution, then 0.3 ml sample solution was added. After a 30min incubation at room temperature in dark, the absorbance was measured at 765 nm. Gallic acid was used as a standard compound for the standard curves. The results were presented in mg gallic acid equivalent GAE/g extract. All the experiments were carried out in triplicate.

Determination of total anthocyanins:

Anthocyanins were determined by the pH differential method of Chew *et al*. (2009), Briefly, 1.0 ml from 0.2 M/L potassium chloride solution (pre-adjusted to pH 1.0 with 1.0M/L Hcl) or 1.0 M/L sodium acetate buffer (pre-adjust to pH 4.5 with 1.0 M/L HCL) was added into 2.0 ml of extracts respectively. The absorbances were then measured at 517 and 700 nm. The concentration of anthocyanin was calculated with the following formula : Conc. of Anthocyanin (mg/L) = Ax L x MWx 10^3 x D where, A is the difference of sample absorbance between pH 1.0 & 4.5. E is molar extinction coefficient for cyanidin – 3 – glucoside (26,900); L is the path lengh of the spectrophotometer cell (1.0 cm); D is the dilution factor; 10^3 is the conversion from g to mg. and MW is moleculer weight of cyanidin – 3 – glucoside (449.2 g/mol). The result was expressed as mg cyanidin – 3 – glucoside equivalent/g extract. All experiments were carried out in triplicate.

Total antioxidant activities:

Antioxidant activities of extracts measured included radical – scavenging activity (PSA). The method was based on procedures described by Chan *et al.* (2007). Radical – scavenging activity was determined using the 2,2 – diphenyl -1- picrylhydrazyl (DPPH) assay. Different dilutions of extracts (1 ml) were added to 2 ml of DPPH (5.9 mg/100 ml methanol). Absorbance was measured at 517 nm after 30 min. Antioxidant activity was presented as IC_{50} u/ml (concentration providing 50% inhibition).

Color measurement:

According to Hunter method (1958), color samples were measured using Hunter Lab colorimeter Ultrascan, model SN 7877 (Hunter Associates Lab Inc, Virginia), Color parameters were measured in the CIE Lab scale : L (lightness, 0 – 100), a (green to red) and b (blue to yellow). Roselle extract (20 ml) was placed in a rectangular

(10 x 53.5 x 54.7) quartz cell, and the color was measured in the transmittance mode, The purity (c), [color saturation] and hue (H) were calculated as follow; $C = \sqrt{a^2 + b^2}$ and $H = \tan^{-1}(b/a)$.

Statistical analysis:

The data obtained from color measurement was statistically subjected to analysis of variance (ANOVA) and means separation was by Snedecor and Cochran (1980). The least significant difference (L.S.D) value was used to evaluate the significance of differences between extraction time, temperature, and solvent ratios. One way analysis of variance was applied for determining differences between results of samples. Values of $p< 0.05$ were considered as significantly different.

Results and Discussion

Acidity, pH and T.S.S:

As a function of time and temperature for different solid – to – solvent ratios, acidity & pH of roselle extracts exhibited no significant varying degree (Table 1) and pH are within the range recommended by the Mexican Standard (a maximum pH 3).

The high acidity in the roselle calyxes extracts is given by organic acids such as citric, malic, tartaric, hibiscus, succinic, oxalic and ascorbic acid (Suliman *et al.,* 2011). Wills *et al.* (1998) reported that the pH value depends on the concentration of free H ions or mirrored the changes in total organic acids. The free state of H ions is due to dissociation of H ions from the carboxylic group (- CooH) of organic acid.

However, the extraction by different solid – to – solvent ratios and by increasing the temperature and time exhibited varying degree of total soluble solids (Table 1). This is because temperature and time increase the efficiency of extraction process which improve the availability of the soluble solids and facilitated the solvent (acidified water) access to the solutes within the solid.

Ascorbic acid:

The results in (Table 1) are consistent with the fact that roselle calyxes are rich in vitamin C (139.87 mg/100g). This may be attributable to the source of calyxes (Wong *et al.,* 2002). This indicated that roselle has a higher content of ascorbic acid than guava, orange and mango (Tee *et al*., 1997). Vitamin C. of roselle calyxes is related to the state of freshness or dryness.

Table (1) shows no significant effect of solid – to – solvent ratios and/or time on ascorbic acid content, but temperature was found to be the most significant factor affecting the ascorbic acid during the extraction. The model failed to show any significant interaction effects between solvent ratios or time with these dependent variables in relationship.

It is well known that heating at a higher temperature would cause the destruction of ascorbic acid. However, Umme *et al.* (1997) found that ascorbic acid concentration remained unchanged after thermal treatment and was still relatively stable at higher temperature for 3.5 h.

In our study, the optimum level of ascorbic acid obtained from roselle was achieved using a combination of solid – to acidified water ratio (1:25) using a combination of 25˚C for 210 min (and/or 90˚C for 210 min which obtained 145.37 mg/100g and 153.72 mg/100g respectively.

Table 1: Physiochemical properties or roselle extracted by different solid – to – solvent ratios, temperatures and times.

Time of extraction (min)	Parameters	Extraction temperature					
		25˚C			90˚C		
		Solid – to – solvent ratio					
		1/5	1/15	1/25	1/5	1/15	1/25
30	pH	2.82	2.82	2.80	2.81	2.80	2.80
	Acidity	11.57	11.57	11.79	11.68	11.79	11.79
	T.S.S (˚Brix)	16.34	16.89	17.30	18.34	18.59	18.77
	V.C mg/100g	139.87	139.95	140.16	143.24	145.07	148.21
90	pH	2.80	2.80	2.80	2.71	2.71	2.71
	Acidity	11.79	11.79	11.79	19.20	19.02	19.02
	T.S.S (˚Brix)	16.64	17.55	17.72	19.09	19.38	19.69
	V.C mg/100g	141.24	142.46	143.44	147.24	149.07	151.21
210	pH	2.73	2.73	2.71	2.70	2.70	2.69
	Acidity	18.85	18.85	19.02	18.93	18.93	18.98
	T.S.S (˚Brix)	17.80	17.88	18.13	20.40	20.68	20.75
	V.C mg/100g	142.55	144.19	145.37	148.22	150.12	153.72

** Titratable acidity was calculated as citric acid % (g/kg) .*

Color properties:

Table 2,3 shows color parameters of roselle calyxes powder and illustrated the effect of solid – to – liquid ratio at 25 ˚C for 210 min. and at 90 ˚C for 30 min respectively .

Natural constituents of organic acid in roselle calyxes such as malic, citric and 3- indlyl acetic acid played an important role in giving brilliant red color of sample extract (Al – Khtani and Hassan, 1990).

Former studies confirmed that distilled water which acidified with formic acid showed that the more the red color intensity observed (Galicia – Flores *et al*., 2008; Sandro and Jose, 2014).

Claudia *et al.* (2012) indicated that differences in color parameter values could be also due to the variety of roselle calyxes, method of extraction and the average particle size.

According our results, we obtained an optimum combination of solid: water ratio, temperature and time of extraction for reselle calyxes extracts using a calyxes: water ratio of 1 : 25 at 25˚C for 210 min which (a) was 49.87 whereas the color purity (c) was 51.39, it presented the highest purity (Table 2), and secondly by using a calyxes : water ratio of 1 : 25 at 90˚C for 30 min. which (a) was = 44.20 whereas the color purity (C) was 45.54.

No significant difference ($P \geq 0.05$) with regard to the color parameter was observed for the other different variable samples.

It can be concluded that, a solid : acidified water ratio (1:25) could be utilized to prepare an extract to be used in food as natural color at 25 ˚C for 3.5h and / or at 90 ˚C for 0.5h. equally.

Table 2: Effect of solid – to- solvent ratio on color properties of roselle calyxes extracted at 25 ˚C for 210 min.

Solid – to solvent ratio			
Parameter	1:5	1:15	1:25
L	31.05 ± 2.38 b	38.36 ± 0.19 a	22.49 ± 1.02 b
a	17.49 ± 2.15 b	22.75 ± 0.29 a	49.87 ± 1.69 b
b	5.54 ± 0.55 b	6.52 ± 0.15 a	12.46 ± 0.67 b
Hue (H)	17.65 ± 0.47 b	15.98 ± 0.12 a	14.04 ± 0.30 b
Purity (c)	18.33 ± 2.23 b	23.67 ± 0.30 a	51.39 ± 1.80 b

Values represent the mean ± standard deviation (n=5). Values that are followed by different letters within the same row are significantly different (P ≤ 0.05)

Table 3: Effect of solid to solvent ratio on color properties of roselle calyxes extracted at 90 ˚C for 30 min.

Solid – to solvent ratio			
Parameter	1:5	1:15	1:25
L	31.08 ± 2.40 b	31.03 ± 2.38 b	20.06 ± 0.44 a
a	17.43 ± 2.13 b	17.44 ± 2.12 b	44.20 ± 0.66 a
b	5.52 ± 0.55 b	5.55 ± 0.53 b	10.99 ± 0.20 a
Hue (H)	17.61 ± 0.47 b	17.65 ± 0.45 b	13.92 ± 0.07 a
Purity (c)	18.36 ± 2.24 b	18.39 ± 2.21 b	45.54 ± 0.78 a

Values represent the mean ± standard deviation (n=5).Values that are followed by different letters within the same row are significantly different (P≤ 0.05).

Extraction Kinetics:

The total anthocyanins extracted were plotted as a function of time for different solid – to – solvent ratios at ambient temperature (25˚C) and at hot conditions (90 ˚C).

The search for optimal operating conditions to maximize the efficiency of the process is necessary for industrial application. So, in this study, it has been found that the solid – to – solvent ratio had a strong effect on both extraction velocity and anthocyanin extraction yield. The main effect of the solid – to – solvent ratio was to increase the anthocyanin yield and decrease the anthocyanin content.

Higher temperature favored extraction by increasing diffusion coefficient on anthocyanins. This enhancement of the mass transfer process resulted in lower extraction time and had no positive influence on the anthocyanin yield (Mady *et al.,* 2012). But had only positive influence on the anthocyanin content.

Anthocyanins and phenols yield increased asymptotically tending to an equilibrium concentration, increasing the solid – to solvent ratio increased its yield.

The search for the solid – to – solvent ratio was the limit that could be used to completely submerge the roselle powder and will confirm, increasing the extraction efficiency. (Cid-Ortega and Guerrero-Beltran, 2014). Thus, in this study ranging from 1/5 to 1/25 were chosen for extraction process.

Total phenols & Anthocyanin content:

Phenolic compounds including hydroxyl benzoic acids, caffeoylquinic acids, flavonols, phenolic acids and anthocyanins are known to be responsible for antioxidant activities in fruits and fruits with higher phenolic contents generally show stronger antioxidant activities such as roselle fruit (Morales-Cabrera, 2013)

Highest ferric reducing ability of plasma values (FRAP) confirmed the antioxidant levels of any extract which due to the amount of anthocyanins and total phenol in the blend (Heba *et al.,* 2014).

The conditions for extraction of bioactive compounds (mainly anthocyanins and phenolic compounds) largely depend on type of sovent, solid – to – solvent ratio, time and temperature of extraction (Abou – Arab *et al.,* 2011) .

Roselle mild (25˚C) and hot (90˚C) water extracts were prepared in various solid-to-solvent ratio, and time – temperature combinations to determine equivalent extraction conditions regarding their physicochemical and phytochemical properties .

In our study, equivalent anthocyanins concentration and phenolic compounds content were obtained (Table 4) at 25 degrees C for 210 min using solid – to – acidified water ratio of 1:25. The contents were 1250 mg cyanidin – 3 – glucoside equivalent/100g dry weight and 4163 mg Gallic acid equivalent/100g dry weight respectively. The results showed that total phenolic and anthocyanins were better extracted with hot water that also resulted in a higher antioxidant capacity these extract. But Cisse *et al*. (2012) showed that the increase of temperature reduced. the extraction time but did not modify the extraction yield. When heated, the extracts were clearly less stable. Their color changed faster during storage especially when hot water were used, therefore temperature greatly affected the stability of the extracts during storage.

Also, Ramirez – Rodrigues *et al.* (2011) found that both cold and hot extractions, yielded similar phytochemical properties, however, under cold extraction, color degradation was significantly lower.

Accordingly, acidified water with 2% citric acid indicating anthocyanins yield of 1250mg/100g might be the best choice and the more preferable solvent compared with other solvents experimented by many solvent compared with other solvents experimented by many anthors, when the extraction process was carried out at 25 ˚C for 210 min (Fig 1).

These extraction conditions giving optimal extraction efficiencies for subsequent bioactivity experiments. It can be observed that the total phenols content showed behavior as the total anthocyanin content (Table 4) regarding to the independent variables. Fig (1, 2) illustrated the phenols & anthocyanin content as a function of temperature, time and solid – to – solvent ratios. This is in agreement with the findings of Salazar-González *et el.* (2012).

Differences in results from this work and other studies are mainly due to the variety of roselle, as well as the type of solvent, sample: solvent ratio and extracting methods.

Fig (3) shows response plots for the anthocyanin yield and concentration as a function of solid – to – solvent ratios ranging from 1/5 to 1/25. As expected, the solid – to – solvent ratio had a positive effect on the anthocyanin content.

All authors also found a linear link between temperature and solid – to – solvent ratio with phenols and anthocyanin extraction yield.

Table 4: Antioxidant activity, total phenols and total anthocyanins obtained under three variable conditions for extraction of roselle juice.

Time of extraction (min)	Parameters	Extraction temperature					
		25˚C			90˚C		
		Solid – to – solvent ratio					
		1:5	1:15	1:25	1:5	1:15	1:25
30	TP	714	995	2164	1450	1907	2218
	AN	518	777	925	610	943	948
	AA	13.07	19.50	22.94	15.36	23.39	23.52
90	TP	1165	1598	3330	2331	3130	3456
	AN	577	817	962	692	995	1000
	AA	18.5	26.0	31.0	22.00	32.00	32.24
210	TP	1540	2039	4163	2831	4046	4329
	AN	925	1012	1250	960	1087	1137
	AA	29.56	32.40	40.50	26.73	35.23	36.45

TP : Results of total phenol expressed as mg Gallic acid equivalent/100g dry weight.
AN : Results of anthocyanins content expressed as mg cyanidin – 3 – glucoside equivalent/ 100g dry weight.
AA : Results of antioxidant activity expressed as EC_{50} (u/ml)

Antioxidant activity:

The stable DPPH radical has been used widely for the determination of primary antioxidant activity that is the free radical scavenging activities of pure antioxidant compounds, plant and fruit extracts (Sukhapat *et al.* 2004). The assay is bassed on the reduction of DPPH radicals in methanol which causes an absorbance drop at 517 nm.

In this study, roselle extracts exhibited varying degrees of antioxidant activity. A higher antioxidant activity 40.50 u/ml was obtained using 1:25 solid – to – water ratio at 25 ˚C after 210 min. (Table 4 & fig 3).

It can be observed that the activity of antioxidant [the lowest of the amount of sample (u/ml) needed for 50% decrease of the initial DPPH concentration (EC_{50})] increased as phenolic compounds and anthocyanin Contents increased. It, thus, confirms that phenolic compounds and anthocyanins have an important role in antioxidant activity (Harborne, 1998). Also can be concluded that acidified water extracts, had a good hydrogen donating abilities, indicating abilities, indicating that they had effective activities as radical scavengers relative to any other solvents.

The sequence for DPPH radical – scavenging abilities were shown in fig (4), it was observed that 1 : 25 ratio of solid : solvent extract had the highest radical – scavenging activities among the three extracts at 25 ˚C

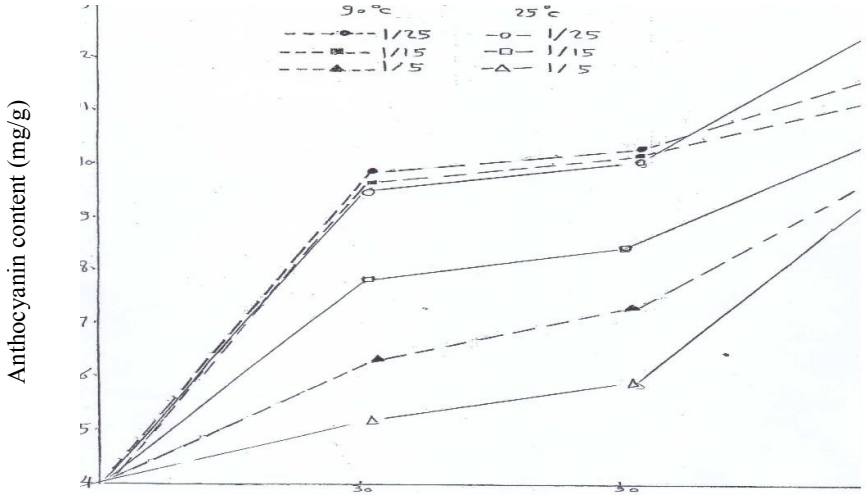

Fig. 1: Anthocyanin content as function of time at 25˚C and 90˚C for different solid – to – solvent ratios

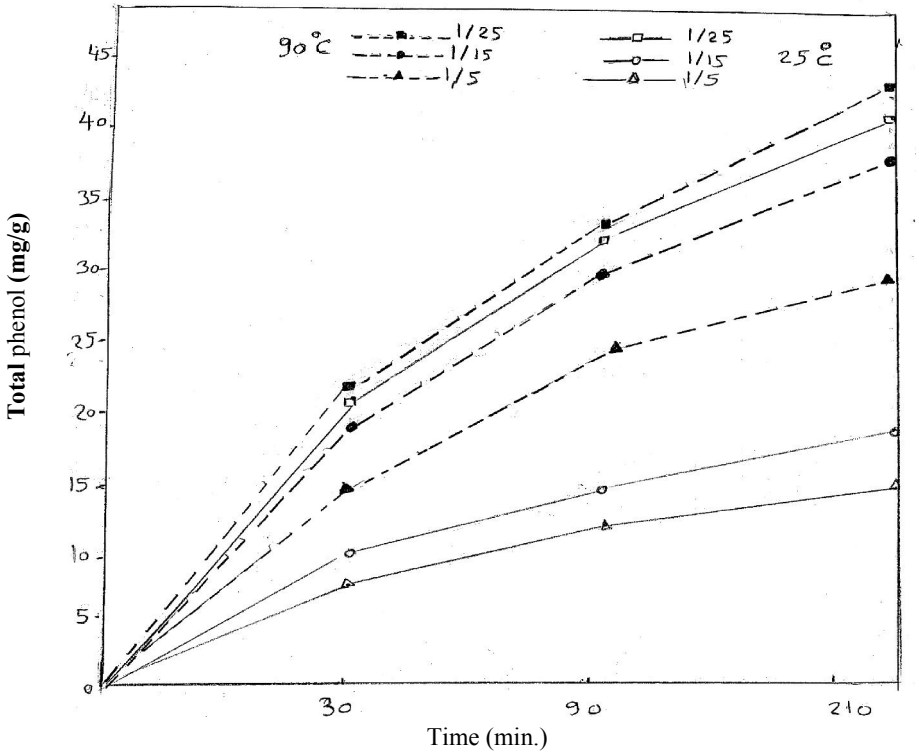

Fig. 2: Phenol compounds as function of time at 25˚C and 90˚C for different solid – to – solvent ratios

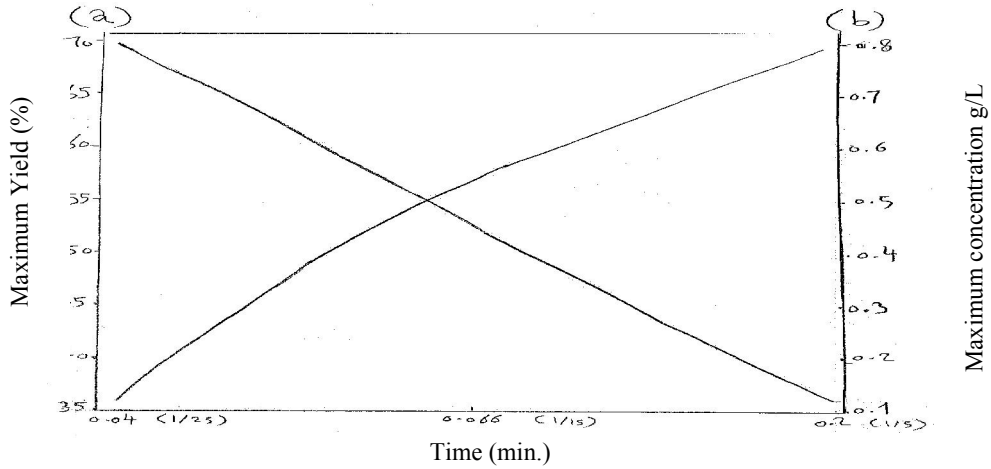

Fig. 3: Simulated values of maximum extraction yields of anthocyanins (a) and maximum anthocyanins content (b) estimated with solid – to – solvent ratios at 25˚C

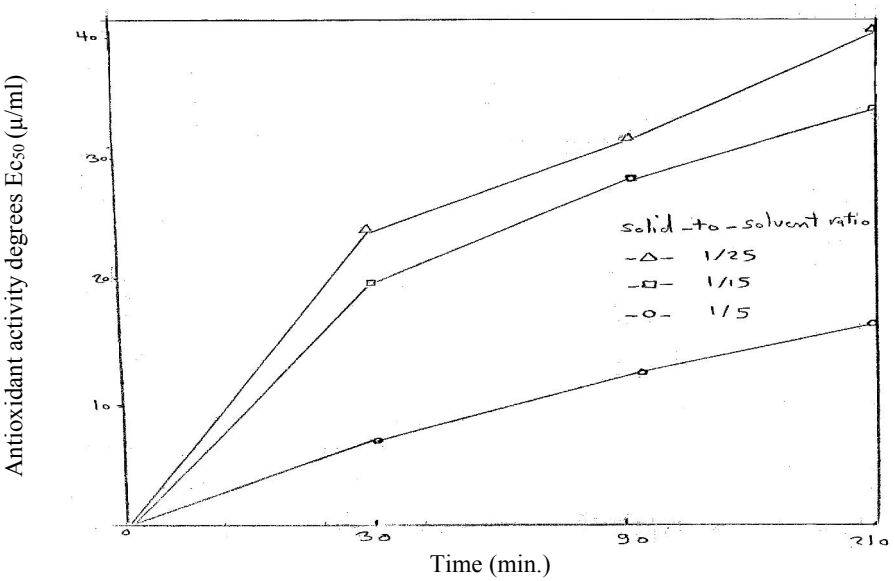

Fig. 4: Antioxidant activity degrees as function of time at 25˚C for different solid – to – solvent ratios

Conclusions

The attempt to study the optimal operating conditions to maximize the efficiency of the extraction process has been successfully carried out. The goal of this work was very close to those determined from the model that coupling the solid – to liquid ratio, retention time and extraction temperature to degrade the anthocyanine, and in the same time increasing the yield and decrease the content. So in this study, results showed that the greater the roselle extracted by water acidified with citric acid 2% the more red color intensity observed. Citric acid could rise the polarity of the aqueous solvent which increase the extractable compounds and extraction yields.

It has been found that the solid – to – solvent ratio (1/25) had a more effective on both extraction velocity and anthocyanin extraction yield indicating anthocyanins yield of 1250 mg/100g dry weight and vitamin C yield of 145 mg/100g dry weight. Also anthocyanin and vitamin C were still relatively stable at low temperature (25˚) for 3.5h . Notably, our correlation study indicates that the total phenol contents and anthocyanins of this extract played major roles in the antioxidant activity.

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