



Total phenolic content and antioxidant capacity of methanolic extracts of ten fruits

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ABSTRACT. The aim of this study is the provision of informations regarding the antioxidant molecules content in methanolic extracts of ten fruits through the correlation of total phenolic compounds with antioxidant capacity. The former was determined through the Folin-Ciocalteu assay while the latter was measured by the DPPH[•] radical inhibition method. It was verified that the fruits possess positive correlation ($R^2 = 0.6169$) of total phenolic compounds in their compositions. Among the analyzed fruits, jambul and acerola showed the greatest values of antioxidant capacity (20.94 and $24.48 \mu\text{g mL}^{-1}$) and total phenolic compounds (635.32 and $675.73 \text{ mg gallic acid equivalent/100 g of sample}$). The results that were obtained in this study are useful for consumers, nutritionists and institutions which formulate food policies.

Keywords: free radicals, gallic acid, UV-Vis espectrophotometry.

Fenólicos totais e capacidade antioxidante em extratos metanólicos de dez frutas

RESUMO. O presente estudo buscou fornecer informações sobre a composição de moléculas antioxidantes em extratos metanólicos de dez frutas por meio da correlação entre a quantidade de fenólicos totais e a capacidade antioxidante. Foram realizados ensaios pelo método de fenólicos totais (Folin-Ciocalteu) e de capacidade antioxidante pela inibição do radical DPPH[•]. Verificou-se que as frutas possuem variação de antioxidante e correlação positiva ($R^2 = 0,6169$) de fenólicos totais em sua composição. Dentre as frutas analisadas, o jambolão e acerola são as que possuem maior capacidade antioxidante: $20,94$ e $24,48 (\mu\text{g mL}^{-1})$ e fenólicos totais $635,32$ e $675,73 \text{ mg}$ equivalente de ácido gálico/100 g de amostra respectivamente. Este estudo forneceu novas informações sobre a composição de antioxidantes em frutas para consumidores, nutricionistas e instituições formuladoras de políticas alimentares.

Palavras-chave: radical livre, ácido gálico, espectrofotometria no UV-Vis.

Introduction

Evidences in epidemiologic studies and other researches suggests that the significative ingestion of foods rich in natural antioxidant compounds, like the ones originated from fruits and vegetables, is associated with the estabilization of peroxides (H_2O_2) and free radicals generated in the human body metabolism such as $\text{O}_2^{\bullet-}$, NO^{\bullet} and OH^{\bullet} , and, therefore, with the reduction of the incidence of degenerative and chronic diseases, such as: cancer, cardiovascular diseases and cerebral disfunctions (ZIBADI et al., 2007). These effects are assigned to a variety of compounds like vitamins (carotenes, ascorbic acid and tocopherols), fibers, minerals and, especially, phenolic compounds such as the flavonoids (KIM et al., 2007; SAURA-CALIXTO; GOÑI, 2006). They are polyphenols with structures

derived from the diphenylpropane ($\text{C}_6\text{C}_3\text{C}_6$), that possess the greatest natural occurrence among the existent phenolic compounds. It is estimated that 2% of all the synthesized carbon by the plants is converted in flavonoids, and studies indicate that this class of molecules possesses allelopathic properties and a greater antioxidant potential than the traditional vitamins (SILVA et al., 2010; VIJAYAKUMAR et al., 2008).

Despite the numerous studies in this area, there is a necessity of obtain data regarding the efficiency, safety and appropriate dosage of antioxidant compounds in a regular diet taken by humans. No daily antioxidant compound ingestion recommendda-tion has been established because the composition data are incomplete, the biologic activities aren't well determined and, especially, the bioavailability and

pharmacokinetic data are inconclusive (HASSIMOTTO et al., 2009).

Furthermore, the accurate total antioxidant capacity quantification is very important, because it's influenced by the substrate, solvent and extraction method, as well by the variables time and temperature (LOCATELLI et al., 2009; OLIVEIRA et al., 2009a). However, in relation to the analysis methods, the following parameters are desirable: the employment of biologically relevant molecules as standards; technical simplicity with final point and well defined chemical mechanisms; easily available instrumentation as well as good repeatability, reproducibility and high yield (HUANG et al., 2005). Thus, the obtainment of data regarding the bioactive compounds content and antioxidant activity (AA) in foods is very important.

The aim of this study was the evaluation of the antioxidant capacity and total phenolic compounds (TPC) values of ten fruits commercialized at the Maringá, Paraná State, city fair, through the employment of a solvent and methods that are significantly reported in the literature and based on the UV-Vis espectrophotometry (OLIVEIRA et al., 2009b).

Material and methods

Sample preparation and antioxidant compounds extraction

The fresh fruits samples were obtained during three consecutive weeks, washed with deionized water, dried at room temperature, milled, homogenized and submitted to methanolic extraction with a 1:10 (sample mass/ methanol volume) proportion, and constant stirring by 4 hours under protection from light, followed by filtration and solvent removal by rotary evaporation.

Antioxidant capacity against DPPH• radical

The antioxidant capacity of the extracts was evaluated through the method proposed by El-Massry et al. (2002), an assay with a methanolic solution of the 2,2-difenil-1-picrilhidrazila (DPPH•) radical, with a concentration of 0.036 mg mL⁻¹. Approximately 2 mL of the DPPH• methanolic solution was added in different aliquots of solutions containing 20 mg of fruit extract and 10 mL of methanol and kept in the dark by 30 minutes at room temperature. The values determination was realized through UV-Vis molecular absorption espectrophotometry, against a blank which contained only methanol, with absorbance scan at 517 nm. The results were expressed through the calculation of the DPPH• inhibition percentage,

which estimates the remaining concentration of the radical after the reaction with the antioxidant compounds from the extract (Equation 1).

$$\% \text{ Inhibition of DPPH} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100 \quad (1)$$

Where A_{control} is the DPPH• radical absorbance without the extract and A_{sample} is the DPPH• absorbance with the extract. The analyses were done in triplicates and the concentration of extract proportional to a 50% inhibition of DPPH• radical (IC_{50}) was obtained through the analysis of the extract solution concentration versus inhibition percentage graphic. Thus, lower extract concentrations ($\mu\text{g mL}^{-1}$) mean greater antioxidant capacity provided by the analyzed fruits.

Antioxidant activity index (AAI) calculus

The antioxidant activity index (AAI) was investigated according with the work of Scherer and Godoy (2009). This parameter measures the antioxidant potential, according to the equation 2.

$$AAI = \frac{\text{Concentration of DPPH} (\mu\text{g mL}^{-1})}{IC_{50} (\mu\text{g mL}^{-1})} \quad (2)$$

Being the AAI the ratio between the DPPH methanolic solution concentration and the IC_{50} of each analyzed fruit. The AAI is calculated considering the masses of the radical and a specific extract. According to the authors this calculation allows the comparison of the results obtained from different samples, despite the variety of proposed methods which employs DPPH, and regardless the concentration of this radical, as well the reaction time.

Total phenolic compounds content

The total phenolic compounds contents were determined according to the method proposed by Shahidi and Nacz (1995). An 0.25 mL aliquot of extract in methanol (2.5 mg mL^{-1}) was mixed with 0.25 mL of the Folin-Ciocalteau reagent previously diluted with water in a proportion of 1:1 (v v⁻¹), followed by the addition of 0.5 mL of a sodium carbonate saturated solution and 4mL of water. The mixture was kept static at room temperature during 25 minutes followed by centrifugation at 3800xg by 10 minutes. The supernatant was separated and measured in a UV-Vis spectrophotometer at 725 nm. The results were determined through the calibration curve established between the relation of the

absorbance in function of various concentrations of gallic acid (standard) diluted in methanol, submitted to the same parameters of the employed method in the samples. Thus, the results were expressed in milligrams of gallic acid equivalent /100 g of sample.

Statistical analysis

The results were expressed with mean and standard deviation in triplicate and submitted to variance (ANOVA) analysis, while their comparison were done through the Tukey's test with $p < 0.05$ significance level on Microsoft Excel 2007 and Statistica 7.0 programs (STATSOFT, 2004). The correlation between TPC and antioxidant capacity was determined through the use of the OriginPro 8 program (ORIGINLAB CORPORATION, 2007).

Results and discussion

Some researches conclude that the methanol is the most effective organic solvent regarding the extraction of bioactive compounds (MOURE et al., 2001; NACZK; SHAHIDI, 2004; OLIVEIRA et al., 2009b; RAZALI et al., 2012; SAHREEN et al., 2010). The authors also report that this solvent possesses high efficiency in the extraction of phenolic compounds. The Table 1 shows the absolute values of the realized analyses for the methanolic extracts of the ten fruits. It can be observed that the jambul and acerola extracts possess greater TPC content and antioxidant capacity.

In relation to the antioxidant molecules composition, previous researches showed that jambul possesses carotenoids and significant amounts of anthocyanins while acerola contains a lesser quantity of this compound, as well as polyphenols and great contents of ascorbic acid (FARIA et al., 2011; MEZADRI et al., 2008; ROSSO et al., 2008). The Figure 1 shows the correlation between the total antioxidant capacity and TPC.

A positive correlation result was observed ($R^2 = 0.6169$), thus the phenolic compounds might be one of the main components responsible by free

radicals inhibition in the analyzed fruits. It's interesting to mention that, despite the significant antioxidant capacity showed by the star fruit, it presented low correlation with TPC, so that upon removing its correlation point, its value becomes more positive ($R^2 = 0.7913$). The obtained results were similar with the ones reported by Fu et al. (2011), Ikram et al. (2009), Vasco et al. (2008) and Almeida et al. (2011) with fruits from different geographic regions. It is very important to relate the polyphenolic molecules composition with their antioxidant activity, which can be defined as chelation of redox-active metal ions, inactivation of lipid free radical chains or prevention of hydroperoxide conversion into reactive oxyradicals (OLIVEIRA et al., 2009a).

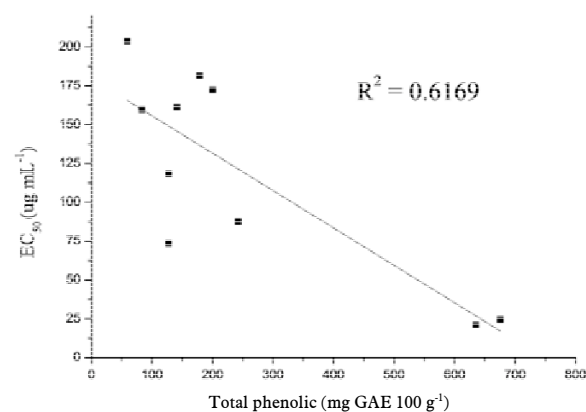


Figure 1. Correlation between the total antioxidant capacity and TPC.

The Figure 2 shows the methanolic extracts AAI of the analyzed fruits. It can be also stated as the comparison of the IC_{50} potential in different samples (SCHERER; GODOY, 2009).

According with the authors when the AAI < 0.5 , the sample possesses a poor AA, between 0.5 and 1.0 an average AA, between 1.0 and 2.0 a strong AA and > 2.0 a very strong AA. In this work, jambul and acerola showed strong AAs.

Table 1. Antioxidant capacity and total phenolic compounds content of methanolic extracts from ten fruits acquired at Maringá, Paraná State, city fair.

| | Popular name | Scientific name | IC_{50} ($\mu g mL^{-1}$) | TPC (mg GAE 100 g sample $^{-1}$) |
|----|----------------|------------------------------|-------------------------------|------------------------------------|
| 1 | Jambul | <i>Syzygium cumini</i> | $20.94^a \pm 0.56$ | $635.32^b \pm 3.82$ |
| 2 | Acerola | <i>Malpighia emarginata</i> | $24.48^a \pm 0.27$ | $675.73^a \pm 3.40$ |
| 3 | Star fruit | <i>Averrhoa carambola</i> L. | $73.48^b \pm 4.53$ | $127.26^g \pm 1.48$ |
| 4 | Mulberry | <i>Morus nigra</i> L. | $87.57^c \pm 2.88$ | $242.60^e \pm 5.57$ |
| 5 | White Guava | <i>Psidium guajava</i> L. | $118.22^d \pm 3.89$ | $127.54^g \pm 2.01$ |
| 6 | Red Guava | <i>Psidium guajava</i> L. | $159.57^e \pm 2.33$ | $83.43^h \pm 2.17$ |
| 7 | Pitanga | <i>Eugenia uniflora</i> L. | $161.02^e \pm 1.37$ | $141.38^f \pm 4.58$ |
| 8 | Blackberry | <i>Rubus procerus</i> | $172.46^f \pm 4.25$ | $200.37^d \pm 1.71$ |
| 9 | Strawberry | <i>Fragaria vesca</i> L. | $181.38^f \pm 3.66$ | $178.56^e \pm 3.40$ |
| 10 | Fuyu Persimmon | <i>Diospyros kaki</i> L. | $203.73^g \pm 4.80$ | $58.97^i \pm 4.62$ |

IC_{50} Antioxidant capacity. TPC total phenolics. GAE Gallic Acid Equivalent. Means with different letters on the same column are significantly different for $p < 0.05$.

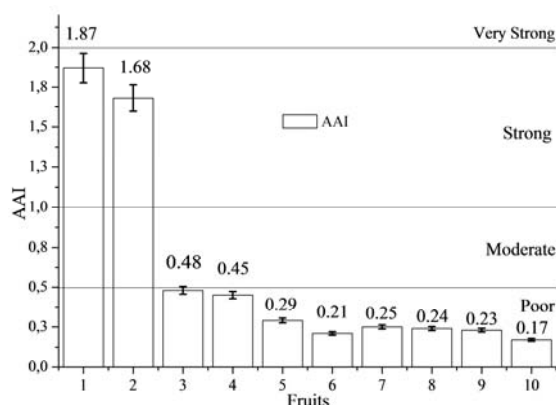


Figure 2. Methanolic extracts AAI of the analyzed fruits. 1- Jambul, 2-Acerola, 3-Star Fruit, 4-Mulberry, 5- White pulp Guava, 6-Red pulp Guava, 7-Pitanga, 8-Blackberry, 9-Strawberry, 10-Fuyu Persimmon.

Conclusion

The results indicate a great variation in the antioxidant capacity among the studied fruits and this characteristic might be related with the TPC composition. Jambul and acerola, in particular, might be great dietetic sources of natural antioxidants aimed to the prevention of diseases related to the oxidative stress. This study provided new informations regarding the antioxidant molecules compositions in fruits for consumers, nutritionists and institutions which formulate food policies.

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