

Flavonoid Contents and Antioxidant Activity in Fruit, Vegetables and Other Types of Food

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Abstract

Flavonoids are a class of natural polyphenolic compounds which cannot be synthesized by humans. These substances possess a series of biological properties, acting on biological systems as antioxidants. The purpose of this study was to analyze the properties of certain foods, determining the total flavonoids as well as their antioxidant activity and fat concentration. We evaluated several foods purchased at the local market, with respect to its antioxidant activity, using two experimental models, the discoloration of DPPH[•] radical and ABTS^{-•}. Some foods such as pitanga showed antioxidant activity. The lipid content of fatty foods like açaí, cacao and cupuaçú was determined. Other foods, including pitanga, açaí, cacao and cupuaçú, were evaluated for flavonoid content and antioxidant activity using multivariate statistical analysis (PCA) as a statistical tool to evaluate the correlation between these two parameters. As samples with ED₅₀ up to 500 µg/mL show promising antioxidant activity, several Brazilian fruit and vegetables could be consumed to this end, with a good correlation between flavonoid content and antioxidant activity in most samples. The daily dose of different types of food for antioxidant activity has been calculated based on these results.

Keywords

Amount of Lipids, Antioxidant Activity, Chemometric Analysis, Fat Content, Flavonoids, Food, Fruit, Vegetables

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1. Introduction

The consumption of nutrients and other biologically active compounds help their consumers lead healthier lives. The antioxidant activity of some foods reduces the risk of certain diseases, as free radicals may affect many biological molecules including lipids, proteins, carbohydrates, and vitamins present in food [1].

The low incidence of disease in some countries called attention to their population's diet. For example, Eskimos have a sea-food based diet (which is rich in omega 3 and 6 lipids) and a low incidence of heart problems. The same tendency is observed in the French who consume red wine, as well as the Oriental populations who consume soybean and have reduced incidence of breast cancer. It has been confirmed that the habit of consuming fruit, vegetables, and certain types of foods help reduce the risk of coronary disease and cancer [2].

Phenolic compounds are commonly found in vegetable based food, and are important components of human diet. They have diverse properties such as antioxidant, anti-aging, anti-cancer, anti-inflammatory activity [3]. Phenolic compounds have been used as antioxidants and mainly inhibit free radicals and block their chain reactions [4].

Free radicals may be related to a series of illnesses, causing tissue damage, arteriosclerosis, rheumatic arthritis, aging, circulatory diseases, neurodegenerative diseases, alteration of DNA, causing different types of cancer and hundreds of other pathologies [5]. Due to the mass production of free radicals during metabolic processes, many innate processes exist to counteract the damage caused by those free radicals; such as those formed by the loss, or addition, of a single electron to a non-radical molecule. The levels of these free radicals fluctuate during various conditions, such as higher oxygenation of the tissues, accumulation of lactic acid (lactoacidosis) and ketones (ketoacidose). Our own bodies generally combat these radicals endogenously. However, when the radical levels rise to high levels or go up too quickly (possibly due to external agents) our bodies are overburdened and this is where food with antioxidant properties enters the scene and helps combat the excess of free radicals [6]-[8]. So, whenever free radicals increase or endogenous antioxidants decrease, oxidative stress ensues, resulting in tissue damage. This process generally leads to direct or indirect inflammatory response which consequently further raises the level of free radicals, maintaining tissue damage. Antioxidants taken in from diet play an important role in the preventions of illness, and there is a direct link between oxidative stress and diet which is important for the antioxidant defense of the organism [9].

Flavonoids are an important class of plant pigments, naturally found in fruit and vegetables. This class of naturally occurring polyphenolic compounds which cannot be synthesized by humans possesses a series of biological properties, acting on biological systems as antioxidants. Flavonoids act as antiviral, anti-inflammatory, and antitumoral agents, affecting capillary permeability and acting as exogenous antioxidants. Flavonoids capture and neutralize the oxidative agents, and quench free radicals, inhibiting several enzymes (ciclo-oxygenase, lipo-oxygenase, NADPH-oxidase, xantine-oxidase, fosfolipase) and stimulating enzymes with antioxidant activity (such as catalase and superoxide dismutase). Therefore flavonoids interfere directly in the formation and propagation of free radicals [4] [7].

Flavonoids present in the diet are directly linked to the prevention of arthrosclerosis, as various studies show that the reduction of total blood cholesterol levels and the antioxidant effect lead to lower risks of arthrosclerosis, teratogenicity, and coronary disease [10].

It has been reported that green vegetables are the source of polyphenolic compounds [10] [11]; so their antioxidant [12] and antimutagenic capacity [13]-[15], effects on apoptosis [16], and antiproliferative mechanisms [17] [18] are related to the presence of these compounds in fruit and vegetables [4].

The objective of this study was to analyze the antioxidant properties of certain foods, determining the total flavonoids as well as their antioxidant activity and fat concentration order to evaluate which types of food can help keep a healthy diet.

2. Materials and Methods

2.1. Plant Material

All foods were purchased at the local market.

2.2. Sample Preparation

All samples were prepared as described in **Table 1**.

Table 1. Conditions of food pre-treatment.

Food	Quantity sample (g)/solvent (mL)	Experimental conditions
Açaí (<i>Euterpe oleracea</i> mart.)	Without extraction	Fruit pulp (Control, Açe) Pulp in oven at 50°C, 24 h (Açe) Pulp 60 min, 100°C (Açf) Pulp left open in a refrigerator for 25 days, (Açg) Pulp + H ₂ O ₂ 10 vol, 20 mL (Açp1) Pulp + H ₂ O ₂ 10 vol, 1mL (Açp2)
Broccolis (<i>Brassica oleracea</i>) raw	13/100 ^w	Turbo-extracted, 3 min. Filtered
Broccolis cooked	21/100 ^w	Boiled, 90 min, 150°C. Filtered
Chocolate-Milk	11/150 ^w	Separately extracted in Soxhlet 4 hours, allowed to cool and filtered to remove the nonpolar fraction.
Chocolate-Bitter	7/150 ^w	
Chocolate-White	9/150 ^w	
Chocolate-Diet	6/150 ^w	
Eggplant (<i>Solanum melongena</i>) raw peel	10/100 ^w	Turbo-extracted, 2 min. Filtered
Eggplant raw pulp	10/100 ^w	Turbo-extracted, 2 min. Filtered
Eggplant cooked peel	10/100 ^w	Boiled 180 min, 100°C. Filtered
Eggplant cooked pulp	10/100 ^w	Boiled, 180 min, 100°C. Filtered
Kale (<i>Brassica oleracea</i> var. <i>acephala</i>) raw	31/100 ^w	Turbo-extracted, 2 min. Filtered
Oregano (<i>Origanum vulgare</i>)	15/200 ^w	Turbo-extracted, 2 min. Filtered
Parsley (<i>Petroselinum crispum</i>)	15/150 ^w	Turbo-extracted, 2 min, Filtered
Pitanga (<i>Eugenia uniflora</i>) green mature leaves seeds industrialized pulp	Without extraction	Turbo-extracted, lyophilized 24 - 48 h. resuspended in water (1 mg/mL)
Squash (<i>Curcubita maxima</i>) raw	30/100 ^w	Turbo-extracted 5 min. Filtered
Squash (cooked)	20/100 ^w	Boiled 120 min, 150°C. Filtered
Soya (<i>Glycine max</i>)	20/200 ^w	Boiled 180 min at 200°C and 45 min, at 300°C. Filtered
Tomato raw (<i>Solanum lycopersicum</i>)	0/150 ^w	Turbo-extracted, 2 min. Filtered
Tomato cooked	40/100 ^w	Boiled 60 min, 150°C. Filtered
Walnuts (<i>Carya illinoensis</i>)	0/100 Hexane	Reflux at 100°C, 6 hours. Apolar moiety and residue separated. Residue extracted with ethanol. Filtered.

Legend: W, water.

The percentage of soluble solids was obtained (% m/V), the flavonoid content and antioxidant activity was determined for all samples. For the pitanga samples (**Pi**), only the antioxidant activity was determined. The fat contents and antioxidant activity was determined for the samples of cacao, cupuaçú, and açaí.

2.3. Antioxidant Activity

2.3.1. DPPH Method

Solutions of the fluid extracts 0.01% (m/V) were prepared, adding ethanol to each of the test tubes to obtain the desired dilution. The volume of DPPH[•] (diphenyl picryl hydrazyl radical, Sigma, USA, 60 µM) was added to the first tube and at 1-minute intervals to all the other tubes. The absorbance at 517 nm was read after 30 minutes in the first tube and at 1-minute intervals for all the other tubes in a spectrophotometer Cary-50 with probe (Varian, USA). The percent of absorbance *versus* concentration of extract were plotted on a graph and the ED₅₀

was calculated by the minimal squares method [19]. All food samples were tested, except for treatments of açai because the sample color was similar to DPPH[•], and both absorbed at the same wavelength.

2.3.2. ABTS- Method

The samples were prepared in the same way as for DPPH[•] and analyzed as described by Pellegrini *et al.* [20]. Water and ethanol extracts of cacao (*Theobroma cacao* L.) (fruit, **Ccf** e pulp, **Ccp**), cupuaçu (*Theobromagrandiflorum*) (fruit, **Cpf** e pulp, **Cpp**), açai (**Table 1: Açc, Açe, Açf, Açg, Açp1 e Açp2**) and pitanga (*Eugenia uniflora*) (**Piv, Pim, Pif, Pis e Pip**) were analyzed in a spectrophotometer Cary-50 with probe (Varian, USA).

2.4. Lipid Content

The method described by Bligh and Dyer [21] was used. Ground raw samples of cacao (fruit, **Ccf** e pulp, **Ccp**), cupuaçu (fruit, **Cpf** and pulp, **Cpp**) e açai (**Table 1: Açc, Açe, Açf, Açg, Açp1 e Açp2**) were analyzed; their lipid contents were extracted and weighed (Ohaus, Analytical Plus 0.00000 g (USA)).

2.5. Flavonoid Content

The determination of total flavonoids was effected using a solution of 100 µL of each sample with a known percentage of soluble solids, in a 10 mL flask, completing the volume with methanol (Merck, Germany, stock solution). All dilutions were prepared in triplicate, parting from this stock solution. An aliquot of 200 µL was transferred to another 10 mL flask, adding 200 µL of a 5% aluminum chloride solution (Synth, Brazil) and completing the volume to nearly 10 mL with methanol. This flask was shaken and heated to a 20°C for 30 min, after which the volume was completed with methanol and the absorbance read at 425 nm (Spectrophotometer Cary 50, Varian, USA). The concentration of flavonoids was calculated based on a calibration curve using quercetin, Sigma, USA).

2.6. Statistical Analysis

Analyzing possible differences between the groups was used analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test (parametric data) and Kruskal-wallis (nonparametric data).

2.7. Multivariate Statistical Analysis

In the multivariate statistical analysis all dates are presented in a matrix with the samples as lines (*scores*) and the columns as the variables (*loadings*) which in this case were the results of the flavonoid contents and antioxidant activity for each sample. In the principal component analysis (PCA) the PC1 × PC2 graph of the scores dividing the samples in groups according to their variables (shown in the loadings graph). For this analysis the Pirouette, version 2.6 (Infometrix, Woodinville, WA, EUA) was used.

3. Results and Discussion

3.1. Antioxidant Activity

The results referring to the antioxidant activity (ED₅₀ in µg/mL) of the açai, cacao, cupuaçu and pitanga samples in water and ethanol are shown in **Table 2**. These results were obtained using both DPPH[•] and ABTS[•] methods by comparison to Vitamin C, a standard antioxidant. In general, the açai samples and some pitanga samples presented high antioxidant activity, in comparison to vitamin C. However the antioxidant activity may be influenced by the solvent and extraction method used.

The antioxidant activity of cupuaçu pulp (**Ccp**) and fruit (**Cpp**) were higher than the commercial pulp of cacao (**Ccp**) and industrialized Pitanga pulp (**Pip**) but lower than all the other samples, considering both water and ethanol extracts analyzed by DPPH. The water extracts of pitanga (*Eugenia uniflora*) analyzed by DPPH, the leaves (**Pif**) and seeds (**Pis**) showed similar ED₅₀ results, having the highest activity. Mature pitanga (**Pim**) and green pitanga fruit (**Piv**) presented intermediary ED₅₀ results. The industrialized pulp of this fruit (**Pip**), had a higher ED₅₀, indicating lower antioxidant activity. The samples extracted with ethanol and analyzed DPPH, and the seeds (**Pis**) presented lower ED₅₀ values, followed by the leaves (**Pif**), indicating a higher antioxidant activity. Mature pitanga (**Pim**) presented moderate activity whereas green pitanga fruit (**Piv**) and industrialized pulp

Table 2. Antioxidant activity expresses as ED₅₀ (µg/mL) of the açai, cacau, cupuaçu, and pitanga samples, determined by the DPPH e ABTS⁻ methods in comparison with vitamin C.

Samples	DPPH*		ABTS*	
	Water	Ethanol	Water	Ethanol
	ED ₅₀ in µg/mL			
Açai (Açc)	-	-	17.00	-
Açai (Açe)	-	-	67.74	-
Açai (Açf)	-	-	16.86	-
Açai (Açg)	-	-	17.52	-
Açai (Açp1)	-	-	16.99	-
Açai (Açp2)	-	-	17.76	-
Cacao fruit (Ccf)	1580.0	7900.0	2630.0	1880.0
Cacao pulp (Ccp)	6110.0	14600	5850.0	6290.0
Cupuaçu fruit (Cpf)	2190.0	4520.0	2590.0	3200.0
Cupuaçu pulp (Ccp)	2320.0	7100.0	3290.0	2410.0
Pitanga green (Piv)	584.0	2354.0	428.0	1197.0
Pitanga mature (Pim)	1466.0	446.0	1258.0	2001.0
Pitanga leaves (Pif)	40.00	114.0	55.00	101.00
Pitanga seeds (Pis)	41.00	32.0	53.00	67.00
Pitanga industrialized pulp (Pip)	7626.0	9720.0	11200	8658.0
Vitamin C	4.00	4.00	4.00	4.00

-: not tested. For abbreviations see **Table 1**. *Material extracted with water or ethanol.

(**Pip**) did not present antioxidant activity. In the water extracts, analyzed by ABTS⁻, the leaves (**Pif**) and seeds (**Pis**) presented similar results with the highest activity. Green pitanga (**Piv**), mature pitanga (**Pim**) and industrialized pulp (**Pip**), showed a lower antioxidant activity. In the samples extracted with ethanol and ABTS, the seeds (**Pis**) and leaves (**Pif**) showed the highest activity, whereas the other samples did not present activity.

The fruit of the açai palm (*Euterpeoleracea*) contains anthocyanins, which are natural antioxidants [22]. This was confirmed by the results of the ABTS⁻ assay. This high antioxidant activity helps to protect the lipophilic fraction of the fruit.

Both cupuaçu and cacao do not have good antioxidant activity; in the majority of cases, the fresh fruit was slightly superior to the industrialized frozen pulp. Although the sample of cupuaçu presented higher activity than the cacao sample, both were produced by the same manufacturer. The transport conditions may be important factors relating to their quality. With respected to pulps, the amount of the lipid is greater in cupuaçu in comparison to cacao, and in both fruits the amount is always higher than in the pulp. Cupuaçu has a higher concentration of lipids compared to *in natura* cacao and processed material. This information must be considered when was evaluated the nutritional potential of both analyzed fruits. Cupuaçu has a higher concentration of lipids compared to *in natura* cacao and processed material. This information must be considered when evaluating the nutritional potential of both analyzed fruits.

Apparently the antioxidant substances present in pitanga are soluble both in water and in ethanol and were extracted in equivalent amounts by both solvents.

3.2. Amount of Lipids

Table 3 shows the percentage of lipids, obtained using the Bligh Dyer method, for açai, cacau and cupuaçu.

Cupuaçu fruit showed the highest amount of lipids, followed by cacao and açaí. These three typical Brazilian fruits have good percentage of lipids. Cupuaçu for example, is used to prepare exotic candies especially in Northern regions of Brazil. In the case of Cacao and Cupuaçu, the industrialized pulp has lower lipid concentration than the whole fruit, indicating that some of the lipids are lost (or diluted) in processing. These pulps are used to make fruit juice.

3.3. Flavonoids and Antioxidant Activity

Polyphenols are secondary metabolites present in foods from plant origin. There are three main types of polyphenols such as, flavonoids, phenolic acids and tannins that are potent antioxidants. These compounds are considered the main substances promoting health benefits as mentioned previously [3] [23].

Table 4 shows the flavonoid content, antioxidant activity and the daily dose of food calculated as necessary to have an antioxidant activity in an adult. For example, people who don't like broccoli or eggplant (daily dose between 20 and 26 g) could eat more chocolate. Oregano and walnuts also present good antioxidant activity, which could please the palate of several people.

Behling *et al.* [24], reported that the flavonoid content in foods consumed daily is: 44 mg in cereals, 45 mg in grains and nuts, and 126 mg in vegetables and herbs. Most flavonoids come from cacao, coffee, black tea, beer and wine, which generates approximately 420 mg/day, an additional 290 mg/day comes from fruit and juices.

Figure 1 shows the flavonoid contents (mean and standard deviation) each for the fruit and vegetables analyzed. The asterisk marks statistically significant differences between foods. Again, several different types of food present important antioxidant activity, showing that it is not necessary to suffer in order to eat healthily.

Table 3. Percentage of lipids in açaí, cacao, and cupuaçu pulp.

Sample	Lipid contents g/3.5g*	Percentage of lipids (%)
Açaí (Aç)	0.14	4.00
Cacao pulp (Ccp)	0.02	0.57
Cacao fruit (Ccf)	0.58	6.82
Cupuaçu pulp (Cpp)	0.10	2.86
Cupuaçu fruit (Cpf)	1.36	38.86

*Sample weight. For abbreviation see **Table 1**.

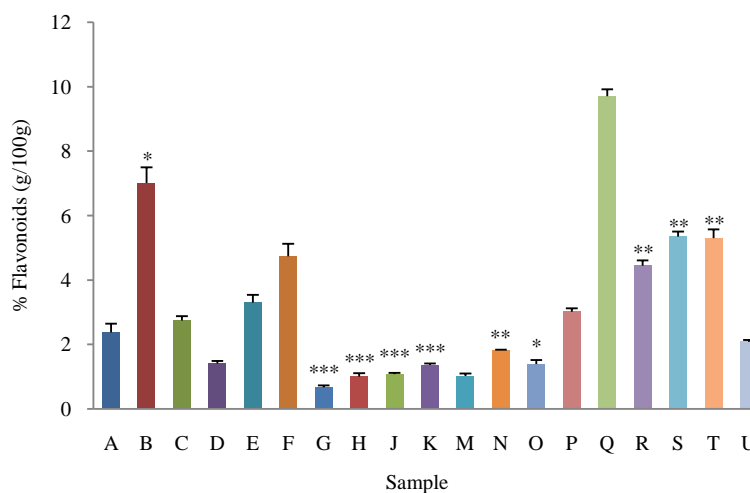


Figure 1. Flavonoids in vegetables and fruit—Mean and Standard Deviation, for each sample, *significant difference $p < 0.05$ (Tukey, T test); **significant difference $p < 0.01$ (Tukey, T test); ***significant difference $p < 0.001$ (Tukey, T test); ****significant difference $p < 0.0001$ (Tukey, T test). For abbreviation, see **Table 1**.

Table 4. Flavonoid contents, concentration that reduces the free radicals in 50 (ED₅₀ in µg/mL) and the necessary daily dose for an antioxidant effect in a 65 kg individual.

	Flavonoids (g/100g)				DPPH ^a (ED ₅₀ µg/mL)	Daily dose (g)
	Average	DP	CV% ^b	uncertainty ^c		
Bitter chocolate (N)	1.83	0.01	0.77	0.31	89.50	8.0
Cooked broccoli (H)	1.01	0.10	9.59	3.91	224.66	20.0
Cooked eggplant peel (D)	1.41	0.08	5.37	2.19	69.88	6.0
Cooked eggplant pulp (F)	4.74	0.39	8.20	3.35	284.33	26.0
Cooked soybean (U)	2.09	0.05	2.30	0.94	156.96	14.0
Cooked squash (B)	7.01	0.49	6.93	2.83	564.68	53.0
Cooked tomato (T)	5.30	0.28	5.27	2.15	115.25	10.0
Diet chocolate (M)	1.03	0.07	6.61	2.70	530.45	48.0
Kale (O)	1.39	0.13	9.19	3.75	221.09	20.0
Milk chocolate (J)	1.08	0.04	3.32	1.36	151.90	14.0
Oregano (P)	3.02	0.11	3.50	1.43	85.87	8.0
Parsley (R)	4.45	0.16	3.66	1.50	132.10	12.0
Raw broccoli (G)	0.68	0.05	7.32	2.99	229.14	21.0
Raw eggplant peel (C)	2.75	0.13	4.67	1.91	171.21	15.0
Raw eggplant pulp (E)	3.30	0.24	7.35	3.00	104.95	9.0
Raw squash (A)	2.38	0.27	11.53	4.71	390.72	35.0
Raw tomato (S)	5.35	0.16	2.98	1.22	147.37	13.0
Walnuts (Q)	9.71	0.21	2.19	0.89	93.11	8.0
White chocolate (K)	1.37	0.04	2.65	1.08	255.75	23.0

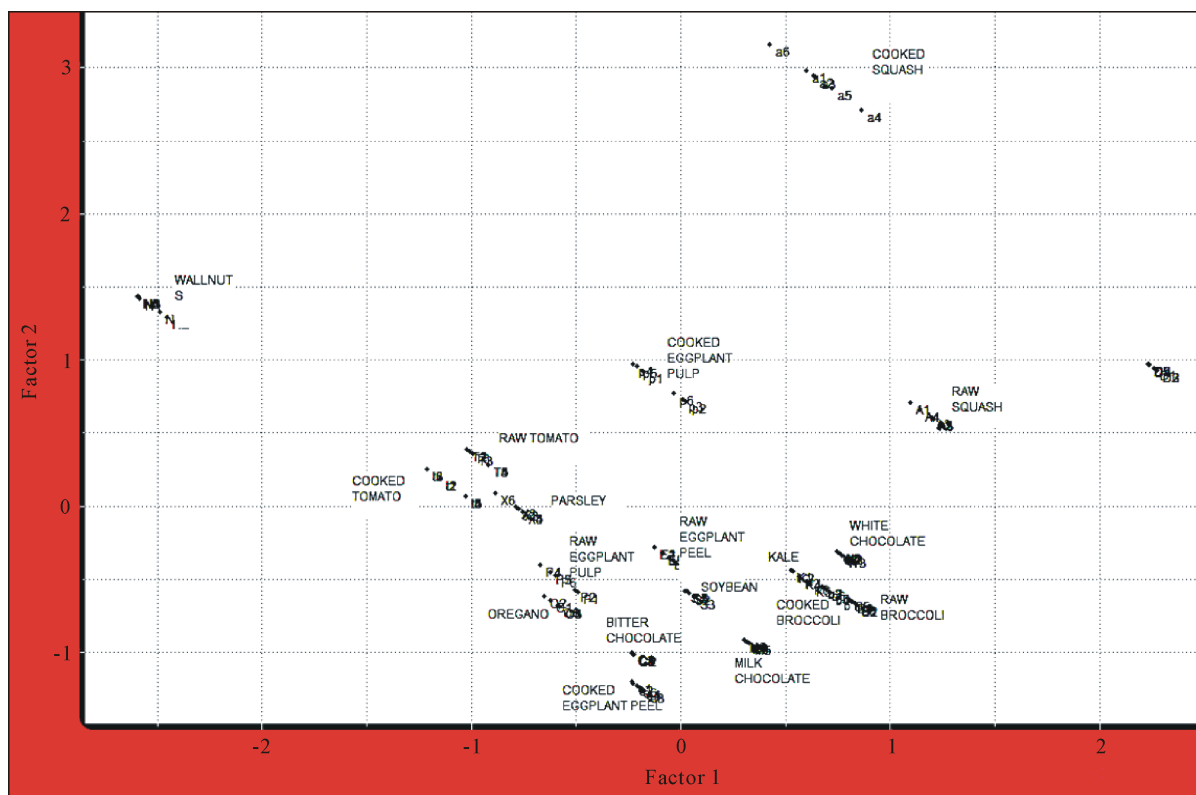
Letter in parenthesis was used in chemometric analysis (PCA). For abbreviation, see Table 1. ^aED₅₀ is the concentration that eliminates 50% of free radicals by minimal squares; ^bCoefficient of variation in percentage; ^cUncertainty simple calculated by the quotient between standard deviation and square root of number of measures; ^dDaily dose calculated in grams for a person of 65 kilogram to obtain the antioxidant effect.

3.4. Principal Component Analysis

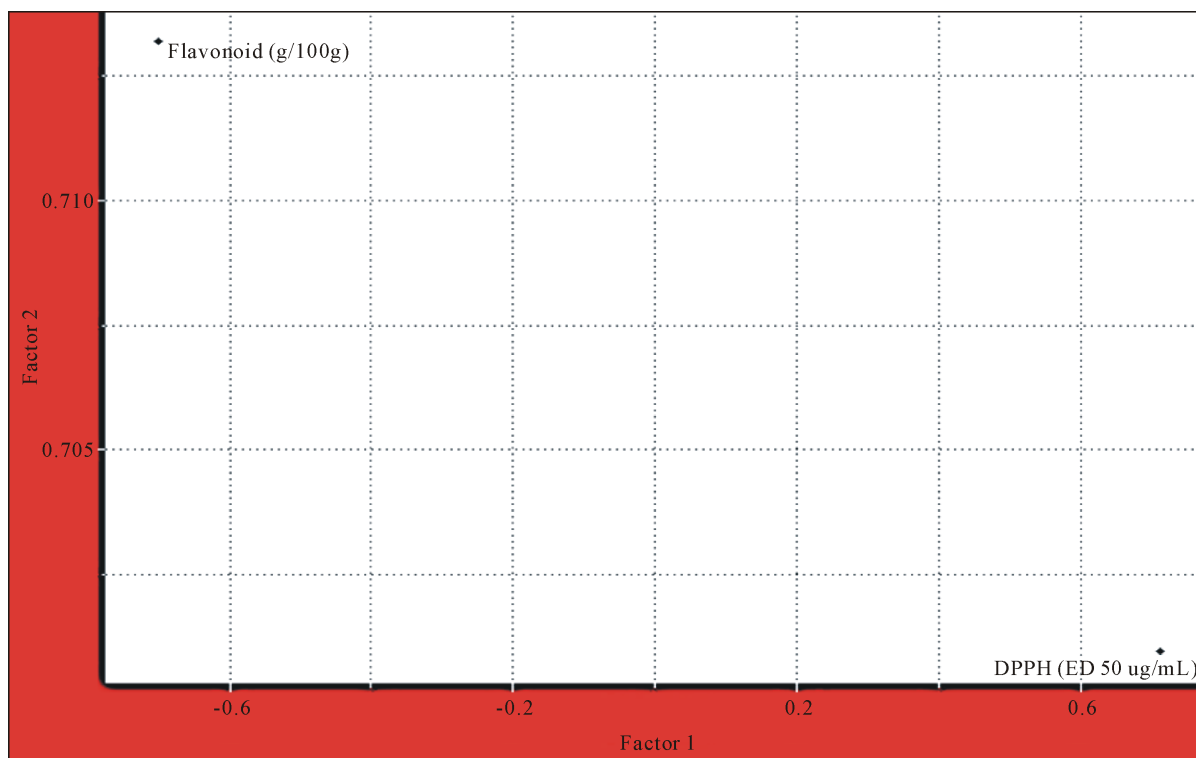
Principal components analysis of the results described in Table 4, is shown in Figure 2(a) (*Scores*) and Figure 2(b) (*Loadings*).

Multivariate statistical analysis is shown in Figure 2. The *loadings* are shown in Figure 2(b). Flavonoids are found in the upper left and antioxidant activity (ED₅₀, µg/mL, DPPH) in the lower right corner. The higher content of flavonoids promotes good antioxidant activity (lower ED₅₀ value). Figure 2(a) must be analyzed together with Figure 2(b), which shows the *Scores*, representing food samples. Most samples are placed in the center of the graph: for amounts of flavonoids between 0.68 to 5.36 g/100g the antioxidant activity ranged from 69.88 to 530.45 µg/g, indicating that there is a linear correlation between flavonoid content and antioxidant activity, as mentioned earlier in this paper; except for the samples of cooked squash and walnuts, the first with a good content of flavonoids and low antioxidant activity and the second, with good content and good flavonoid antioxidant activity. These results suggest that flavonoids present in walnuts are responsible for the antioxidant activity but in cooked squash other substances are probably involved in this activity.

Free radicals are produced more rapidly and abundantly in old people, in both young and elderly people, the consumption of food and drinks increase the appearance of many diseases such as obesity, cardiovascular disorders, diabetes and cancer [3]. Many food have been recognized as beneficial to health because they have antioxidant characteristics and break the oxidative chain, decreasing the concentration of free radicals in organisms [9].



(a)



(b)

Figure 2. Principal component analysis ((a) Scores, and (b) Loadings). Loadings represent analyzes (flavonoid content and antioxidant activity) and Scores food samples and their treatment. For description of samples, see [Table 1](#).

Based on these results, it was defined that samples with ED₅₀ up to 500 µg/mL show antioxidant activity, but higher values are not indicative of this activity. Thus, most of the food samples analyzed showed antioxidant activity, especially bitter chocolate, baked eggplant peel, oregano and walnuts. Azevedo and Rodriguez-Amaya [25] reported that the squash is rich in carotenoids which have antioxidant action. Míean and Mohamed [26] demonstrated that broccoli has a high amount of quercetin, with important antioxidant activity. Chocolate contains catechins and epicatechins, a type of flavonoid which confers antioxidant activity to bitter chocolate [27]. Parsley contains apigenin, a flavonoid with antioxidant activity [28].

Flavonoids are the main class of chemical compounds found in with antioxidant activity [9]. Most of the food samples showed presented flavonoids in their composition. The analysis of total flavonoids suffer influences of many factors, including handling, storage, preparation, processing, climate and season [29].

4. Conclusion

The present study analyzed the antioxidant properties and chemical composition of diverse foods, cupuaçu fruit showed the highest amount of lipids, followed by cacao and açaí. According to the results, some types of food presented important antioxidant activity and flavonoid content, justifying the importance of their presence in human diet, aiding in the reduction of free radicals and reducing the related diseases. However, not enough is known about the biodisponibility of flavonoids and their chronic ingestion. Further studies on the biodisponibility and mechanisms of action will be necessary, as well as their antioxidant activity and possible synergy with other dietary components.

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