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ORIGINAL ARTICLE

POTENTIAL NUTRITIONAL VALUES OF SKIN, PULP AND SEED OF MIRACLE FRUIT (*SYNSEPALUM DULCIFICUM)*

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ABSTRACT

Background The miracle fruit or berry is a wild fruit so far known for the unusual taste modifying properties of its pulp to induce a sweet flavor to acidic products. Consequently, much research on this fruit has focused on the pulp and its properties to the detriment of the other constituent parts of the fruit which have nutritional potentials that deserve to be investigated. **Objectives**: This study aims to determine the physicochemical characteristics of the fruit, taking into account all its constituent parts, namely skin, pulp and seed. **Methods**: The methods used to characterize these parts are visual observation, chemical analyses and atomic absorption spectrophotometry. **Results**: Investigations revealed that the miracle berry is a small oval shaped wild berry with an average weight of about 0.94 to 1.28 g and an average length of about 2.12 to 2.40 cm. The fresh fruit consists of 23.74% skin, 35.45% pulp and 41.60% seed. The skin and the pulp are acidic (pH = 3.16-4.02). The skin, pulp and seed contain varying proportions of proteins (15 - 22%), fats (2 - 12%) and carbohydrates (66 - 84%). Major minerals such as K, P, Ca, Na and Mg are present and traces of heavy metals such as Pb, Cu, Cd and Ni have been noted. These different parts of the fruit also contain 0.6 - 1.16% dietary fibers and 27.42 - 72.16 mg/g of highly reactive polyphenol compounds. **Conclusion:** This study showed that all the different parts of the miracle fruit are rich in proteins, fats, carbohydrates, vitamin C, mineral elements and polyphenolic compounds. The skin as well as the seed can be used as supplements in human and animal food. Their significant levels of highly reactive polyphenols give them the quality of ingredients that could be used in the formulation of nutraceutical preparations for the protection of human health.

Keywords: *Miracle berry, constituent elements, chemical composition, food.*

1. INTRODUCTION

The savannahs and forests of tropical countries are full of important plant species. However, most of these species are poorly known and less valued [1]. Among these species is *Richa(r)della dulcifica* also known as *Synsepalum dulcificum*, a shrub belonging to the family of Sapotaceae [2, 3] and originating in West Africa. Its geographical area extends from Ghana to Congo. The growing interest in this shrub is due to its fruits. These very perishable ones are called "miracle fruits" or "miracle berries" because of their extraordinary property to change an acid flavor into a sweet one [4, 5]. The main virtue of this fruit is that it removes acid sourness from what one eats. So this fruit allows eating a slice of lemon without shuddering. Although not sweet, this fruit has the property of changing flavors and its consumption results in a loss for almost two hours of sensitivity to acidity or bitterness and everything that is tasted thereafter has a sweet taste. Indigenous people suck the fruit before consuming stale and sour corn bread, sour palm wine or beer to make them sweet [6]. This sugaring inducing property is due to a fruit glycoprotein called "miraculin" [7, 8] contained in the pulp of the fruit [9]. In order to make it more available and more accessible in time and space like so many other molecules or proteins of interest, miraculin has been the subject of several works of genetic engineering for its transgenesis in more widely cultivated and consumed crop species. Early production trials of miraculin were carried out on the microbial strain *Escherichia coli* [10] followed by transgenic yeast and tobacco [5]. The results of this work were not very successful because the recombinant miraculin obtained did not induce the sweet taste in its hosts. The work of Masuda et al. (1995), which resulted in isolation and sequencing of the gene coding for miraculin, were a turning point for the first successes in the production of recombinant miraculin from transgenic plants [11]. Sun et al. (2007) manage to produce miraculin in transgenic lettuce and tomato [12]. Thus, many works carried out on the miracle berry relate almost exclusively to miraculin. It is recently that some researches have been somewhat interested in the composition of the fruit in organic constituents, mineral elements [13, 14, 15] and phytochemical elements and their antioxidant properties [13-16, 17, 18]. These previous studies have focused all their attentions on the pulp containing the miraculin to the detriment of the other parts of the fruit. This work has the merit of expanding the field of knowledge on the fruit as a whole by presenting the physicochemical characteristics and the chemical constituents of each part namely the skin, the pulp and the seed in order to determine their nutritional and bioactive potentials.

2. MATERIALS AND METHODS

2.1 Materials:

The study focused on miracle berries that were collected in the maritime and plateaus regions (Togo) during the maturity and harvest season (September-November 2015). These wild fruits were picked from the homes of private individuals in Lomé and in Noépé and Vogan areas. Others were bought at the market of Lomé (Agoè-Asiyéyé) which is supplied by localities like Kpalimé, Azanhoun and Aflao.

The *S. dulcificum* fruits of interest in this study was small bright red berries similar in size to olives. The fruits were cleaned and washed and skin, pulp and seed were manually isolated from the fruits. The skin was obtained by exerting finger pressure on the apex of the fruit after having caused an opening at the receptacle by the hand or a blade. The pulp was obtained by scraping with a blade all the tissue surrounding the seed. The almond of the seed was obtained by breaking the shell of the seed by hand. These various parts were dried in an oven at 50 °C for 48 hours and then placed in jars and stored at 4 °C for the various tests.

2.2 Methods:

The shape and color were determined by visual observation. The weigh was determined by weighing and the size by measurement of the length. The acidity of the berries was determined on fruit extracts using the digital pH meter (HANNA HI 98127). The determination of soluble solids was carried out by refractometry. Water content was determined by the oven drying method [19] (AFNOR, 1988). Ashes were determined by incineration of a test sample in a muffle furnace at 550 °C for 6 hours. Proteins were assayed by the Kjeldahl method [20] and the fats were extracted with the ether-type Rafatec II Sohxlet [21]. The rate of carbohydrates was obtained by the difference between the dry sample and the sum of moisture, proteins, fats and ashes. The spectrophotometric and colorimetric determination of minerals was carried out after mineralization of the samples and the slurry was then solubilized by acid etching on a sand bath using concentrated nitric acid and hydrogen peroxide. The concentrations of potassium, calcium, sodium, copper, magnesium, manganese and zinc were determined by atomic absorption spectrophotometry and those of phosphorus and iron by colorimetry [22]. The vitamin C content was determined by the method described by Deymie et al. (1981) [23]. Dietary fibers were determined according to the AOAC method [20]. The content of total phenolic compounds was determined by the Folin-Ciocalteu method [24] and the proanthocyanins were assayed by spectrophotometry at 517 nm after extraction with butanol-HCl. The antiradical activity was measured by the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay.

2.3 Statistics:

The results are presented as mean ± standard deviation of three trials and are statistically treated by the Microsoft Excel 2007 program. One-factor ANOVA and Fisher's Least Significant Difference (LSD) method were used for comparison of results. Similarly, the T-test was used to compare two sets of observations ($p < 0.05$).

3. RESULTS

3.1 Physical characteristics of the miracle berry:

Physical characterization revealed that the miracle berry is a small oval shaped wild fruit of about 0.94 to 1.28 g and 2.12 to 2.40 cm long. Visual observation also identified two types of fruit, one with a pointed apex and the other with a rounded apex. The isolation of the different parts of the fruit showed that the miracle fruit consists from the outside towards the inside, of the skin, the pulp, the shell and the almond. Pulp constitutes the predominant part of the fruit (36.19% of the fresh weight) followed by almond or seed (33.65%), skin (23.69%) and shell (6.47%). The unripe fruit is green in color and at the stage of ripeness, sharp-pointed apex berries are light red and those with rounded apex become bright red. The pulp appears whitish in the sharp-pointed fruit while it varies from light pink to red in the rounded apex fruit. The almond or seed of whitish color in the fruit with pointed apex becomes greenish in the fruit with rounded apex.

3.2 Chemical characteristics:

The miracle fruit had a very slightly sweetened pulp with a higher soluble dry matter content (Table 1) (19.6±3.21 Brix) in sharp-pointed fruit than in rounded apex fruits (12.0±1.41 Brix). Extracts of the fruit skin and pulp were found to be acidic (Table 2) with a pulp pH (3.30±0.14) slightly more acidic than that of the skin (3.95±0.07).

Table 3 shows the composition of organic constituents of the different parts of the combined different types of miracle fruits. The results of this table showed that the water contents of dried skin, pulp and seed were respectively 12.00±0.22%, 16.44±0.28% and 6.70±0.09% while the moisture content of the whole fresh fruit was 63.11±0.40%.

The mean total protein content of the skin (20.81±2.16%) was relatively but not significantly ($p<0.05$) higher than that of the pulp (15.33±4.5%) and the seed (16.62±0.26). The skin was the richest part in fat contents (11.76±0.67%) followed by the seed (9.39±0.26%) and the pulp (1.83±0.48). The total carbohydrate contents of the seed (66.90±1.15%) and the pulp (65.07±3.95%) were significantly higher than that of the skin (54.35±2.79%).

Table 1: The table presents the dry matter contents (degree Brix) of whitish and reddish miracle berry pulps

Fruit pulp	Dry matters (Brix)
Whitish pulp	19.6±3.21^{a}
Reddish pulp	12.0±1.41^{b}

Values are mean ± standard deviation of triplicate determination. Values with different superscripts in the same column are significantly different ($P<0.05$).

Table 2: The table presents pH values of miracle berry skin and pulp extracts.

Component extracts	pH
Skin extracts	3.95±0.07^{a}
Pulp extracts	3.30±0.14^{b}

Values are mean ± standard deviation of triplicate determination. Values with different superscripts in the same column are significantly different ($P<0.05$).

Table 3: The table presents the chemical composition of each component of miracle fruit.

Fruit components	Moisture	Proteins	fats	Carbohydrates	Ashes	Dietary fibers	Vitamin C (mg/00g. wb)
Skin	12,00±0,22^{a}	20,81±2,16^{a}	11,46±0,67^{a}	54,35±2,79^{a}	1,38±0,02^{a}	1,08±0,01^{a}	23,0±0,13^{a}
Pulp	16,44±0,28^{b}	15,33±4,51^{a}	1,83±0,48^{b}	65,07±3,95^{b}	1,33±0,02^{a}	1,16±0,01^{a}	5,63±0,00^{b}
Seed	6,70±0,09^{c}	16,62±0,26^{a}	9,39±0,26^{a}	66,90±1,15^{b}	0,39±0,01^{b}	0,57±0,06^{b}	16,89±0,00^{a}

Values are mean ± standard deviation of triplicate determination. Values with different superscripts in the same column are significantly different ($P<0.05$). Wb = wet basis

Table 4: The table presents the levels of some mineral element compositions of each component of miracle fruit.

Fruit components	Na	K	Ca	P	Fe	Mg	Ni	Zn	Pb	Cu	Cd
Skin	626,67±66,58	1296,67±268,39	303,33±5,67	106,59±10,28	23,33±11,54	230,00±113,58	9,15±0,24	12,39±2,10	1,42±0,53	0,01±0,00	0,07±0,00
Pulp	646,67±55,08	1463,33±306,00	423,33±17,90	120,96±4,61	54,33±14,01	293,33±25,17	16,10±0,68	48,53±4,69	1,92±0,01	0,02±0,00	0,11±0,00
Seed	580,00±40,00	805,00±77,78	480,00±30,00	29,29±8,30	12,50±2,50	260,00±80,00	10,21±0,27	6,94±0,26	2,54±0,03	0,04±0,00	0,05±0,00

Values are mean ± standard deviation of triplicate determination; Na = sodium; K = potassium; Ca = calcium; P = phosphorus; Mg = magnesium; Fe = iron; Zn = zinc; Ni = nickel; Cu = copper; Pb = lead; Cd = cadmium.

Table 5: The table presents total polyphenolic compound and proanthocyanin contents and the half maximum effective concentrations of polyphenols in each component of miracle fruit.

Fruit components	Total Polyphenols	Proanthocyanins	CE_{50} (%)
Skin	72,16±24,56^{a}	53,92±4,92^{a}	0,03±0,00
Pulp	41,26±2,37^{b}	22,80±1,67^{b}	0,05±0,00
Seed	27,42±5,46^{c}	11,50±3,87^{c}	0,04±0,00

EC_{50}: Half maximum effective concentration or concentration of polyphenolic compounds at which 50% of its maximum response is observed. Values are mean ± standard deviation of triplicate determination. Values with different superscripts in the same column are significantly different ($P<0.05$).

The composition in mineral substances (ashes) gave values for the skin (1.38±0.02%) and the pulp (1.33±0.02%) higher than those of the seed (0.39±0.01%). The dietary fiber contents of the skin (1.08±0.01%) and the pulp (1.16±0.01%) were substantially equal but greater than those of the seed (0.57±0.06%). On the basis of the fresh material, the miracle berry skin was richer (23.0±0.13 mg/100 g) in vitamin C, followed by the seed (16.89±0.00 mg/100g) and the pulp (5.63±0.00 mg/100g).

Table 4 illustrates the composition in mineral elements of the various components of the miracle fruit. Data in this table showed that skin, pulp and seed analyzed contain the same minerals but in varying amounts. Except for calcium, lead and copper contents in the seed, sodium (646.67±55.08 mg/100 g), potassium (1463.33±306.00 mg/100 g), calcium (423.33±17.90 mg/100 g), phosphorus (120.96±4.61 mg/100g), magnesium (293.33±25.17 mg/100 g), iron (54.33±14.01 mg/100 g), nickel (16.10±0.68 mg/100 g), zinc (48.53 ± 4.69 mg/100 g), lead (1.92±0.01 mg/100 g), copper (0.02±0.00 mg/100 g) and cadmium (0.11±0.00 mg/100 g) in the pulp were higher compared to those estimated respectively in skin and seed at 626.67±66.58 and 580.00±40.00 mg/100 g for sodium, 1296.67±268.39 and 805.00±77.78 mg/100 g for potassium, 303.33±5.67 and 480.00±30.00 mg/100 g for calcium, 106.59±10.28 and 29.29±8.30 mg/100 g for phosphorus, 230.00±113.58 and 260.00±80.00 mg/100 g for magnesium, 23,33±11,54 and 12,50±2,50 mg/100 g for iron, 9,15±0,24 and 10,21±0,27 mg/100 g for nickel, 12.39±2.10 and 6.94±0.26 mg/100 g for zinc, 1.42±0.53 and 2.54±0.03 for lead, 0.01±0.00 and 0.04±0.00 for copper and 0.07±0.00 and 0.05±0.00 for cadmium.

The results of the measurement of the phenolic contents and the antioxidant activity of the various parts of the miracle berry (Table 5) showed that the skin was richer in polyphenolic compounds (72.16±24.56 mg/g) and proanthocyanins (53.92±4.92 mg/g) than the pulp (respectively 41.26±2.37 mg/g; 22.80±1.67 mg/g) and the seed (27.42±5.46 mg/g; 11.50±3.8 mg/g). As a result, the effective polyphenol compounds concentration to produce 50% reduction of DPPH (EC_{50}) for the skin extracts was lower (0.03%) and hence higher antioxidant activity than those of the seed (0.04%) and pulp (0.05%).

4. DISCUSSION

Fruits of *Synsepalum dulcificum* have two main harvest seasons in Togo: March-April and September-October. On the other hand, studies of the physical characteristics of these wild fruits identified at least two types of fruit: one with a pointed apex and the other with a rounded apex confirming the finding made by other previous works [6]. Observations of the physical characteristics also revealed that the miracle fruit is a small, ovoid-shaped berry with an average weight of 1.11±0.17 g and an average size of 2.26±0.14 cm. The previous characteristics are similar to those described earlier by other authors [25, 26, 27]. The constituent parts of the fruit are from the outside towards the inside: the skin, the pulp, the shell and the kernel of the seed. The skin that accounts for about 24% of the weight of the fresh fruit is the envelope of the fruit. It is green in color and when the fruit is ripe, it becomes light red in fruits with sharp apex and bright red or purple in fruits with rounded apex. This difference in color at maturity of the fruit led previous authors [28] to identify in Nigeria two varieties of yellow and red fruits.

The pulp is the fleshy part of about 36% of the fresh fruit, which covers the seed. It is whitish in berries with pointed apex and pale pink to red in berries with rounded apex. Each fruit contains one seed [29] consisting of an almond protected by a shell. The hull of 6% of the weight of the fresh fruit is dark brown and made of a smooth area and a whitish rough area. The latter is traversed in its length by a ridge [25] which divides it into two equal parts. The seed of about 34% of the fresh weight is composed of two juxtaposed cotyledons. It has a whitish color in fruits with pointed apex and greenish in fruits with rounded apex. The significantly ($p<0.05$) higher dry matter content (19.6±3.21 Brix) in the whitish pulps compared to that (12.0±1.41 Brix) in the red pulps might be due to variety, edaphic and climatic factors. The pH value (3.30±0.14) recorded for the pulp is comparable to that of the literature (3.2) [15] and shows a high acidity of the pulp. Some previous studies have shown that miraculin is more soluble and keeps its stability andtaste-modifying ability at low pH [30, 31].

The high moisture content of the whole fresh fruit (63.11±0.40%) indicates that this fruit is a perishable commodity justifying its rapid degradation after harvesting. However, this moisture content remains above the values measured by other research works in Nigeria [13, 14, 15].

The mean total protein contents of the skin (20.81±2.16%), the pulp (15.33 ± 4.5%) and the seed (16.62±0.26) are practically close to the values recorded for the seed by certain authors [14] whereas they remain significantly ($p<0.05$) higher than determined for the pulp by other studies [13-15]. According to some authors [13] all amino acids are present in the pulp proteins at variable rates with a predominance of tryptophan. The yield of extracting the fat contained in the various components of the miracle fruit showed some variability. Fat contents were (1.83±0.48%) for the pulp, (9.39±0.26%) for the seed and (11.76±0.67%) for the skin which appears to be the richest part in fat while the pulp is less affluent. These results are consistent with those of the literature for the pulp [13-15] and the seed [14]. The total carbohydrate in the skin (54.35±2.79%), pulp (65.07±3.95%) and seed (66.90±1.15%) are significantly higher than those of the literature [13, 14, 15]. The skin and pulp have substantially equal levels of dietary fibers (1.08±0.01% and 1.16±0.01%, respectively) but these values are greater than those of the seed (0.57±0.06%). These values are close to those of the literature for the seed [14] but lower for the pulp [13]. Dietary fibers are essential in the daily diet because they have a laxative action, play an important role in the intestinal transit of food and have a beneficial effect against obesity and cardiovascular diseases with a probable role in the prevention of colon cancer [32] however the miracle berry does not appear to be a good source. The seed is poorer in ashes (0.39±.01%) compared to the skin (1.38±0.02%) and the pulp (1.33±0.02%). These levels are lower than recorded by other studies [13, 14] carried out on pulp and seed. On wet material basis, the skin of the miracle berry is the richest in vitamin C (23.0±0.13 mg/100g), followed by the seed (16.89±0.00 mg/100g) and the pulp (5.63±0.00 mg/100g). Vitamin C or ascorbic acid is an antioxidant absolutely necessary for living beings. It is synthesized by all animals except the primates, therefore its contribution by diet is indispensable for human being. It plays a vital role in the growth, resistance and health of body tissues. A deficiency of vitamin C causes affections of which the most serious is the scurvy, with severe manifestations. The richness of the miracle fruit in vitamin C has already been reported by other investigations [13] making this fruit a good remedy against diseases related to vitamin C dietary deficiency.

Concerning mineral elements, the results showed that the different components of the miracle fruit contained in varying quantities the same elements (sodium, potassium, calcium, phosphorus, magnesium, iron, zinc, nickel, copper, lead, cadmium). Compared to the other components of the fruit, the pulp included, with exception for three mineral elements: calcium (423.33±117.90 mg/100g), lead (1.92±0.01 mg/100g) and copper (0.02±0.00 mg/100g), the highest proportions (mg/100g) in sodium (646.67±55.08), phosphorus (120.96±4.61), magnesium (293.33±25), iron (54.33±14.01), nickel (16.10 ± 0.68), zinc (48.53±4.69), cadmium (0.11±0.00) and potassium (1463,33±306,00). The latter appears as the most representative element in all parts of the fruit. Other authors [13] were unable to detect minerals as magnesium, potassium, sodium, manganese and lead in the pulp, and the concentrations recorded for the other minerals were lower. The skin is the part that comes second with respect to mineral content with proportions (mg/100g) slightly lower in sodium (626.67±66.58), potassium (1296.67±268.39), calcium (303.33±5.67), phosphorus (106.59±10.28), magnesium (230.00±113.58), iron (23.33±11.54), nickel (l9.15±0.24), zinc (12.39±2.10), lead (1.42±0.53), copper (0.01±0.00) and cadmium (0.07±0.00). Finally, the seed is the richest fraction of the fruit in calcium (480.00±30.00 mg/100g), lead (2.54±0.03 mg/100g) and copper (0.04±0,00 mg/100g) but also the least concentrated (mg/100g) in sodium (580.00±40.00), potassium (805.00±77.78), phosphorus (29.29±8.30), iron (12.50±2.50), nickel (10.21±0.27), zinc (6.94±0.26) and cadmium (0.05±0.00). Almost all the aforementioned elements were also detected in the seed of the miracle fruit by other authors [14] but at lower proportions and always with a predominance of potassium. The diversity of results compared to the literature may be related to the state of maturity of the fruits and the climatic and edaphic conditions of the environment of culture. The presence of traces of heavy metals such as lead and cadmium which are toxic to the biological system [33] may cause concern and could be explained by soil and air pollution. Indeed most of the analyzed miracle berries come from urban areas where the human polluting activity is increasing.

The different constituent parts of the miracle fruit were found to be rich in polyphenolic compounds and proanthocyanins or flavonoids with respective contents (mg/g) of 72.16±24.56 and 53.92±4.92 for the skin, 41.26±2.37 and 22.80±1.67 for the pulp and 27.42±5.46 and 11.50±3.87 for the seed. The phenolic compounds were concentrated to 51.23% in the skin, 29.29% in the pulp and 19.47% in the seed. Furthermore, these data revealed that the polyphenols in the skin were 74.69% of the proanthocyanins, those of the pulp were at 55.25% and those of the seed at 41.93%. Concerning the anti-free radical activity (EC_{50}), the extracts of the skin appeared to be the most reactive (0.03±0.00%) followed by those of the seed (0.04±0.00%) and the pulp (0.05±0.00%). These findings support those of previous authors [16, 17, 18] who all agreed on the richness of the miracle fruit in polyphenolic antioxidant compounds that most of which are flavonoids with high antioxidant reactivity. It should be noted that polyphenols are very powerful antioxidants. They are effective against neurodegenerative diseases [34] related to the aging process, inflammation [35], cardiovascular diseases [36] and certain cancers [37] in humans.

5. CONCLUSION

The research revealed that the miracle berry previously known for the unusual property of its pulp to induce a sweet flavor to acid products, also contains in its various constituent parts, organic and mineral elements of considerable nutritional and functional values. The skin and the seed are particularly rich in crude protein, crude fat, total carbohydrate, vitamin C and polyphenolics while the pulp includes the highest levels of mineral elements with a predominance of potassium. The high nutrient contents in the components of miracle fruit offer other opportunities for use. The richness of the skin and the seed in organic nutrients makes them an ideal food for animals. This seed can also be roasted as peanuts for human consumption. The skin, the pulp and the seed could be used as a dietary supplement in feed. The high contents in vitamin C and phenolic molecules with high antioxidant reactivity in the skin make the latter a good functional food candidate and could serve as an ingredient in the formulation of nutraceutical preparations for the protection of human health.

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