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*Chemistry*

# STUDY OF ANTIOXIDANT PROPERTIES OF SOME TYPES OF PACKAGED COFFEE

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In this work, several varieties of instant and ground, roasted black packaged coffee were studied. Various extraction methods were used to obtain extracts. The caffeine content was investigated by UV-Vis absorption spectroscopy and HPLC chromatography. The antioxidant properties of the extracts were studied by the method of competitive reactions.

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***Keywords*:** instant coffee, ground, roasted black coffee, caffeine, antioxidant properties, photochemistry, PNDMA.

**Introduction.** In the cellular metabolism of the human body, the formation and accumulation of reactive oxygen forms take place. These molecules exhibit detrimental effects on cellular integrity and normal physiological processes, leading to oxidative stress. An essential role of antioxidants is to bind and remove free radicals from the body. Numerous food types encompass compounds with inherent antioxidant properties, offering the potential to enhance and rehabilitate the endogenous antioxidant defense system [1].

There are more than 100 types of coffee in the world classified by their origin, of which 98% are Arabica and Robusta. At the same time, there are two types as an end-product: instant and ground, roasted coffee [2].

The antioxidants like phenolic compounds, caffeine, chlorogenic acid, caffeic acid and other organic acids can be contained in coffee [3–7].

Some physiological properties of coffee are determined by the content of purine alkaloids: caffeine, theobromine and theophylline. The listed alkaloids actively affect the central nervous system (CNS), initiate cardiac activity, improve mental and physical abilities of a person. Caffeine (1,3,7-trimethylxanthine) has the greatest effect on the CNS. The main sources of caffeine in the human diet are coffee and tea [8].

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The purpose of this work is to study the antioxidant properties of different samples of instant and ground, roasted black coffee on sale, to find out which samples contain the highest amount of caffeine and have the strongest antioxidant activity. The experiments were carried out using UV-Vis absorption spectroscopy and HPLC.

**Materials and Methods.** The samples of instant and ground, roasted black coffee on sale are presented in Tab. 1.

*Table 1*

*List of samples*

| Type of coffee | Sample No | Brand, name |
|---|---|---|
| Instant | 1 | Jockey Favorite strong and rich Arabica |
| | 2 | Nescafe Gold golden roast, with ground Arabica |
| | 3 | Tchibo Gold Selection natural instant sublimated coffee |
| | 4 | Jacobs Monarch |
| | 5 | Café Pelé |
| Ground, roasted, black | 6 | Jacobs Monarch natural roasted ground coffee |
| | 7 | Jockey Arabica ground roasted coffee |
| | 8 | Le Café de Paris (80% Arabica,20% Robusta) |
| | 9 | "Royal Armenia" Classic Arabica and Robusta |
| | 10 | Latino ground coffee Robusta |

***Methods.*** 1 *g* of the selected sample was extracted with 200 *mL* of distilled water in the presence of a reflux refrigerator on a water bath at 90℃ for 20 *min*. After the extraction was completed, the extracts were brought to room temperature, transferred to 250 *mL* volumetric flasks and brought to the mark. The standardized solutions were filtered and kept at 4℃ throughout the study. The resulting standard solutions were diluted 100 times and subjected to UV-Vis spectral analysis.

***Instrumentation.*** The studies were carried out by UV-Vis spectrometer (PG-instruments, model T60) in the wavelength range of 220–600 *nm*.

Quantitative and qualitative analysis of caffeine was carried out using the HPLC method. The conditions of this method are presented in Tab. 2.

*Table 2*

*HPLC conditions for used method*

| Column | Nucleosil C18 250×4.6 *mm*, 5 $\mu m$ |
|---|---|
| Wavelength of the detector | 275 *nm* |
| Flow velocity | 1 *mL*/*min* |
| Column temperature | 40℃ |
| Injected volume | 10 $\mu L$ |
| Mobile phase | 60% $H_2O$ / 40% methanol |

A caffeine standard solution was used as a reference: "Standard sample 250 *mg*/*L*, Shimadzu corp., Japan". Samples were injected without dilution.

***Free Radical Scavenging Ability Using Hydroxyl Radical.*** In the evaluation of antioxidant properties within the p-nitrosodimethylaniline (PNDMA) assay, the focus lies on analyzing the competitive reaction kinetics between PNDMA and hydroxyl radicals. When exposed to UV light at 313 *nm*, hydrogen peroxide undergoes irradiation, leading to the formation of hydroxyl radicals. These radicals, in turn, interact with PNDMA, resulting in a reduction in its color intensity. The rate of this interaction between hydroxyl radicals and PNDMA is determined by measuring the absorption at 440 *nm* through the employment of spectrophotometry.

The introduction of coffee extracts significantly alters this reaction due to the competition between antioxidants and PNDMA, which are the targets for hydroxyl radicals. This competition leads to a decelerated fading of the decolorization of PNDMA. The rate constant of the reaction between antioxidants, derived from coffee extracts and hydroxyl radicals can be calculated using the equation [1, 9, 10]:

$$k_{\mathrm{OH+antioxidant}} = 1.25 \cdot 10^{10} \frac{[\mathrm{PNDMA}]}{[\mathrm{Antioxidant}]}\left(\frac{W_1}{W_2} - 1\right).$$

The factor $1.25 \cdot 10^{10}$ signifies the rate constant for the PNDMA and hydroxyl radical reaction, measured in units of, $mol^{-1} \cdot L \cdot s^{-1}$. $W_1$ and $W_2$ correspond to the slopes observed in the plots depicting the changes in absorption of PNDMA over time during hydrogen peroxide irradiation, both in the absence and presence of coffee extracts, respectively. A methodology similar to this was employed for determination of the rate constant of the PNDMA and hydroxyl radical reaction in the presence of the widely recognized antioxidant, ascorbic acid ($9.45 \cdot 10^9\ M^{-1} \cdot s^{-1}$) [10].

**Results and Discussion.**

***Spectral Analysis.*** UV-Vis spectra of instant coffee extracts are shown in Fig. 1.

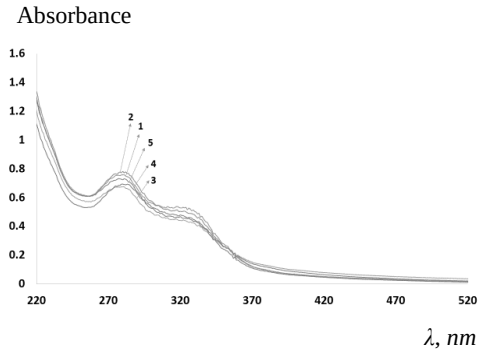


Fig. 1. UV-Vis absorption spectra of extracts prepared from instant coffee samples No 1–5.

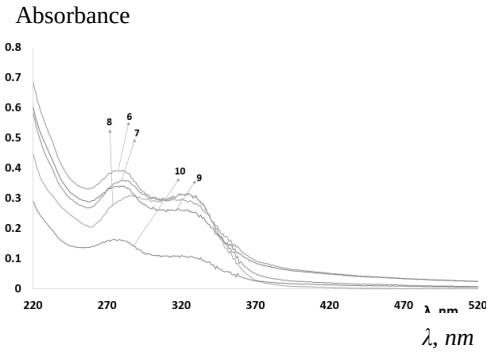


Fig. 2. UV-Vis absorption spectra of extracts prepared from ground, roasted coffee samples No 6–10.

UV-Vis spectra of coffee extracts are characterized by two bands: at 278 *nm* and 317 *nm*, which indicates the presence of caffeine and other substances in trace amounts in the extracts. UV-Vis spectra of ground, roasted black coffee extracts are shown in Fig. 2.

The comparison of the obtained spectra with literature data [11] indicates that two bands at 278 *nm* and 317 *nm* are due to the trace amounts of caffeine and other substances in the extracts. From the obtained spectra of all extracts can be noticed

the bathochromic and hypsochromic shifts of peaks, which are due to the different composition of extracts [12].

***HPLC Chromatography.*** In order to determine the amount of caffeine, the chromatographic analysis of the samples was carried out. The retention times of the separated substances in the chromatograms, along with the quantitative data acquired for caffeine, are shown in Tab. 3.

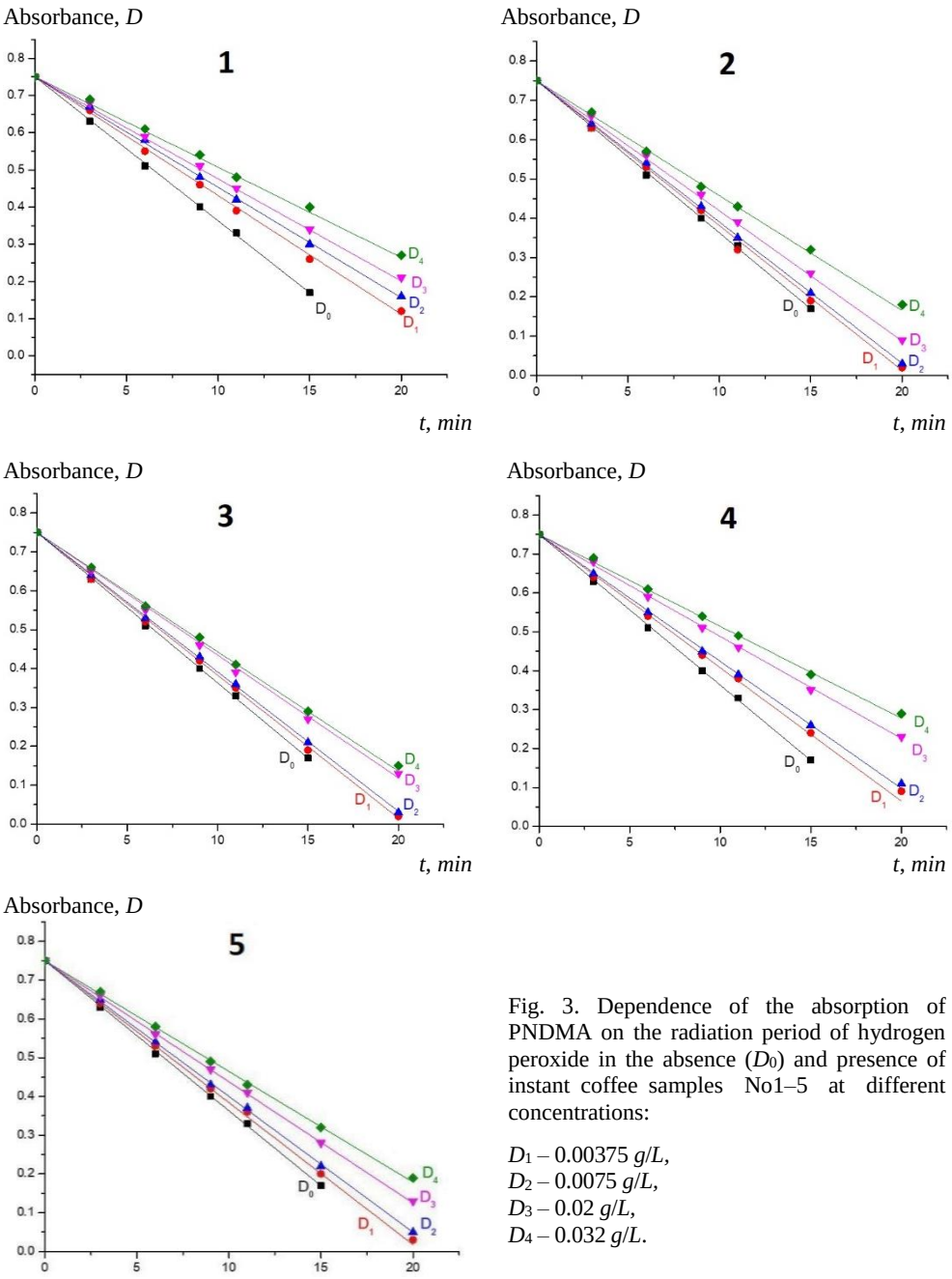


Fig. 3. Dependence of the absorption of PNDMA on the radiation period of hydrogen peroxide in the absence ($D_0$) and presence of instant coffee samples No1–5 at different concentrations:

$D_1$ – 0.00375 *g/L*,
$D_2$ – 0.0075 *g/L*,
$D_3$ – 0.02 *g/L*,
$D_4$ – 0.032 *g/L*.

According to the results of chromatographic analysis, the highest amount of caffeine was found in samples No 5 (instant coffee) and No 6 (ground, roasted black coffee).

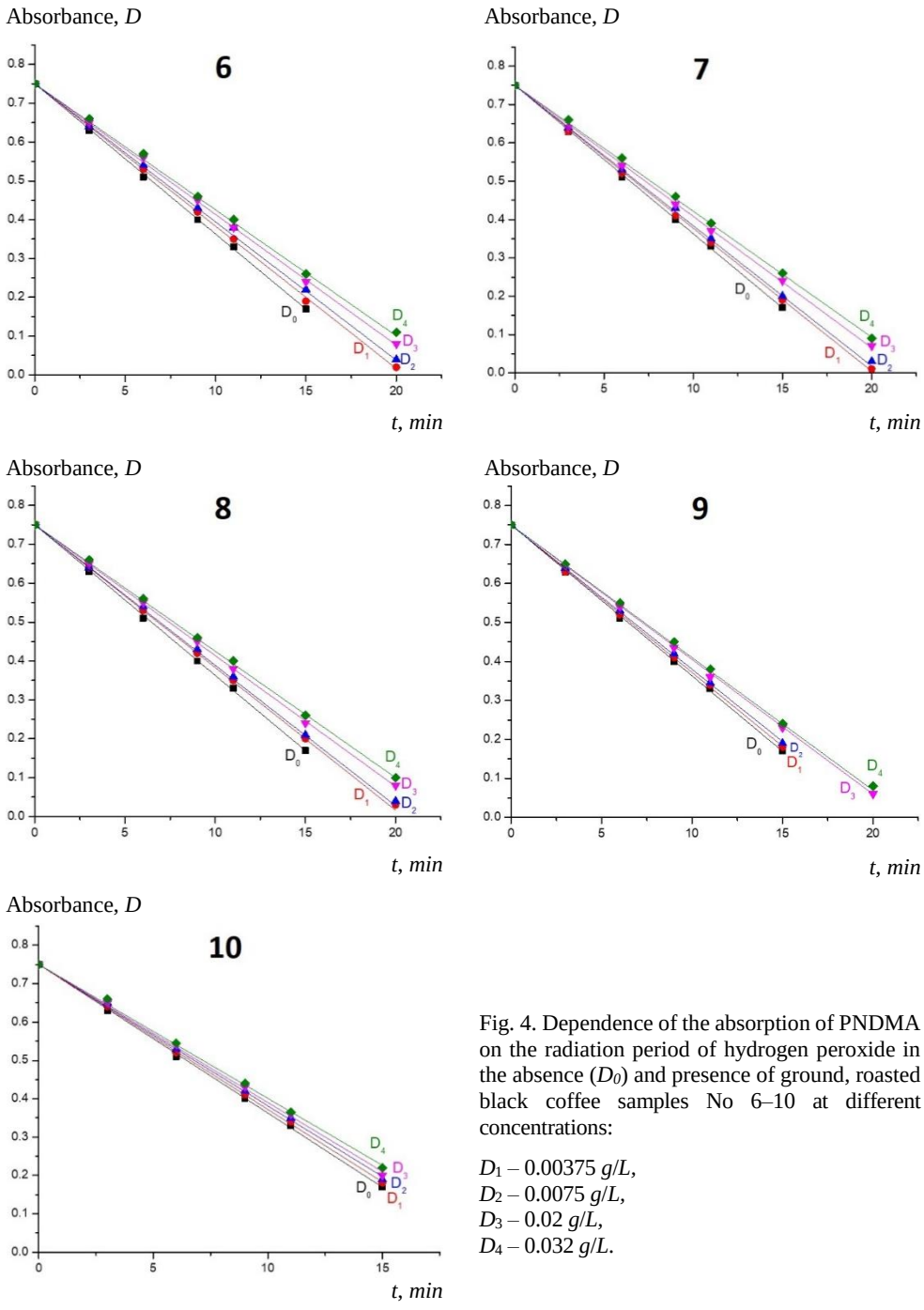


Fig. 4. Dependence of the absorption of PNDMA on the radiation period of hydrogen peroxide in the absence ($D_0$) and presence of ground, roasted black coffee samples No 6–10 at different concentrations:

$D_1$ – 0.00375 *g/L*,
$D_2$ – 0.0075 *g/L*,
$D_3$ – 0.02 *g/L*,
$D_4$ – 0.032 *g/L*.

To demonstrate the antioxidant properties of mixtures obtained, the method of competitive reactions was carried out. For research purposes, measurements were performed for each sample extract in systems with concentrations of 0.004, 0.008, 0.02, and 0.032 *g*/*L*. Measurements were carried out using a photocolorimeter at a wavelength of 440 *nm*.

Fig. 3 and Fig. 4 represent the dependence of the absorption of PNDMA on the radiation period of hydrogen peroxide in the absence and presence of instant coffee extracts, and ground, roasted black coffee extracts, respectively.

*Table 3*

*Results of HPLC analysis of caffeine*

| No | Retention time, *min* | Amount of caffeine, *mg*/*mL* |
|---|---|---|
| Standard | 6.489 | 0.25 |
| 1 | 6.558 | 0.001825 |
| 2 | 6.552 | 0.001235 |
| 3 | 6.550 | 0.001572 |
| 4 | 6.540 | 0.001166 |
| 5 | 6.536 | 0.002501 |
| 6 | 6.531 | 0.001367 |
| 7 | 6.537 | 0.00089 |
| 8 | 6.535 | 0.000579 |
| 9 | 6.524 | 0.000921 |
| 10 | 6.535 | 0.000535 |

*Table 4*

*Rate constants of the reaction between coffee extract and hydroxyl radicals*

| Type of coffee | Sample No | Antioxidant activity, $k \cdot 10^{8}$, $mol^{-1} \cdot L \cdot s^{-1}$ |
|---|---|---|
| Instant | 1 | 1.372 |
| | 2 | 0.334 |
| | 3 | 0.366 |
| | 4 | 0.858 |
| | 5 | 0.449 |
| Ground, roasted, black | 6 | 0.438 |
| | 7 | 0.280 |
| | 8 | 0.377 |
| | 9 | 0.223 |
| | 10 | 0.204 |

The reaction rate curves were constructed for instant (Fig. 5, a) and ground, roasted black coffees (Fig. 5, b).

Based on the data obtained and using the equation, the relative values of the reaction rate constants were calculated, which determine quantitatively the antioxidant activity. The results obtained are given in Tab. 4.

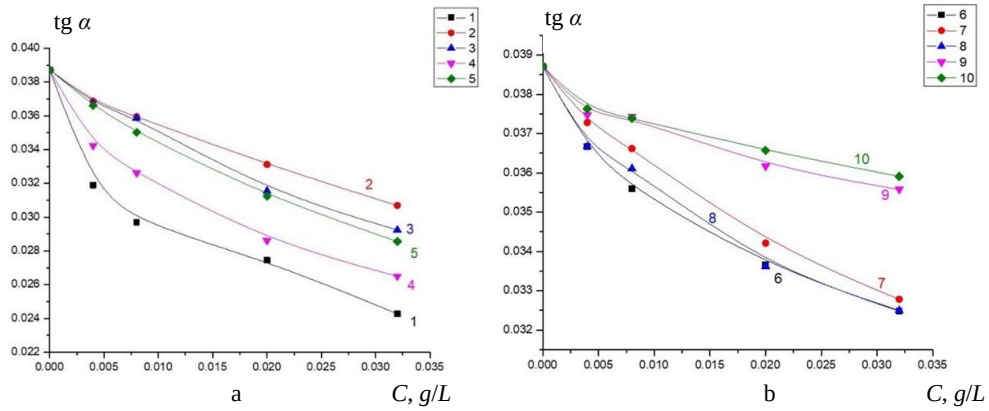


Fig. 5. Dependence of the reaction rate on the concentrations of extracts of the studied instant coffee (a) and ground, roasted black coffee (b) samples.

**Conclusion.** The highest content of the caffeine was found in samples No 5 (instant coffee) and No 6 (ground, roasted black coffee). Instant coffee was found to contain more caffeine in comparison with ground, roasted black coffee.

The study of antioxidant activity revealed that extracts No 1 (instant coffee) and No 6 (ground, roasted black coffee) have the highest antioxidant activity. The antioxidant activity of instant coffee was found to be higher in comparison with the ground roasted black coffee. This can be explained by the presence of relevant amounts of caffeine in the samples.

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## ՓԱԹԵԹԱՎՈՐՎԱԾ ՍՈՒՐՃԻ ՈՐՈՇ ՏԵՍԱԿՆԵՐԻ ՀԱԿԱՕՔՍԻԴԱՆՏ ՀԱՏԿՈՒԹՅՈՒՆՆԵՐԻ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆ

Այս աշխատանքում ուսումնասիրվել են լուծվող և աղացած, բոված սև փաթեթավորված սուրճի մի քանի տեսակներ: Լուծամզվածքներ ստանալու համար օգտագործվել են էքստրակցիայի տարբեր մեթոդներ: Կոֆեինի պարունակությունը հետազոտվել է կլանման էլեկտրոնային սպեկտրա-սկոպիայի և ԲԱՀՔ մեթոդների միջոցով: Էքստրակտների հակաօքսիդիչ հատկություններն ուսումնասիրվել են մրցակցային ռեակցիաների եղանակով:

В. В. ВАРДАПЕТЯН, М. С. ТОРОСЯН, Г. А. ШАГИНЯН, А. И. МАРТИРЯН

## ИЗУЧЕНИЕ АНТИОКСИДАНТНЫХ СВОЙСТВ НЕКОТОРЫХ ВИДОВ УПАКОВАННОГО КОФЕ

В данной работе были изучены несколько сортов растворимого и молотого, обжаренного черного упакованного кофе. Для получения экстрактов использовались различные методы экстракции. Содержание кофеина было исследовано УФ-спектроскопическим и ВЭЖХ методами. Антиоксидантные свойства экстрактов были исследованы методом конкурентных реакций.