# Contents of Phenolics and Flavonoids and Antioxidant Activities in Skin, Pulp, and Seeds of Miracle Fruit

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**Abstract:** Miracle Fruit (*Synsepalum dulificum*) has been studied because of its unique taste modifying properties. This study investigated contents of phenolics, flavonoids, and antioxidant activities in skin, pulp, and seeds of Miracle Fruit. The free phenolic content in skin was almost 3 times of that in pulp and 4 times of that in seeds. Skin contributed 43.96% of free phenolic compounds with 15.91% of freeze-dried solids due to its high phenolic content. As the trend observed for phenolic content, the free flavonoid content in the skin was tremendously higher than that in the seed and pulp. The skin contributed about 52% of total flavonoid with 15.91% of dried solids. On other hand, the differences in the bound phenolic contents were not so distinct among the 3 components. The free antioxidant activities in skin and pulp were comparable, and were significantly higher than that in seeds. Although the antioxidant activities in seeds was considerably lower than that in skin, 49.45% free antioxidant activity, 76.41% bound antioxidant activity, and 58.56% of total antioxidant activity were contributed by seeds due to about 66% of solid of total solids. In general, the results of antioxidant activities using sequential methods were higher than that using direct method. This study suggests that Miracle Fruit is a good source not only for flavor and color, and also antioxidant activity for functional food applications.

**Keywords:** antioxidant activity, flavonoids, Miracle Fruit, phenolic

## Introduction

The Miracle Fruit shrub, *Synsepalum dulificum* (Daniell 1852), also *Richardella dulcifica* (Brouwer and others 1968) is indigenous to tropical West Africa. The Miracle Fruit plant was introduced to the U.S. Dept. of Agriculture at Federal Experiment Station in Puerto Rico (Inglett and May 1968). The 6 to 15 ft height shrub yields ripe red fruits (Figure 1) from December to June. Miracle Fruit berries have unusual taste modifying properties of allowing sour substances to taste amazingly excellent sweetness after the inside of the mouth has been thoroughly exposed to the fruit's mucilaginous pulp (Inglett and others 1964; Brouwer and others 1968). The reduction in sourness may be the result of mixture suppression (Bartoshuk and others 1974).

Research on Miracle Fruit was initiated by Inglett (1964) while searching for natural sweeteners to replace saccharin and cyclamate at the research center of International Minerals and Chemicals Corporation (IMC). The unique taste-modifying principles of the freeze–dried pulp were investigated by mild extractive procedures and polar–nonpolar extractions (Inglett and others 1965). The Miracle Fruit concentrate gave a tan-colored fraction of colloidal materials composed of mucilages, proteins, lignins, and cellulosic materials (Inglett and others 1965). The active principle could be a glycoprotein with a molecular weight of 44000 (Kurihara and others 1968). Acid hydrolysis of the insoluble colloidal fractions gave amino acids (arginine, histidine, and lysine) as well as sugar degradation products (Inglett and others 1965). Miraculin, a taste-effecting glycoprotein from Miracle fruit, was purified by ion exchange column chromatography (Giroux and Henkin 1974).

Besides Miracle Fruit's unique potential to make sour food taste sweet, the pigment from red-colored skin could be a natural color food ingredient. Anthocyanin and flavonol pigments of miracle fruit (*Synsepalum dulificum*, *Schum*) were isolated and identified as cyaniding-3-monogalactoside, cyaniding-3-monoglucoside, cyanidin-3-monoarabinoside, delphinidin-3-monogalactoside, and delphinidin-3-monoaabinoside by paper chromatography and spectral analysis (Buckmire and Francis 1976). Miracle fruit has been studied to improve insulin resistance induced by fructose-rich chow in rats (Chen and others 2006). Other research on the extraction of phenolic compounds from wine by-products has resulted in commercial extracts of grape seeds and/or skins. Also, considerable attention has been given to the consumption fruit for health purposes because of the fruit extract (*Vitis vinifera*) influence on serum lipids, low, and high density lipoproteins-cholesterol, and also blood glucose (Jassim and others 2010). Research on alternative antioxidants sources from various fruit products is considered important. The antioxidant activity profile of miracle fruit has not been studied, so this research was conducted to investigate the free and bound antioxidant activities and flavonoids in each Miracle Fruit component portion.

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## Materials and Methods

### The source of Miracle Fruit sources

Fresh Miracle Fruit berries, *Synsepalum dulificum (Richardella dulcifica)*, were contributed from Curtis Mosie and Jose Fernando Aristizabal of the Miracle Fruit Exchange, Southwest Ranches, Florida.

The skin was separated from pulp and seeds of Miracle Fruit, and skin and pulp-seeds were freeze dried separately. Dried pulp-seeds were separated into pulp and seeds after freeze–drying. The skin, pulp and seeds were ground individually to fine powder before analysis.

### Extraction methods

**Sequential extraction.** The sequential extraction including 2 steps was modified upon a procedure by Adom and others (2002).

(1) Double extraction for free compounds

Freeze–dried samples (100 mg) were extracted twice with 10 mL of 50% ethanol by mixing with a vortex mixer. Each extraction step was followed by centrifugation at 1462 × *g* for 10 min. Combined supernatants were used for free phenolic and antioxidant activity measurements.

(2) Alkaline extraction for bound compounds

The solid residue from double extraction was hydrolyzed with 5 mL of 2 *N* sodium hydroxide for 1 h under $N_2$ by shaking in the dark at room temperature. The alkaline extracts were neutralized by 5 mL 2 N HCl and centrifuged at and centrifuged at 1462 × *g* for 10 min. The supernatants were used for bound phenolic and antioxidant activity measurements.

**Direct extraction.** Freeze–dried sample (10 mg) was transferred to a centrifuge tube. Total of 1 mL of 50% ethanol was added and vortexed. The reaction was started by adding 1 mL of 200 $\mu$M 2,2-diphenyl-1-picryl-hydrazyl (DPPH) as described by Serpen and others (2008) and subsequently. The mixture was vortexed every 5 min until centrifugation at 1460 × *g* for 10 min. The supernatants were used for antioxidant activity measurements.

### Phenolic content analysis

Phenolic content was determined by the Folin–Ciocalteau colorimetric method as described previously with minor modifications (Waterhouse 2001; Yu and Zhou 2004). Briefly, 7.9 mL of deionized water and 0.5 mL of Folin–Ciocalteau reagent (F9252, Sigma Aldrich, and St. Louis, Mo., U.S.A.) were added to 100 $\mu$L of extract, mixed on a vortex mixer, and 1.5 mL of 1.85 *M* $Na_2CO_3$ was added after 15 min. Absorbance of samples was measured at 765 nm after 2 h using gallic acid as a standard. Results were expressed as milligram of gallic acid equivalents per gram d.m.

Figure 1–Miracle Fruit shrub with ripe berry.

### Antioxidant activity analysis

Antioxidant activity was determined by reacting 1 mL of the extracts with 1 mL of 200 $\mu$M 2,2-diphenyl-1-picryl-hydrazyl (DPPH) as described by Şensoy and others (2006). Absorbance was measured at 515 nm wavelength after 40 min reaction in dark. Cloudiness occurred after mixing the reagent with extracts; therefore, tubes were centrifuged for 10 min at 1462 × *g* prior to reading the absorbance at 515 nm. Results were expressed as micromol of 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) equivalents per gram d.m.

### Flavonoid analysis

Method was modified based on the previous procedure (Hung and Morita 2008). A portion of 0.5 mL of extract was mixed with 1.5 mL of 95% ethanol, followed by the addition of 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water made up to 5 mL. Absorbance was measured at 415 nm wavelength after 30 min reaction in dark. Cloudiness occurred after mixing the reagent with extracts; therefore, tubes were centrifuged for 10 min at 1462 × *g* rpm prior to reading the absorbance at 415 nm. The flavonoid contents were expressed as microgram of rutin equivalent per gram d.m.

### Statistical analysis

Duplicated extractions were analyzed in triplicate. Data were analyzed using SAS software (version 8, SAS Inst., Cary, N.C., U.S.A.) using analysis of variance (ANOVA) with Tukey's (1993) multiple comparison adjustment to determine significant differences ($P < 0.05$) between treatments.

## Results and Discussion

### Freeze–dried solid contents

Lyophilization of frozen skin, pulp, seeds gave 3.9, 4.4, 16.2 g of dried materials, respectively, from per 100 g of fresh Miracle Fruit (Table 1). About 16%, 18%, and 66% of freeze–dried solids were contributed by skin, pulp, seeds, respectively (Table 1).

### Phenolic contents

The free phenolic content (Table 2, 52.72 mg/g) in skin was almost 3 times of that found in pulp (16.95 mg/g) and 4 times of that the seeds (11.56 mg/g). On other hand, the differences in bound phenolic contents were very distinct among the 3 components. The skin contributed 43.96% of free phenolic compounds in the 16% of freeze–dried Miracle Fruit solids due to its high phenolic content (Table 1 and 2), while seeds contributed 68.69% of bound phenolic content because of high solids (66.01%).

**Table 1–The percentage of weights and solids were contributed by each component of Miracle Fruit.**

| | Weight% (wet basis) | Solid% (dry basis) |
|---|---|---|
| Seeds | 16.23 | 66.01 |
| Pulp | 4.44 | 18.07 |
| Skin | 3.91 | 15.91 |
| Water | 75.22 | |
| Whole | 100.00 | 100.00 |

### Flavonoid contents

A similar trend to phenolic contents was observed for flavonoid contents of Miracle Fruit (Table 3). The free flavonoid content in the skin was significantly higher than that in the seed and pulp. The differences in bound flavonoid among the skin, pulp, and seeds were not as great as for free flavonoid. The skin having only 15.91% of the solids contributed about 52% of total flavonoid content. The decrease in cholesterolemia was 14% for the animals treated with the fruit skin extract and 12.8% for the animals that received seeds extract, while that for LDL-cholesterol and triglycerides after the administration of extract from grape peel was 12% and 12.4%, respectively (Jassim and others 2010). These results provide strong suggestive clinical data that fruits with high antioxidants activity, including Miracle Fruit, could be good for health.

### Antioxidant activities

The free antioxidant activities from skin (17.95 $\mu$mol/mg) and pulp (19.55 $\mu$mol/mg) were similar, and both were higher than that found in seeds (9.47 $\mu$mol/mg, Table 4). The sulfur-containing amino acids were oxidized during the isolation procedures to cysteic acid and methionine sulfone (Inglett and others 1965). This could explain lower antioxidant activity in skin compared to the phenolic content in skin. Some antioxidant activity may be lost during double extraction and freeze–dry procedure, or they are not completely released in the analysis and still remained in the solids. The antioxidant activity in the skin was not tremendous high but it was significantly higher than that in seeds and pulp. The bound antioxidant activity in pulp was extremely low suggesting that antioxidants in the pulp were not easy to bind possibly due to pulp structure.

The free antioxidant activity for seeds was about a half compared with the skin and pulp. Extremely lower bound antioxidants (0.33 $\mu$mol/g) were found in pulp (Table 4). Although the antioxidant activity in seeds was lowest among the 3 components, approximate 49% free antioxidant activity, 76% bound antioxidant activity, and 58% of total antioxidant activity were contributed by seeds based on its' 66% of the total solids. Thus, the seeds could be a good resource to be utilized for antioxidant activity.

The free antioxidant activity of seeds using direct methods was comparable to that using double extraction (Table 4). Free antioxidant activities from pulp and skin using direct method were significantly lower than that from pulp and skin using double extraction (Table 4). The free antioxidant activity was possibly not completed by single extraction using direct method. Again, it suggests that the antioxidant activity in skin maybe bound to cell walls and was not easily extracted by single extraction. In general, the antioxidant activities using sequential methods were higher than that using direct method since bound antioxidant activities were added to total antioxidant activities. Therefore, the direct method does not appear to be suitable for measuring antioxidant activity of the Miracle Fruit.

The high antioxidant activity in Miracle Fruit skin could be related to the high total phenolic and flavonoid contents. Similar results were found in a previous publication (Molina-Quijada and others 2010) where high contents of gallic acid and flavonoids found in the grape skin extracts had high antioxidant activities.

## Conclusions

The portions of the Miracle Fruit skin and pulp have all valuable antioxidant activities with the largest quantities found in the seeds. This study suggests that the Miracle Fruit could have some

**Table 2–The free, bound, total phenolic contents in each component of Miracle Fruit (mean ± standard deviation; $n = 3$) and percentage contributions to total phenolic content.**

| | Phenolic content (mg/g) | | | Phenolic compounds (%) | | |
|---|---|---|---|---|---|---|
| Sample | Free | Bound | Total | Free | Bound | Total |
| Seeds | 11.56 ± 0.01 c[A] | 6.99 ± 0.54 a | 18.55 ± 0.68 c | 39.99 | 68.69 | 47.46 |
| Pulp | 16.95 ± 0.00 b | 5.63 ± 0.00 b | 22.58 ± 0.00 b | 16.05 | 15.15 | 15.82 |
| Skin | 52.72 ± 0.47 a | 6.82 ± 0.11 ab | 59.54 ± 0.36 a | 43.96 | 16.16 | 36.72 |
| Whole | | | | 100.00 | 100.00 | 100.00 |

[A]Values with different letters denote the significance ($P < 0.05$) for each comparison among treatments in the respective column.

**Table 3–The free, bound, total flavonoid contents in each component of Miracle Fruit (mean ± standard deviation; $n = 3$) and percentage contributions to total flavonoids.**

| | Flavonoid content ($\mu$g/g) | | | Flavonoids (%) | | |
|---|---|---|---|---|---|---|
| | Free | Bound | Total | Free | Bound | Total |
| Seeds | 0.04 ± 0.02 c[A] | 0.84 ± 0.24 a | 0.88 ± 0.27 b | 2.91 | 78.43 | 36.02 |
| Pulp | 0.79 ± 0.05 b | 0.28 ± 0.01 a | 1.07 ± 0.04 b | 15.76 | 7.16 | 11.99 |
| Skin | 4.63 ± 0.29 a | 0.64 ± 0.01 a | 5.27 ± 0.28 a | 81.32 | 14.41 | 51.99 |
| Whole | | | | 100.00 | 100.00 | 100.00 |

[A]Values with different letters denote the significance ($P < 0.05$) for each comparison among treatments in the respective column.

**Table 4–The free, bound, total antioxidant activities in each component of Miracle Fruit (mean ± standard deviation; $n = 3$) and percentage contributions to total phenolic content; compared antioxidant activity from Sequential method to Different Methods.**

| | Sequential method ($\mu$mol/g) | | | Antioxidant activity (%) | | | Direct ($\mu$mol/g) |
|---|---|---|---|---|---|---|---|
| Sample | Free | Bound | Total | Free | Bound | Total | Total |
| Seeds | 9.47 ± 0.08 c[A] | 7.47 ± 0.11 b | 16.94 ± 0.18 c | 49.45 | 76.41 | 58.56 | 9.47 ± 0.15 a |
| Pulp | 19.55 ± 0.04 a | 0.33 ± 0.08 c | 19.88 ± 0.12 b | 27.95 | 0.92 | 18.82 | 9.57 ± 0.12 a |
| Skin | 17.95 ± 0.00 b | 9.19 ± 0.01 a | 27.15 ± 0.00 a | 22.60 | 22.66 | 22.62 | 7.88 ± 0.14 b |
| Whole | | | | 100.00 | 100.00 | 100.00 | |

[A]Values with different letters denote the significance ($P < 0.05$) for each comparison among treatments in the respective column.

unique applications in functional foods for improving American health. Its antioxidant activity, particularly in seeds, could perhaps find some potential industrials applications. Future research using fresh Miracle Fruit for identifying phenolic compounds will be considered.

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