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Arab Univ.
J. Agric. Sci.,
Ain Shams Univ.,
Cairo, 18(1), 155-163, 2010

UTILIZATION OF ACEROLA FRUIT AS A SOURCE OF POWERFUL ANTIOXIDANT FOR ENRICHMENT OF SOME PROCESSED FOODS

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Keywords: Acerola, Ripening stages, Drying, Phytochemical compounds, Jam

ABSTRACT

Four stages of maturity acerola fruits (*Malpighia glabra L*.) namely green, mature green/yellow, pale red and ripe mature were analyzed in fresh and dried at 50˚C under vacuum. Also, fresh fruits were used to prepare jam and mixed with different ratios of fig fruit. Results showed that protein content was gradually decreased from 1.12 for green to 0.82% for ripe mature stage of acerola, while, the titratable acidity was decreased gradually with increasing the maturity stage while, the pH and soluble solids were gradually increased. The highest percentages of reducing and total sugars were observed for pale red stage and the lowest were showed in green stage. Also, ascorbic acid was higher in green/yellow mature while in pale red and ripe stages were lower. Phenolic compounds were analyzed by HPLC in acerola fruit. The detected phenolic compounds were gallic, protocatechuic, catchein, catechol, chlorogenic, caffeic, syringic, ellagic, ferrulic, coumarine and cinnamic in both fresh and dried acerola. The green/yellow maturity of acerola extract recorded the highest percent of total antioxidant activity (74.46 %) while the lowest total antioxidant activity was observed in the ripe mature acerola (20.75%). Sensory evaluation of jam prepared from acerola and mixed with fig 1:1 recorded the highest scores compared to other prepared jams. Meanwhile, the content of ascorbic acid and total antioxidant activity were increased by increasing the percent of acerola fruit.

INTRODUCTION

Acerola *(Malpighia glabra L.),* originally from Antilles, was cultivated in Brazil nowadays, the major worldwide producer, consumer and exporter **Vera de Rosso *et al* (2008).**

Acerola, also known as cherry in West Indian, Barbados cherry or Antilhas Cherry *(Malpighia glabra L., Malpighia punicifolia L. or Malpighia emarginata DC.)* and grows also in the northeast of South America and in Central America. It is round shape, with diameter varying from 3 to 6 cm. Its fleshy and succulent pulp surrounded by a very thin protection peel which helps for quickly ripening. At the initial stage of ripening, the fruit is a full green color, changing to yellow-reddish and finally to red or purple when completely ripened **(Assis *et al* 2001).**

Acerola rich in other nutrients such as carotenes, thiamin, riboflavin, niacin, proteins, and mineral salts, mainly iron, calcium and phosphorus **(Assis *et al* 2001)**. The composition of acerola fruit depends on some factors such as climatic conditions, the culture treatments and geographic location also stage of maturation or processing and storage **Hanamura *et al* (2005).**

Nowadays, Acerola plants were introduced to Egypt through Horticulture research institute, Agricultural Research Center in September, 2003. Acerola is a potential crop in Egypt which has perfectly environmental conditions climate and soil appropriate for the acerola culture and also need to low water requirement, low labor input and low crops management.

Acerola is recognized as a functional food due to its main appealing feature as high Vitamin C content, which might vary from 1247.10 to 1845.79 mg/100 g **(Lima *et al* 2005)**; the consumption of

(Received October 10, 2009)
(Accepted November 2, 2009)

three fruit units for an adult per day satisfies the recommended dietary allowance of vitamin C. **(Mezquita and Vigoa, 2000)**.

The Vitamin C contents in the concentrated immature, the immature, and the mature acerola juices were 4.80, 1.90 and 0.97 g/100 g for respectively. While, the total phenol contents decreased during ripening, from 3.8 mg of catechin/g for immature acerola juice to 1.4 mg/g for mature acerola juice. The concentrated immature juice had a content of 9.2mg/g of juice **(Righetto *et al* 2005)**.

The physico-chemical characteristics of mature acerola fruits contained total anthocyanins from 3.81 to 47.4 mg/ 100 g of pulp, total flavonols from 7.00 to 18.5 mg quercetin/ 100 g pulp, titratable acidity (TA) from 1.04 to 1.87 g of malic acid/ 100 g of pulp, total soluble solids from 7.00 to 8.43 Brix/ TA ratio from 4.36 to 6.86 and pH from 3.11 to 3.41 **(Lima *et al* 2005)**.

Recently, much attention has been paid to their content in carotenoids and bioflavonoids for their antioxidant properties**.** Carotenoids are present at levels between 3.2 and 406mg/kg **(Lima *et al* 2005). Mezadri *et al* (2005)** found that, acerola contained highest content of β-carotene was (40-60% of total carotenoids). The main components of acerola fruit from flavonoids are anthocyanins (3.79-59.74mg/100g) and flavonols (7.0-18.5 mg/100g) **(Mezadri *et al* 2008)**. Besides the colorant property, **(Seeram & Nair, 2002)** found that the anthocyanins have the colorant property and also as an exhibit potential therapeutic effect for anti-inflammatory, radiation-protective, chemoprotective, vasoprotective, inhibition of LDL oxidation and decrease the risks of cardiovascular diseases.

The association between the consumption of fruit and vegetables affects for reducing the risk of cardiovascular disease and cancer which are supported by considerable epidemiological evidence **(Hertog *et al* 1995 and WHO, 2003).** This beneficial effect is due to the action of antioxidant compounds, which are capable of neutralizing free radicals and reduce oxidative damage in the body **(Clifford, 1995).**

The low stability of the acerola anthocyanins represents a problem during the storage of the pasteurized juice and frozen pulp of this fruit. The presence of ascorbic acid (AA) has shown a negative impact on anthocyanin stability, leading to the mutual degradation of these compounds **(Garzon & Wrolstad, 2002 and Brenes *et al* 2005).**

The aim of this study is to determine the content of natural antioxidants of acerola such as vitamin C, total carotenoids and anthocyanin at different maturity stages for fresh and dried acerola, as well as to evaluate the polyphenols compounds by using HPLC which also considered as effective antioxidant. The acerola fruit behavior and quality attribute of jams prepared with mixing of acerola and fig fruit was also undertaken.

MATERIALS AND METHODS

1- Sample preparation

Acerola fruits (*Malpighia glabra L.*) at different maturity stages were carefully harvested from plants grown in Horticulture research institute, Agriculture research center, Giza, Egypt at season 2008 in March and April. Twenty kilograms of sampling acerola fruit were divided to four mature stages first stage (green), second (mature green/yellow), third (pale red) and fourth (ripe mature). These samples were performing to dried or used as fresh then press crushing. Care was taken to have homogeneity in each lot. Once harvested, the fruits were frozen and stored at −18 ˚C until analysis was performed. Then they were unfrozen and immediately dried at 50˚C under vacuum.

2- Preparation of mixed jam

One kilogram of jams was prepared by mixing acerola fruit pulp and fig fruit 1:1 and 1:2 and sucrose was added 1:1 W/W then homogenized and cooked in stainless vessel until 60% of dry matter was achieved.

3- Sensory evaluation

The sensory evaluations of acerola jam were carried out with three replications by 10 trained panelists. The quality attributes of acerola jam were organized into flavour (odour and taste), texture, colour and over all acceptability. All organized attributes were scored between 0 (the worst) and 10 degree.

4- Chemical analysis

Moisture, protein, ash, titratabe acidity, pH, reducing sugar, non reducing sugar and total sugars were determined according to **AOAC (1990).**

Total soluble solids were determined by using refractometer at ~ 25˚C and the result expressed as Brix.

Pectin was determined according the method described by **Shelukhina and Fedichkina (1994).**

Determination of phenolic compounds

Extraction, separation and quantification of phenolic compounds were determined according to the method described by **Goupy *et al* (1999).**

Determination of total antioxidant activity of acerola

Antioxidant activity was determined according to the method of **Brand-Williams *et al* (1995)** and modified by **Zhang & Hamauzu (2004)** as follows: Five grams of acerola fruit pulp at different ripening stages was extracted by 100mL 80% methanol. Different concentrations 10 to 50 µmL were used to determine the percents of antioxidant activity using 2, 2-diphenyl-1picryl hydroxyl (DPPH) purchased from Sigma Co.

Total phenolics were estimated with the method described in **(AOAC 1970)** by using photometric method with folin's reagent**.**

Flavonoids were extracted and determined according **Zhuang *et al* (1992).**

Carotenoids and ascorbic acid were determined according to **(AOAC 1990).**

Total anthocyanins were determined according to the method described by **Colin and Peter (1980)**.

5- Statistical analysis

All data were recorded as means and analyzed by SPSS Windows (ver.10.1.). One-way analysis of variance (ANOVA) and Duncan comparisons were tested to signify differences between raw and different treatments of acerola.

RESULTS AND DISCUSSION

Results in **Table (1)**. Shows the chemical composition of acerola fruit at different stages of maturation (green, green/yellow, pale red, ripe mature). Protein content was gradually decreased from 1.12 for green to 0.82% for ripe mature. No significant decrease in ash content was observed at different maturity stages of acerola from green to ripe mature. Meanwhile, the titratable acidity was gradually decreased with increasing maturity stages of acerola from 14.17% for green mature stage to 8.00% in ripe mature, calculated with NaOH 0.1N/ 100g of fresh acerola, On the other hand, the pH and soluble solids were gradually increased with increasing the maturity stage. Where, the TSS were 7.99 in green, 8.44 in green/yellow, 8.83 in pale red and 8.21 in ripe mature while pH ranged from 3.03 to 3.27, respectively. The highest reducing and total sugars were observed with green/yellow stage of acerola. The corresponding results were 2.72 and 4.3%. Meanwhile the lowest percentages of both reducing and non reducing sugars were in green stage, 1.63 and 2.32%, respectively. Results also show that the pectin content was 0.49% in the green stage and decreased to 0.32 % through increasing the maturity stages.

The phytochemical compounds in fresh acerola in comparison with dried acerola are shown in **Table (2)**. Results reveal that the contents of ascorbic acid in green, green/yellow, pale red and ripe mature was observed 1430, 1568.67, 1317.33 and 842.16 mg/100g, respectively calculated on fresh weight. However the highest content of ascorbic acid was observed in (green/yellow), but decreased in ascorbic acid was observed in ripe mature. Ascorbic acid was greatly reduced from the green to the red fruit with a loss of about 50%due to the biochemical oxidation. This is supported by the appearance of 3-hydroxy-2-pirone which was found only in ripe acerola as result of the oxidative breakdown of ascorbic acid. **(Vendramini & Trugo, 2000).** The corresponding contents of ascorbic were 552.66, 582.92, 547.09 and 366.82 after drying acerola at 50˚C under vacuum, respectively, while phenols and flavonoid contents had the highest content in green/yellow stage. The content of both phenols and flavonoids were 2935.5 and 14.21 mg/100g, while the lowest content was 2259.99 and 10.88 mg/100g which showed in ripe mature stage. Both phenols and flavonoids content were significantly decreased to 25.17 and 26.56% in (green), 21.68 and19.35% in (green/yellow), 22.37 and 26.82% in (pale red) and 27.53 and 34.19 % in (ripe mature). Carotenoids content was determined and the results are illustrated in **Table (2)**. The total Carotenoids content was increased by increasing the maturity stage. The total carotenoids content ranged from 1010.1µ/100g in green stage increased to 1924.0µ/100g in ripe mature stage while, these contents were decreased to 745.6 and 1467.05 µ/100g, respectively after drying samples. On the other hand, total anthocyanins was drastically increased from 2.1 in green acerola to 14.79mg/100g in ripe mature stage of acerola and the corresponding contents were decreased after drying of acerola to 1.07 and 5.36mg/100g, respectively. The rich of acerola fruits in these phytochemical compounds play an important role as a beneficial healthy effect owing the action of antioxidant compounds, which are capable of neutralizing free radicals and reduce oxidative damage in the body **(Clifford, 1995).**

Table 1. Chemical composition of acerola at different maturity stages depending on colour

	green	green/yellow	pale red	ripe matue
Moisture	88.12c	89.22b	9.04a	90.21a
Protein	1.12a	1.07a	0.95b	0.82c
Ash	0.54a	0.53a	0.53a	0.52a
Titratabe acidity *	14.17b	15.67a	9.5c	8d
pH	3.03b	3.1ab	3.18ab	3.27a
Soluble solids	7.99b	8.44ab	8.83a	8.21b
Reducing sugar	1.63d	2.32b	2.72a	1.76c
Non-reducing sugar	0.69d	1.88a	1.58b	1.37c
Total sugar	2.32c	4.2a	4.3a	3.13b
Pectin	0.49a	0.41b	0.36bc	0.32c

*** in ml of NaOH 0.1 N/ 100 g of sample**

Table 2. Phytochemicl compounds at different maturity stages in fresh and dried acerola

Constituents	Fresh acerola				Dried acerola			
	Green	Green/ yellow	Pale red	Ripe mature	Green	Green/ yellow	Pale red	Ripe mature
Vitamin C mg/100g	1430b	1568.67a	1317.33c	842.17d	552.47b	582.83a	547.07b	366.83c
Polyphenols mg/100g	2752.13b	2953.5a	2533.97c	2259.99d	2059.42b	2313.18a	1967.12c	1637.81d
Flavonoids	12.5c	14.21a	13.76b	10.88d	9.18c	11.46a	10.07b	7.16d
Carotenoids µ/100g	1010.1d	1226.6c	1346.8b	1924a	745.6d	801.7c	1034.2b	1467.05a
Total anthocyanin mg/100g	2.1d	4.42c	7.75b	14.79a	1.07d	2.29c	4.36b	5.36a

Eleven compounds of phenols were identified in the second stage of mature (green/yellow) for either fresh or dried by HPLC analysis. The detected phenolic compounds were gallic, protochatechuic, catchein, chatechol, chlorogenic, caffeic, syringic, ellagic, ferrulic, coumarine and cinnamic as shown in **Table (3) and Figs. (1 and 2).** The highest content of phenolic compounds was protocatechuic 34.77 mg/g then decreased to 27.54 mg/g after drying under vacuum at 50˚C followed by ferrulic 10.12 in fresh ripening acerola mg/g decreased to 2.19 after drying while catechol and cinnamic had the lowest acerola phenolic compounds recorded (1.21 and 0.42mg/g) in fresh acerola and (0.40 and 0.18mg/g) after drying, respectively. These results were agreement with that obtained by **Righetto et al (2005)** which identified some phenolic compounds such as catechin, gallic

Table 3. Phenolic compounds mg/100gm of fresh and dried acerola

	Fresh acerola	Dried acerola
Gallic	3.19	1.77
protocatechuic	34.77	27.54
Catchein	7.26	0.98
Catechol	1.21	0.4
Chlorogenic	5.08	3.59
Caffeic	1.74	2.01
Syrngic	3.46	0.81
Ellagic	8.61	4.56
Ferrulic	10.12	2.19
Coumarine	1.81	0.53
Cinnamic	0.42	0.18

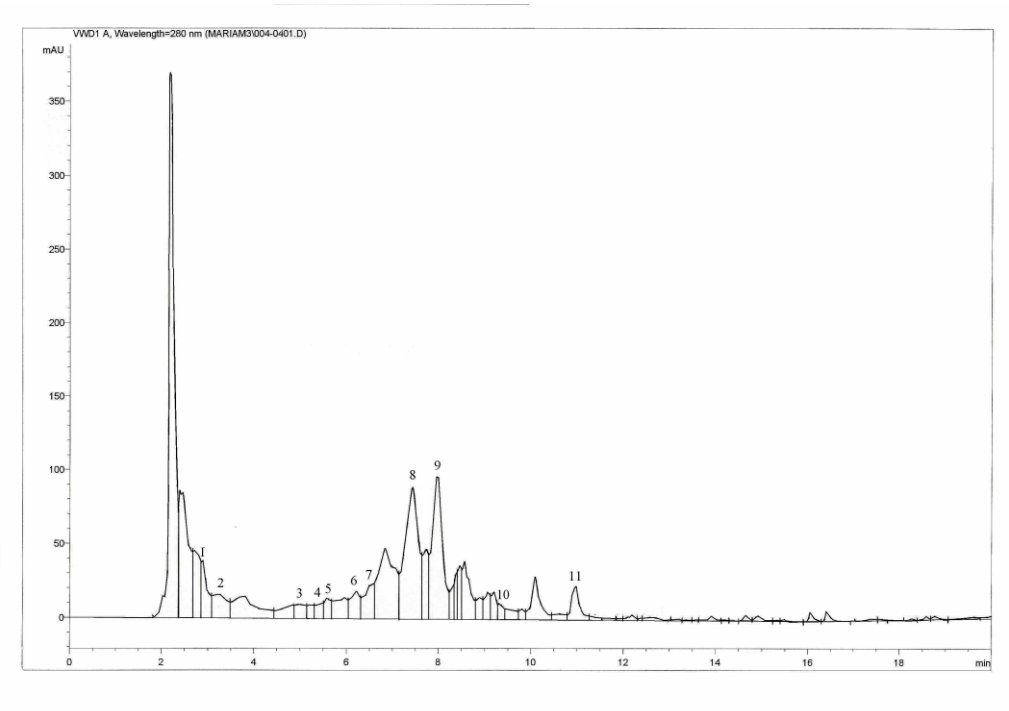

Fig. 1. Chromatogram of phenolic compounds in fresh acerola 1. gallic, 2. protochatechuic, 3. catchein, 4. chatechol, 5. chlorogenic, 6. caffeic, 7. syringic, 8.ellagic, 9. ferrulic, 10. coumarine and 11. cinnamic

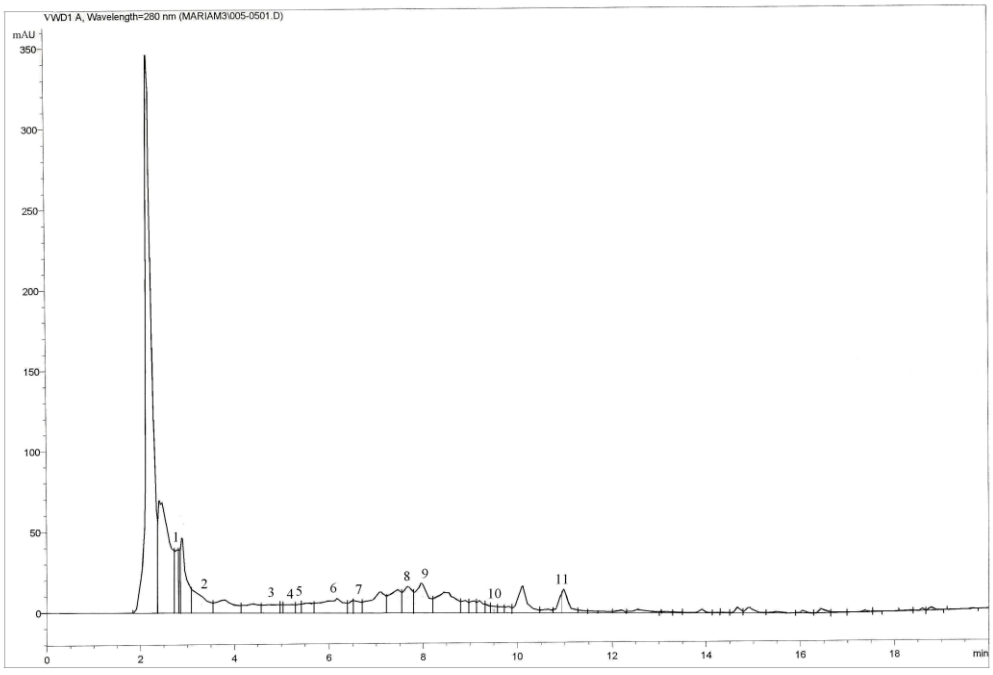

Fig. 2. Chromatogram of phenolic compounds in dried acerola 1. gallic, 2. protochatechuic, 3. catchein, 4. chatechol, 5. chlorogenic, 6.caffeic, 7.syringic, 8.ellagic, 9. ferrulic, 10.coumarine and11.cinnamic

acid, coumaric acid, syringic acid, caffeic acid and ferrulic acid in prepared immature acerola juice by HPLC analysis.

Results in **Table (4)**. Shows that total antioxidant activity (TAA) of acerola in different maturity stages. One hundred ml 80% methanol concentrations was used to extract 5gm fresh acerola. After that, 10 to 50µmL were taken to determine the total antioxidant activity (TAA). These results revealed that antioxidant activity of acerola was gradually increased by increasing the extracted concentration. On the other side, the second stage (green/yellow) had the highest percentage of total antioxidant activity was found at 50µmL which was 74.46 %, while the lowest activity was obtained in ripe mature stage which recorded 20.75% of TAA. These results indicating that the antioxidant compounds present in acerola such as ascorbic acid, phenols, flavonoids and anthocyanins with high percentages which acts together as an important role for antioxidant activity. Also, it contains highest antioxidant activity of both gallic and protochatechuic acid and our results correlated with the results obtained by **Scherer & Godoy (2009)** which used as a new antioxidant activity index AAI determined by DPPH method and they found the gallic acid was higher value of AAI (27) followed protochatechuic acid (20) and these two compounds may play an important role as strongly antioxidant compounds in acerola fruit.

Sensory evaluation of acerola jam prepared by acerola fruit pulp as will as mixing acerola with fig fruit 1:1 and 1:2 (w/w) is illustrated in **Table (5)**. Results revealed that no significant differences in the colour and odor in all prepared jams. While, acerola pulp was mixed with fig fruit 1:1 w/w recorded significant increased in taste and texture comparing with other jam samples. Meanwhile, the lowest score was observed in jam prepared by fig fruit. Also, acerola was mixed with fig 1:1 recorded the highest score compared with other prepared jam. At the same time, jam prepared by acerola pulp and acerola mixed with 1:2 fig recorded moderate score while the lowest score was obtained with fig fruit jam. Conclusively, acerola fruit improved the quality of fig jam comparing with fig jam without acerola.

Results in **Table (6)**. Show the content of ascorbic acid, phenolic compounds and antioxidant activity in acerola fruit jam and jam prepared from acerola and mixing with fig fruit 1:1 and 1:2 w/w. Results show that the content of ascorbic acid was increased by increasing the percent of acerola fruit in prepared jam mixed between acerola and fig caused by the high content of ascorbic acid. The contents of ascorbic acid were 286.14, 220.84 and 178.95 mg/100g in acerola jam, acerola mixed with fig (1:1) and acerola mixed with figs (1:2), respectively, while the lowest content was 3.08 mg/100g jam in fig fruit jam. Also, phenolic compounds were 109.79mg/100gm in fig fruit jam and increased to 384.62 mg/100gm with acerola jam. At the same time, it could be noticed that there were a gradually decreased from 346.15 and 283.22mg/100g for prepared acerola jam mixed with fig (1:1) and acerola mixed with fig (1:2), respectively. Results indicated also, the highest percent of total antioxidant activity in acerola fruit jam 89.17% (by extracting with 100ml methanol 80% from 5gm acerola jam), while the lowest percentage was 3.02% for fig fruit jam.

Table 4. Antioxidant activity (%) at different maturity stages of fresh acerola

Extracted acerola* µmL	Green	Maturity stages Green/yellow	Pale red	Ripe mature
10	4.94	8.5	7.11	4.55
20	9.49	17	12.06	7.71
30	16.6	20.95	22.6	11.6
40	24.31	44.47	34.39	16.21
50	66.01	74.46	55.99	20.75

*** 5gm fresh acerola extracted by 100mL 80% methanol**

Table 5. Sensory evauation of jam prepared from acerola fruit and mixed with fig fruit

	Colour	Taste	Odor	Texture	Over all acceptability
Fig fruit	7.5a	7b	7a	6.44b	6.83b
Acerola fruit	7.66a	7.28ab	7.61a	7.72a	7.44ab
Acerola with fig 1:1	7.88a	8.17a	7.67a	8.06a	8.28a
Acerola with fig 1:2	7.94a	7.78ab	7.39a	7.44ab	7.61ab

Table 6. The content of ascorbic acid, phenolic compounds and antioxidant activity in acerola jam and mixed with fig fruit

	Fig fruit	Acerola fruit	Acerola with fig 1:1	Acerola with fig 1:2
Ascrbic acid mg/100gm	3.08d	286.14a	220.84b	178.95c
Phenolic compounds mg/100gm	109.79d	384.62a	346.15b	283.22c
Total antioxidant activity %	3.02	89.17	60.43	45.28

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