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INVESTIGATION ON THE CONTENT OF PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY OF INSTANT COFFEES FROM COFFEA ARABICA

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Abstract
Coffee is one of the most popular and consumed beverage worldwide both for the organoleptic characteristics and for the bioactive compounds' content. Previous studies have shown that despite some risks it brings to health, it is useful in preventing cardiovascular diseases, liver diseases and hyperglycemia, and adding creams based on milk proteins helps to preserve its antioxidant activity after digestion.
The content of total phenolics, flavonoids, tannins and the antioxidant activity were assessed in ten commercially soluble coffee samples. The results showed that the total phenolics content ranged from 1783.45 to 2061.30 mg gallic acid 100^{-1} g, the total flavonoids content ranged from 7637.55 to 13373.67 mg quercitin 100^{-1} g and condensed tannins level ranged from 1701.21 to 2176.57 mg catechin 100^{-1} g. The total antioxidant activity varied from 5513.84 to 7905.11 mg ascorbic acid 100^{-1} g. The differences found among the investigated samples containing 100% Coffea arabica might be due to the different bean processing. Despite these variations, instant coffees represent an additional source of bioactive compounds with antioxidant properties.

Keywords: antioxidant activity, flavonoids, instant coffee, phenolics, tannins.

1. INTRODUCTION

There is a common agreement among researchers that a diet rich in fruits and vegetables has beneficial effects on human health, based on the phytochemical composition of plant foods. Other foods, such as beverages (herbal tea, specific teas, coffee and wine) may contribute to the intake of antioxidant compounds (Ricci et al., 2018).
Coffee is one of the world's most famous beverage (Alves et al., 2021). Beside caffeine, coffee beans (*Coffea arabica, Coffea canephora*) are rich sources of valuable compounds, such as minerals, glucans, mannans, lignin, pectins, proteins, oils, enzymes and polyphenols, in particular chlorogenic acid (Trugo, 2003, Hutachok et al., 2021), but also gallic acid, rutin and caffeic acid (Hutachok et al., 2021).
According to Tsirimiagkou et al. (2021), a moderate consumption of coffee can support the elasticity of the arteries, reducing the chances of developing diseases of the circulatory system, but also to protect the body from developing chronic liver disease (Kennedy et al., 2021).

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Instant coffee represents a dried water-soluble fraction of roasted coffee, containing mainly soluble and aromatic substances of coffee. It was shown that instant coffees contain higher amounts of total phenolics and caffeine compared to green Robusta coffee (Pérez-Hernández et al., 2012). Gajić et al. (2022) concluded that in order to promote its sale among the people interested in health or who are more powerful from a financial point of view, the emphasis should be placed on the content of natural ingredients beneficial to the well-being, but also on finding a sustainable way of packaging coffee.

The presence of bioactive compounds in coffee, in particular polyphenolic ones, determines beneficial human health effects, such as protective role against oxidative stress, hypoglycemic, antiviral and hepatoprotective properties (Farah and Donangelo, 2006). Instant coffee with added chlorogenic acid proved to have a stronger effect in reducing glucose adsorption than the classic one (Thom, 2007).

Despite the benefits it could have, coffee consumption also raises some health issues. For example, the coffee contamination with the mycotoxin ochratoxin A (OTA) is associated with a higher risk of cancer development, instant coffee showing a greater nephrotoxic effect than the classic one (Yazdanfar et al., 2022). Zapaśnik et al. (2022) observed that instant coffee also presents an isomer of OTA, namely 2′R-OTA (2′R-ochratoxin A), both compounds being identified in higher amounts than in roasted coffee or cocoa. Furthermore, women's consumption of instant coffee could increase the risk of developing breast cancer (Lee et al., 2019).

Instant coffee will never be able to reach the quality of natural coffee, which is much safer for regular consumption due to its lower cadmium and lead content (Winiarska-Mieczan et al., 2023).

Yu et al. (2021) observed that without the addition of skim milk or creams based on milk proteins, instant coffee loses a considerable part of its antioxidant potential after digestion.

According to Wuerges et al. (2020), depending on the procedure of harvesting and preparation of coffee beans, but also on the technique of obtaining the drink and the characteristics of the production process (Alves et al., 2021, Bastian et al., 2021), the bioactivity of coffee and the content of unwanted compounds can be different (Wuerges et al., 2020, Alves et al., 2021). Thus, instant coffee proved to have a lower content of diterpenes than other types of coffee (Wuerges et al., 2020).

The processing of green beans highly influences the content of specific compounds, which may decrease during roasting (Cortés-Macías et al., 2022). Also, other compounds (volatiles, polymers) may occur as a result of the Maillard reactions (Starowicz and Zieliński, 2019). During brewing of ground roasted coffee, polar compounds including volatiles responsible for taste and flavor are extracted (Cordoba et al., 2020). Depending on the brewing conditions, in particular temperature, the final beverage may present different bioactive composition (Cordoba et al., 2020, Bastian et al., 2021).

The aim of the present study was to investigate the content of phenolics, flavonoids and tannins of ten different commercially soluble coffee samples. The total antioxidant activity, measured by ferric reducing antioxidant power (FRAP) assay was also performed.

2. MATERIALS AND METHODS

Sample preparation

A number of 10 samples of instant coffees produced by different manufacturers and purchased from local markets, was investigated. The analyzed samples were selected from the following brands:

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Doncafé (1-3), Jacobs (4-5), Nescafé (6), Amigo (7), Fortuna (8), Globo Café (9) and Tchibo (10). The commercial soluble coffee samples were dissolved in water at 85°C just before analysis.

Determination of total phenolics

The total phenolics content of instant coffees was determined by Follin-Ciocalteu assay (Singleton and Rossi, 1965). Values were calculated based on a standard calibration curve of gallic acid (GAE) and expressed as mg GAE $100g^{-1}$ DM.

Determination of total flavonoids

The total flavonoids content of instant coffees was determined by colorimetric method (Bag et al., 2015). Values were calculated based on a standard calibration curve of quercetin and expressed as mg quercetin $100g^{-1}$ DM.

Determination of total condensed tannins

The tannin content of instant coffees was determined by the method of Broadhurst et al. (Broadhurst and Jones, 1978). Values were calculated based on a standard calibration curve of catechin and expressed as mg catechin $100g^{-1}$ DM.

Antioxidant activity

The antioxidant activity of instant coffees was determined by ferric reducing antioxidant power (FRAP) assay according to Benzie and Strain (Benzie and Strain, 1996).

Statistical analysis

All measurements were performed in duplicate. Results were calculated and expressed as mean ± standard deviation. The existence of possible correlations and glm models between variables were analyzed using R 4.2.0 software.

3. RESULTS AND DISCUSSIONS

The results regarding the total concentration of significant antioxidant compounds of the polyphenolic structure (flavonoids, phenolics, tannins) of ten instant coffee samples from commercially coffees prepared in water, as described on the label of manufacturer, are shown in Table 1.

Table 1. Total content of polyphenolic-based bioactive compounds in instant coffee samples (mean value±SD).

Sample	Total phenolics (mg GAE $100g^{-1}$)	Total flavonoids (mg quercetin $100g^{-1}$)	Total tannins (mg catechin $100g^{-1}$)
1	2015.18±8.13	9963.55±37.07	1984.41±3.07
2	1880.92±5.72	10548.11±3.09	1874.21±2.14
3	1839.11±4.25	7953.69±71.96	2053.78±7.65
4	1961.39±9.00	9636.80±106.74	2075.05±6.13
5	1841.67±2.04	8758.99±5.27	1963.75±8.87
6	1849.22±6.87	8343.65±24.71	1851.26±3.72
7	1854.20±5.84	13373.67±83.10	2031.44±2.95
8	1783.45±5.34	10373.15±225.69	2176.57±1.38
9	1839.12±12.70	10489.11±1.06	1701.21±3.11
10	2061.30±10.25	7637.55±7.07	1948.69±3.32

Coffee pulp and seed contain high amounts of non-flavonoid compounds (chlorogenic acids) and lower amounts of flavonoids (Farah and Donangelo, 2006). These authors reported a large variation of the content of total chlorogenic acids (CGA) from 0.6 to 10.7 g CGA $100g^{-1}$ DM, in 13

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commercial instant coffees, due to their composition (blends) and processing (roasting degree). Other authors reported high content of total phenolics in instant coffee from Robusta beans (143.99±9.92 mg chlorogenic acid g-1) (Pérez-Hernández et al., 2012). The analysis of Arabica instant 100% coffee showed a content of 57.47±11.24 µg GAE ml^{-1} total phenolics and 1.02±0.05 µg quercetin ml^{-1} total flavonoids which were higher than Arabica instant coffee Crema Gold (31.24±18.07 µg GAE ml^{-1} total phenolics and 0.91±0.01 µg quercetin ml^{-1} total flavonoids) (Hudáková et al., 2016). These authors found that lower contents of flavonoids and phenolics are extracted in instant coffee samples compared to roasted/green ground coffees. Regarding the tannins content, it was reported that a cup of instant coffee may provide between 111 and 128 mg of tannins (Janssen et al., 1996). Other authors reported a content of 4.3% tannins in instant coffee (Berdanier, 2011). Our results showed much lower amounts of total tannins below 2% (mean value 1966.04 mg catechin $100g^{-1}$) than the other reported studies. According to Thakur et al. (2019), the presence of a low level of such anti-nutritional compounds can have beneficial effects on human health such as reducing blood glucose. According to Thakur et al. (2019), the presence of a low level of such anti-nutritional compounds can have beneficial effects on human health such as reducing blood glucose.

The results regarding the total antioxidant activity of investigated samples as measured by FRAP and presented in Figure 1, showed high values in samples 3, 8 and 10 (between 7830.92 and 7905.11 mg ascorbic acid $100g^{-1}$). The lowest total antioxidant activity was registered in sample 6 (5513.84 mg ascorbic acid $100g^{-1}$).

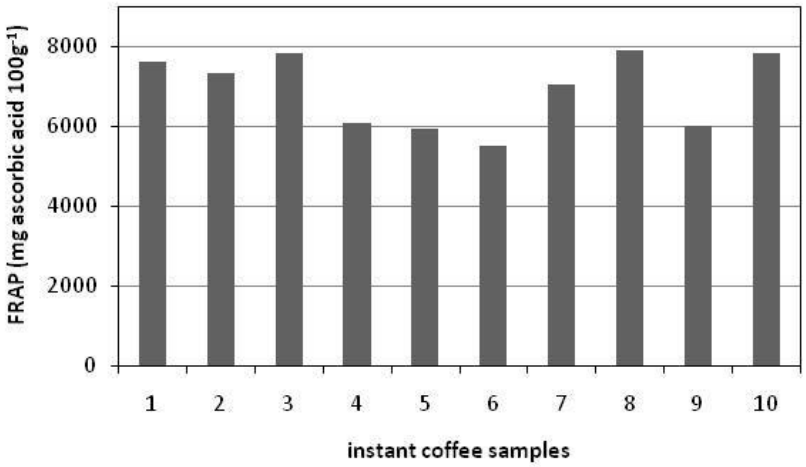

Figure 1. The total antioxidant activity of instant coffees, as measured by FRAP assay.

The antioxidant activity of instant coffees was investigated by other authors, using different assays than the FRAP used in our study. A research on 33 samples of Brazilian commercial soluble coffees showed high radical scavenging activity as measured by ABTS method, in particular in regular samples compared to Gourmet coffees (Marcucci et al. 2017). Another study described higher antioxidant activity by ABTS assay of instant coffees than the green Robusta beans (Pérez-Hernández et al., 2012).

We found a moderate positive correlation between the content of total tannins and FRAP activity (R= 0.5011), but the result is not significant at $p<0.05$.

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In order to analyze the correlations between the content of polyphenols, tannins and flavonoids of the samples, the correlation matrix, found in Table 2, was built.

Table 2. The correlation matrix between the content of total phenolics, tannins and flavonoids

	Total phenolics	Total tannins	Total flavonoids
Total phenolics	1.00	-0.03	-0.29
Total tannins	-0.03	1.00	0.07
Total flavonoids	-0.29	0.07	1.00

All three variables presented a normal distribution, therefore the Pearson correlation was used. The correlations between the amount of total phenols and tannins (p = 0.9384), between total flavonoids and tannins (p = 0.8560) and between flavonoids and phenols (p = 0.4232) proved no significance for the investigated samples. Instead, the GLM analysis, with Gamma distribution and "log" link function, showed a significant relationship between the antioxidant activity analyzed by FRAP of the coffee samples and the flavonoid content, the first one increasing by 1.000083 for each mg quercetin/100g DW added. . Luximon-Ramma et al. (2005) reported a weak relationship between the two variables. The total phenolics and the tannin content do not significantly explain the variation in the antioxidant activity in this case, compared to other reported studies (Luximon-Ramma et al., 2005, Jeng et al., 2010).

4. CONCLUSIONS

Instant coffees may constitute an important source of antioxidant compounds of polyphenolic structure.

Ten samples of commercially instant coffee were investigated for the content of total phenolics, flavonoids and tannins, and total antioxidant activity as measured by FRAP assay. The results showed an average content of 1892.56 mg GAE $100g^{-1}$ phenolics, 9707.82 mg quercetin $100g^{-1}$ flavonoids and 1966.04 mg catechin $100g^{-1}$ tannins. Doncafé and Tchibo samples showed the highest phenolics content, while Amigo samples registered the highest level of flavonoids. The sample Globo Café showed the lowest tannin content. The mean value of the total antioxidant activity in all samples was 6914.78 mg ascorbic acid $100g^{-1}$. Fortuna, Doncafé and Tchibo samples showed the highest antioxidant activity.

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