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

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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, catechin, 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

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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 9.2 mg/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 406 mg/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 (3.79-59.74 mg/100 g) and flavonols (7.0-18.5 mg/100 g) (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 Bienes 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 attributes 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 maturity 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 homogenity 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 panellists. 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, titratable 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 µL were used to determine the percents of antioxidant activity using 2, 2-diphenyl-1-pircyl 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 acid 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

| Constituent            | Green  | Green/yellow | Pale red | Ripe mature |
|------------------------|--------|--------------|----------|-------------|
| 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       |
| Titrateable acid *     | 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. Phytochemical 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 maturation (green/yellow) for either fresh or dried by HPLC analysis. The detected phenolic compounds were gallic, protocatechuic, catechin, 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

| Phenolic Compound | Fresh acerola | Dried acerola |
|------------------|---------------|---------------|
| Gallic           | 3.19          | 1.77          |
| Protocatechuic   | 34.77         | 27.54         |
| Catechin         | 7.26          | 0.98          |
| Catechol         | 1.21          | 0.4           |
| Chlorogenic      | 5.08          | 3.59          |
| Caffeic          | 1.74          | 2.01          |
| Syringic         | 3.46          | 0.81          |
| Ellagic          | 8.61          | 4.56          |
| Ferrulic         | 10.12         | 2.19          |
| Coumarine        | 1.81          | 0.53          |
| Cinnamic         | 0.42          | 0.18          |

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

Sensory evaluation of acerola jam prepared by acerola fruit pulp as well 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 increased 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.

| 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

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

|                | Colour | Taste | Odor  | Texture | Overall 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 |
|----------------|-----------|--------------|----------------------|----------------------|
| Ascorbic 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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