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Comparação entre o conteúdo de fenólicos totais e a capacidade antiradical de produtos de chocolate derivados de cacau e de cupuaçu

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Abstract

Polyphenolic compounds seem to be related to the health benefits produced by the cocoa due to their antioxidant properties. Cupuassu powder, prepared from *Theobroma grandiflorum* seeds, is a very promising cocoa powder substitute. In order to assess the potential health benefits of the cupuassu powder, a comparison was performed between cocoa, chocolate, and cupuassu powders in relation to the content of total phenolic compounds, flavonoids and DPPH scavenging capacity of methanolic extracts. Cupuassu "chocolates" (milk, dark, and white) were also analyzed. Mineral, lipid, protein, and moisture determinations were made in cocoa and cupuassu powders and in cupuassu "chocolates". Results showed that the phenolic contents of cocoa and chocolate powders are more than three times higher than those of cupuassu powder; however, flavonoid contents were significantly lower. The DPPH scavenging capacity varied hugely among the different samples, from 0.5 (white cupuassu "chocolate") to 120 (cocoa powder) μg of Trolox equivalent per 100 g (FW), and presented a significant correlation (r = 0.977) with the total phenolic contents but not with the flavonoid contents (r = –0.035).

Keywords: *DPPH scavenging capacity; total phenolic content; cupuassu "chocolate"; cupuassu and cocoa powders.*

Resumo

Os compostos polifenólicos têm sido implicados nos efeitos benéficos à saúde promovidos pelo cacau devido às suas propriedades antioxidantes. Cupuaçu em pó, preparado a partir de sementes de *Theobroma grandiflorum,* é um substituto promissor para o cacau em pó. Com a finalidade de verificar os benefícios potenciais para a saúde deste produto, foi efetuada uma avaliação do cacau e cupuaçu em pó, bem como de "chocolates" de cupuaçu, com relação ao conteúdo de compostos fenólicos, flavonóides e capacidade anti-radical livre dos extratos metanólicos. Os conteúdos de minerais, lipídeos, proteínas e umidade foram determinados em cacau e cupuaçu em pó, assim como nos "chocolates" de cupuaçu. Os resultados mostraram que os conteúdos de compostos fenólicos no cacau e no chocolate em pó são mais que três vezes maiores que do cupuaçu em pó; contudo, o conteúdo de flavonóides é significativamente menor. A capacidade seqüestradora do DPPH variou enormemente entre as diferentes amostras, de 0,5 ("chocolate" de cupuaçu branco) a 120 (cacau em pó) μg de equivalentes de Trolox por 100 g (FW), e apresentou correlação significativa (r = 0,977) com o conteúdo de fenólicos totais, porém não com o conteúdo de flavonóides (r = –0,035).

Palavras-chave: *capacidade seqüestradora do DPPH; fenólicos totais; "chocolate" de cupuaçu; cupuaçu e cacau em pó.*

1 Introduction

Cupuassu is a popular fruit from Amazonian region. It originated in south and southeastern Amazonia in Brazil, and it is also native to the states of Para and Maranhao (VASCONCELOS et al., 1975). Each fruit contains about 50 seeds which are surrounded by a mucilaginous pulp with an acid taste and a strong "goaty" aroma (VENTURIERI; ALVES; NOGUEIRA, 1985). The cupuassu seed is very rich in fats (± 60% dry weight), which are 91% digestible by humans. The processing of the seed is similar to the processing of cocoa beans: fermentation, roasting, milling, and pressing to obtain fat. From the defatted mass, the cupuassu powder is obtained (LANNES, 2003). During the fermentation of the cupuassu seed, the cotyledon's pH increases from 5.2 to 6.8, the phenolic compounds from 1.12 to 1.34 mg.100 g^{-1}, the fats remain unchanged at 63.5% dry weight, and the color changes from a beige cream to a dark reddish caramel. The flavor and aroma of the fermented seed is indistinguishable from that of fermented cocoa seed (ARAGÃO, 1992).

The seeds can be used to make chocolate and show a great potential to substitute cocoa in chocolate products**.** Cupuassu fat is very similar to cocoa butter, although with a different fatty acid profile, and its application for confectionery industry is very promising (LANNES; AMARAL; MEDEIROS, 2002; LANNES, 2008; MEDEIROS, 2006).

Cocoa (*Theobroma cocoa* L.) is an important product and highly consumed in several countries. Othman et al. (2007) reported that the phenolic content and antioxidant capacity of cocoa beans (dried and fermented) could vary according to the country of origin.

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Cocoa beans and their derivatives, such as cocoa powder and chocolate, are important sources of polyphenols (LEE et al., 2003). Cocoa nibs have a high phenolic content of about 12-18% (dry weight) and 95% are flavanol monomers (epicatechin and catechin) and procyanidin oligomers (dimmer to decamer) (LAMUELA-RAVENTÓS et al., 2005). These compounds were reported to be potential candidates to combat free radicals, which are harmful to our body and food systems (OTHMAN et al., 2007).

Epidemiological studies show that many polyphenolic compounds present in fruits, vegetables, nuts, cocoa, wine, and tea are partly responsible for their beneficial health effects. Phenolic compounds are secondary metabolites with considerable physiological and metabolic importance in plants. These compounds play an important role in growth and reproduction providing protection against pathogens and predators (BRAVO, 1998).

For humans, one of the most important biological functions is their antioxidant capacity. Antioxidants are compounds that hinder oxidative processes and thereby delay or prevent oxidative stress. Oxidative stress is caused by the excess of free radicals due to lifestyle and pathological situations, and it has been related to cardiovascular diseases, cancer and other chronic diseases that account for a major portion of deaths today (WILLCOX et al., 2004).

According to this, the objective of this study was to evaluate the content of total phenolics and flavonoids and the DPPH scavenging capacity of cupuassu powder and "chocolates", as well as to compare them to the products obtained from cocoa.

2 Materials and methods

2.1 Samples

The cupuassu "chocolates" (3 kg/each type) were prepared according to Lannes, Amaral, Medeiros (2002). The cupuassu powder was obtained from fermented cupuassu seeds (Chocam – Manaus). The cocoa powder (Impact Lec-AW 70 – Cargill) was obtained directly from the industry, and the chocolate powders (soluble powder chocolate – Nestlé) were bought from a local market, three packages (200 g).

All samples were thoroughly homogenized by powdering in liquid nitrogen, when necessary, and stored at –18 ºC under N_2 until analysis.

2.2 Chemicals

The 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), gallic acid, rutin, and the Folin-Ciocalteu reagents were purchased from Sigma Co (St. Louis, MO). The hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Aldrich (Milwaukee, WI). All chemicals and solvents used were analytical grade.

2.3 Moisture, minerals and proteins determination

The determinations were conducted according to the AOAC Methods (1995). The results were expressed as a percentage. The protein content was determined using N × 6.25.

2.4 Lipid determination

The lipid content was determined according to Lannes (2008) using ether as the solvent. The analysis were made in triplicate and the results expressed as a percentage (g.100 g^{-1} in fresh weight).

2.5 Sample extraction for total phenolics/flavonoids and DPPH scavenging capacity assays

The samples (10 g) were extracted three times using a Brinkmann homogenizer (Polytron-Kinematica GmbH, Kriens-Luzern) with methanol/water (70:30) or pure methanol (100 mL the first time, 50 mL the next two times), in an ice bath at speed five for 1 minute, and filtered under reduced pressure through filter paper (Whatman Nº 1), according to Arabbi et al. (2004).

2.6 Total phenolics

The analysis was performed according to Singleton et al. (1999) with some modifications. A 0.25 mL aliquot of the extract obtained above was mixed with 0.25 mL of the Folin-Ciocalteu reagent and 2 mL of distillated water. After 3 minutes at room temperature, 0.25 mL of a saturated sodium carbonate (Na_2CO_3) solution was added and the mixture placed at 37 °C in a water bath for 30 minutes. The absorbance was measured at 750 nm using a model Ultrospec 2000 UV/visible spectrophotometer (Amersham Biosciences, Cambridge, U.K.). the gallic acid was used as the reference standard, and the results were expressed as mg of gallic acid equivalent (GAE)/100 g sample in fresh weight (FW).

2.7 Total flavonoids

The total flavonoid content was determined by a reaction with $AlCl_3$ and quantified by absorbance measurement according to Woisky and Salatino (1998), with minor modifications. The appropriately diluted extract in methanol was mixed with 2% $AlCl_3$ solution in methanol. After 1 h at room temperature, the absorbance was measured at 420 nm. The amounts of flavonoids were determined from a standard absorbance plot, using rutin as standard and calculated as mg rutin equivalent (RE).100 g^{-1} sample FW.

2.8 DPPH radical scavenging capacity

The anti-radical capacity was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging method according to the method of Brand-Williams et al. (1995) with some modifications (DUARTE-ALMEIDA et al., 2006). A 50 µL aliquot of the extract previously diluted and 250 µL of DPPH (0.5 mM) was mixed, and after 20 minutes the absorbance was measured at 517 nm using a Microplate Spectrophotometer (Benchmark Plus, BioRad, Hercules, CA). The control consisted of a methanolic solution of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) at different concentrations. The decrease of absorbance caused by the sample, compared to a blank (50 µL methanol), was related to those of the control. The DPPH scavenging capacity was expressed as µmoles Trolox equivalent/g sample in fresh weight (FW).

2.9 Statistical analysis

All analyses were carried out in triplicate and the results were expressed as mean ± standard deviation (SD). The statistical analysis was made using the Statistic software package version 5.0 (StatSoft, Inc., Tulsa, OK). The differences between the means were first analyzed by the ANOVA test and then Least Significant Difference (LSD) test ($p < 0.05$). The data were subjected to Pearson correlations.

3 Results and discussion

3.1 Total phenolic content

The identification of new dietary sources of phenolic compounds is of great interest to public health. Cocoa is consumed all over the world, mainly as chocolate, and the similar cupuassu "chocolate" has been explored locally, as an economic substitute, and represents good perspectives for agro industrial purposes. The content of total phenolics, however, was more than three times higher for cocoa and chocolate powders compared to cupuassu powder. Also, a much higher phenolic amount was extracted in 70% methanol compared to pure methanol for all samples analyzed. Not all chocolates are equal sources of polyphenols; dark chocolate is formulated with a higher percentage of cocoa bean liquor than the milk chocolate, and, therefore, it often contains greater amounts of flavonoids. As expected, the phenolic content of white cupuassu "chocolate" was much lower than those of milk and dark cupuassu "chocolate" (Table 1). The levels of phenolic compounds in plants are influenced by genetic and environmental factors, soil composition, and maturation level (BRAVO, 1998). Although belonging to the same genera and being subjected to similar conditions of processing, it is likely that the polyphenolics composition and levels of cocoa and cupuassu seeds differ. The total polyphenolics contents ranging from 650 (Brazilian origin) to 2000 (USA origin) mg equivalent of gallic acid.100 g^{-1}, estimated by the same methodology used here, were previously reported for cocoa powders (WOLLGAST; ANKLAM, 2000a).

Table 1. Total phenolic content (mg GAE.100 g^{-1} FW) and total flavonoid content (mg RE.100 g^{-1} FW) of pure methanolic and 70% aqueous methanolic extracts from cupuassu, cocoa and chocolate powders and of cupuassu "chocolates".

Samples	Total phenolics	Total flavonoids
Cupuassu powder		
Pure methanol extract	284 ± 12^{b}	299 ± 18^{d}
70% aqueous methanol	977 ± 44^{e}	590 ± 24^{i}
Cocoa powder		
Pure methanol extract	1132 ± 74^{f}	90 ± 5^{d}
70% aqueous methanol	3647 ± 63^{h}	120 ± 6^{e}
Chocolate powder		
Pure methanol extract	1158 ± 52^{f}	69 ± 3^{bc}
70% aqueous methanol	3322 ± 109^{g}	185 ± 10^{f}
White cupuassu "chocolate"		
70% aqueous methanol	96 ± 8^{a}	22 ± 1^{a}
Milk cupuassu "chocolate"		
70% aqueous methanol	540 ± 25^{c}	61 ± 4^{b}
Dark cupuassu "chocolate"		
70% aqueous methanol	680 ± 38^{d}	73 ± 3^{c}

Results are expressed as means ± SD for triplicates. Means in the same column with common letters are not significantly different ($p < 0.05$).

3.2. Total flavonoid content

Contrary to what was observed for the phenolic contents, the flavonoid content of cupuassu powder was more than three times higher than those of cocoa and chocolate powders (Table 1). Flavonoids were also better extracted in 70% methanol compared to pure methanol. The cupuassu "chocolates" presented flavonoid contents ranging from 22 to 73 mg.100 g^{-1} FW showing that they could also represent a good source. The primary flavonoids in cocoa and chocolate are the flavan-3-ols catechin and epicatechin (monomeric units), and proanthocyanidins, which are polymeric compounds comprising catechin and epicatechin subunits (WOLLGAST; ANKLAM, 2000b). For milk and dark chocolate, a content of 70 and 170 mg (flavanols plus procyanidins).100 g^{-1} was reported, respectively (STEINBERG et al., 2003). In addition, chocolate products have a higher total flavan-3-ol concentration on a per weight basis than that found in most plant-based foods and beverages that contain flavan-3-ols (STEINBERG et al., 2003). Altogether, these results show that cupuassu products can also provide significant amounts of these compounds to the diet.

3.3 DPPH scavenging capacity

The 70% methanolic extracts derived from cocoa, chocolate, and cupuassu powders and cupuassu "chocolates" were evaluated for antiradical capacity by the DPPH radical scavenging method, which is based on the measurement of the reducing ability of antioxidants toward the radical DPPH$^{\bullet}$. In this reaction, the methanolic solution of DPPH changes from purple to light yellow and the degree of discoloration, evaluated spectrometrically at 517 nm, indicates the free radical scavenging potential of the antioxidant compound (BRAND-WILLIAMS et al., 1995). The DPPH scavenging capacity varied significantly among the different samples, from 0.5 (white cupuassu "chocolate") to 120 (cocoa powder) µg of Trolox equivalent per g (FW), and presented a significant correlation (r = 0.977) with the phenolic contents but not with the flavonoid contents (r = –0.035). (Table 2).

Table 2. DPPH radical scavenging activity of 70% aqueous methanolic extracts from cupuassu, cocoa and chocolate powders and of cupuassu "chocolate".

Samples	µmoles Trolox equivalents/g sample (FW)
Cupuassu powder	13.0 ± 0.5^{d}
Cocoa powder	120 ± 5^{f}
Chocolate powder	80 ± 2^{e}
White cupuassu "chocolate'	0.51 ± 0.02^{a}
Milk cupuassu "chocolate"	4.5 ± 0.2^{b}
Dark cupussu "chocolate"	7.8 ± 0.4^{c}

Results are expressed as means ± SD for triplicates. Means in the same column with common letters are not significantly different ($p < 0.05$).

Tabela 3. Moisture, minerals, lipids, and protein contents of cupuassu "chocolates", cupuassu and cocoa powders.

	Milk cupuassu "chocolate"	Dark cupuassu "chocolate"	White cupuassu "chocolate"	Cupuassu powder	Cocoa powder
Moisture (%)	2.35 ± 0.00	1.93 ± 0.00	2.05 ± 0.00	3.05 ± 0.01	2.68 ± 0.07
Minerals (%)	1.80 ± 0.01	1.30 ± 0.00	1.36 ± 0.00	3.80 ± 0.42	9.7 ± 0.1
Proteins (%)	7.9 ± 0.1	5.55 ± 0.05	6.2 ± 0.2	17.2 ± 0.4	24.8 ± 0.1
Lipids (%)	32.3 ± 1.0	29.0 ± 0.4	36.3 ± 1.5	28.2 ± 0.2	14.21 ± 0.03

Results are expressed as means ± SD for triplicates.

Similarly to our results, a good correlation between total phenolics and DPPH scavenging activity was previously found for Brazilian native fruits (r = 0.997) (GENOVESE et al., 2008) and dry beans (*Phaseolus vulgaris*) cotyledons (RANILLA et al., 2007). Besides, almost double the value of antioxidant activity of the milk chocolate (67 μmol/100 g) was previously reported for dark chocolate (131 μmol/100 g) (STEINBERG et al., 2003).

3.4 Chemical properties

The fat content of cocoa powder in industry is around 10-12%. The cupuassu powder used in this study was only partially defatted with 28.16% and the cocoa powder had 14.21% fat. The fat content of "chocolates" was around 29 to 36%. The protein contents were higher in the cocoa powder (24.84%) than in the cupuassu powder (17.23%), and the "chocolates" showed values varying from 5.55 to 7.88%. Moisture varied from 1.93 to 3.05%; and the minerals contents were higher in the cocoa powder (9.69%), probably due to the presence of some seed coats (Table 3).

4 Conclusion

The findings of this work showed that although the cupuassu powder may represent a better flavonoid source than cocoa powder, the total polyphenol content and *in vitro* antiradical activity of cocoa is much higher.

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