

## NUTRITIONAL PROFILE ASSESSMENT AND ANTIOXIDANT CAPACITY OF SOME FUNCTIONAL BEVERAGES COMMERCIALIZED IN ROMANIA

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*The purpose of this paper is to estimate the nutritional value and antioxidant capacity of 10 functional waters with vitamins and minerals, compared to 10 juices with 10-30% fruits extract, commercialized in Romania. The mineral content was determined by atomic absorption spectrometry method, the total polyphenol content by Folin-Ciocalteau method and the antioxidant capacity by photochemical fluorescence method and DPPH method. The obtained results classify the functional waters in the "significant sources of minerals" category, with a polyphenol content between 10.55-69.10 mg/100 mL (gallic acid) and an antioxidant capacity between 1.875-5.267 (µg Trolox/mL), respectively an IC<sub>50</sub> between 93-893 µg/mL. The results obtained for juices with 10-30% fruits extract highlight a higher content of polyphenols (35.85- 136.07 mg/100 mL gallic acid) and an increased antioxidant capacity, compared to functional waters (6.457 µg Trolox/mL, respectively an IC<sub>50</sub> between 93-242 µg/mL). Compared to fruits, in which the content of all nutrients is much higher, including polyphenols correlated with antioxidant activity, commercial fruit juices must be the secondary choice, only if fresh fruits are not available, and the consumption of water with addition of minerals and vitamins does not contribute significantly to the daily intake of phenolic compounds.*

**Keywords:** functional waters, juices, mineral content, polyphenols, radical scavenger activity

### 1. Introduction

Intense pollution, daily stress, metabolic imbalances and inappropriate eating habits are some of the determining factors for many of the consumers who consume food supplements/functional foods, wanting to improve the quality of life. Health safety is today a priority for most consumers.

The body needs a balanced supply of nutrients and compounds with a physiological role, which are mostly provided by food and supplemented by food

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supplements or functional foods. Food supplements, according to EC Directive 46/2002 [1], do not replace a balanced diet and may contain vitamins, minerals and substances with a nutritional and physiological effect (amino acids, enzymes, essential fatty acids, pre- and probiotics, antioxidants, medicinal plants and plant extracts). Functional food was defined for the first time by the Japanese academic society in 1980, at the same time implementing the FOSHU (Food for Specific Health Use) legislation [2]. Thus, functional food is a conventional or enriched food, containing nutrients (vitamins, minerals, essential fatty acids, essential amino acids, enzymes) with a physiological role that provides a beneficial effect to the body. Contrary to food supplements, functional food is not presented in any pharmaceutical form, but as natural food [3]. Many consumers are turning towards the consumption of natural supplements/functional foods more and more, to prevent and correct biological imbalances, to reduce oxidative stress or to support health and increase the quality of life [4]. Researchers in the field draw attention to the inappropriate consumption of food supplements or functional foods, because it increases the risk of unknown reactions, as a result of some properties of the active compounds, interactions with some foods or drugs. These unwanted reactions must be reported, evaluated and monitored by agencies specialized in nutrивigilance [5].

Functional foods include functional drinks, pre- and probiotic foods, omega-3 acids, oats - a valuable source of  $\beta$ -glucan, tomatoes as lycopene source, as well as many other vegetable sources of antioxidants (turmeric, grapes, tea, coffee). In the consumption top, functional drinks occupy a special place, which includes: energy drinks, sports drinks, functional juices, functional waters, functional coffee and tea. Depending on the nutritional or physiological ingredient contained, functional drinks provide a series of beneficial effects for the body, such as: energy, relaxation, immunity, concentration power, weight and cholesterol/glycemia control, digestive, joint, eye, cognitive health and a healthy sleep, stress relief and healthy aging [6].

The increases in the consumption of food supplements will be maintained in the coming years. If in three years (2018-2020), North America achieved an increase of approximately 16% (from 42.6 to 50.7 billion dollars), it is estimated that in the following years (until 2026) an increase of 40% compared to 2018, respectively by 29.6% compared to 2023. Europe will not register increases, and the market in Asia Pacific will be smaller by 20 billion dollars, compared to the counterpart in North America (which in 2026 will reach a sale of 72 billion dollars) [7]. Regarding the functional drinks market, globally, it is held by the USA. Euromonitor estimated that it was worth USD 48.4 billion in 2020 and will grow at a CAGR of 6.6% during 2020-2025 [8].

The World Health Organization - WHO, as well as the French Agency for Food, Environment, Health and Optional Safety - ANSES, are responsible for the regulation of nutrивigilance and inform consumers of unwanted reactions

following the inappropriate consumption of nutraceuticals. There are reported cases of nutravigilance, when consuming functional drinks, being mainly caused by caffeine. There are numerous data in the literature that draw attention to the uncontrolled consumption among young people of these energy drinks, in which caffeine is accumulated with taurine and glucuronolactone, especially if they are consumed together with alcohol, having as consequences: cardiovascular disorders, psycho-compartmental disorders or neurological (anxiety, nervousness, panic attacks, hallucinations, sleep disorders) [9, 10]. The extract of green tea (*Camellia sinensis*) used a lot to reduce body weight, combined with warfarin causes liver damage, and the consumption of *Aloe vera* (*Aloe barbadensis* Miller), can cause gastrointestinal disturbances, arrhythmias, nephropathy and edema, and when it is administered together with antidiabetic drugs causes hypoglycemia [11]. Many of them functional drinks also contain a series of synthetic additives (preservatives, dyes, acidity correctors) that reduce the quality and safety of the product. The potentiation of Attention-Deficit/Hyperactivity Disorder (ADHD) syndrome is demonstrated as a result of the use of some dyes (carmoisine, ponceau red, tartrazine, orange yellow, blue blue) in combination with sodium benzoate, additives that are found in many of the functional drinks [12, 13]. The main argument for this nutravigilance is that any internally administered substance, whether it serves an explicit medical purpose or not, must be evaluated for both efficacy and safety before reaching the hands of a consumer [14].

With regard to the consumption of soft drinks among young people, the data from the specialized literature highlight a high consumption of two categories of functional drinks - waters that contain some vitamins and some minerals (Mg, Zn, Ca) ± fruit extracts, respectively juices of fruit. These functional drinks are preferred over fresh fruits, which are a natural source of vitamins, minerals, antioxidants and carbohydrates [15]. Recent studies on the nutritional profile of these drinks highlight an unsatisfactory profile with zero values for the SAIN score and high values for the Lim score, classifying them in the category of products consumed occasionally [9].

Polyphenols are compounds with antioxidant properties and prevent coronary diseases, inflammatory processes, diabetes, cancers and aging [16, 17]. Numerous studies confirm the presence of polyphenols in amounts between 100-1000 mg/100 g, correlated with an increased antioxidant activity in blueberries, currants, raisins, cherries, black grapes, red wine, plums, bitter cherries and mulberries, as well as a content below 65 mg/100 g in apple, pomegranate, orange and lemon juice; peaches, black beans, white wine [16, 18, 19, 20]. It was found that tomatoes have an antioxidant activity correlated with the content of lycopene and polyphenols, higher if they are thermally processed [21].

The purpose of this work was to evaluate 20 functional drinks (10 functional waters, respectively 10 fruit juices), marketed in Romania, in terms of the energy value and the quality of the additives, the determination of the mineral

content (Ca, Mg and Zn) and the content of total polyphenols, correlated with their antioxidant activity. The paper aims to contribute to the estimation of nutrients intake from vitaminizing and mineralizing waters, compared to the fruit juices. It is also desired to inform consumers about paying more attention to the labeling of these products, to increase their safety and health security.

## Experimental

### 2.1. Materials

The samples analyzed are of 2 categories (Table 1): category A - waters enriched with vitamins, minerals and fruit concentrate 2 - 3.5%; category B - juices with fruit extract 10-30%, specific compositions reported on each labels by producers.

### 2.2. Evaluation of nutritional labeling and quality of additives

The labeling of the products requires the nutritional declaration, based on the chemical composition of the nutrients, expressed in appropriate mass units by calculating the energy value, the degree of coverage expressed in percentages of DZR per 100 mL of the product, respectively on the portion recommended by the manufacturer and the ingredients in the product, according to the legislation in force [22, 23].

### 2.3. Determination of minerals content

For determination of Ca, Mg and Zn concentration, the Atomic Absorption Spectrometry method, acetylene flame technique was used, with HR-CS AAS Spectrometer ContrAA 700, Analytik Jena AG, Germany, at metals primary line wavelengths, for calcium (422 nm), magnesium (285 nm) and zinc (213 nm) [24]. An aliquot of 0.5 mL of each sample were digested in 5 mL 67% HNO<sub>3</sub> and boiled at 150 °C for one hour, on the sand bath. After cooling, volumes of 2 mL 30% H<sub>2</sub>O<sub>2</sub> were subsequently added, followed by one hour boiling at 150 °C. Samples were diluted to 50 mL each with ultrapure deionized water and 0.2% CsCl and 0.2% LaCl<sub>3</sub> were used, to reduce interferences and to ensure a useful working range. The samples were filtered at normal pressure, through Whatman quantitative filter paper. The calculation of the mineral elements amount (mg/mL) was performed based on the corresponding calibration curves (for calcium R<sup>2</sup>= 0.9994; for magnesium R<sup>2</sup>= 0.9999; for zinc R<sup>2</sup>= 0.9954) [37, 38, 39].

### 2.4. Determination of total polyphenol content

The Folin – Ciocâlteu (FC) spectrophotometric method was used, according to the European Pharmacopoeia 9<sup>th</sup> Edition, adapted [25]. The standard solution of gallic acid of 50 mg/100 mL is used, and each sample was diluted accordingly. The 25 mL mixture was made consisting of 5 mL diluted sample/standard solution + 1 mL FC reagent + 10 mL distilled water and supplemented with Na<sub>2</sub>CO<sub>3</sub> 290 g/L. The samples are left to rest for 30 min., and the absorbance reading is done at 760 nm wavelength with the UV-Vis 6300 PC double beam spectrophotometer,

Japan. The content of total polyphenols is expressed in % gallic acid, using the formula: % total polyphenol =  $[62.5 \times A_1 \times m_2] / [A_2 \times m_1]$ , where:  $A_1$  = sample absorbance,  $A_2$  = standard absorbance,  $m_1$  = mass of the sample used, in g,  $m_2$  = mass of gallic acid, in g, [36-38, 40, 41].

## **2.5. Determination of antioxidant capacity using the photochemiluminescence method**

Total antioxidant capacity (TEAC) of the samples was determined by the photochemiluminescence method, through Antioxidant Capacity of Lipid Soluble Substances (ACL) procedure, using Photochem apparatus, Analytik Jena AG, Germany, with Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as a standard substance. All measurements were made in triplicate and the values are given as average ones and expressed as Trolox equivalents, based on Trolox calibration curve ( $R^2=0.9998$ ). Before the measurements, each sample was rapidly homogenized using a Vortex mixer, Velp Scientifica, Italy, and 10  $\mu$ L aliquot from the supernatant were withdrawn. Each determination lasted 120 sec. [26, 36, 37, 39].

## **2.6. Determination of antioxidant capacity using the DPPH radical scavenging assay**

The phenolic compounds in the analyzed samples reduce the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (purple compound) to a pale yellow compound, and this free radical scavenging power is measured spectrophotometrically at a wavelength of 517 nm wavelength, using the method used in the studies published by Brand et al. [27] and Sharm et al. [28] adapted to the own analysis conditions. A series of 5 different concentrations (0.1 - 0.02 mg/mL) in 90% methyl alcohol is prepared from each sample. The solutions of different concentrations in a volume of 0.1 mL are mixed with 3.9 mL of the 0.004% DPPH methanolic solution. After 20 minutes of incubation in the dark and at room temperature, the absorbance of the solutions is measured at 517 nm wavelength, with the UV-Vis 6300 PC double beam spectrophotometer, Japan. The decrease in the absorbance of the DPPH solution (control solution) indicates the increase in free radical scavenging activity. The 100  $\mu$ g/mL gallic acid solution was used as a standard solution, which was processed identically to the samples to be analyzed. The absorption value reduction (% Inhibition) for the DPPH solution is calculated according to the equation: % Inhibition =  $[(A_{control} - A_{sample})/A_{control}] \times 100$ , where:  $A_{control}$  = Absorbance of DPPH and  $A_{sample}$  = Absorbance of the reaction mixture (in the presence of sample). For each sample, the antioxidant activity was graphically represented as a function of concentration, and the  $IC_{50}$  value (the concentration of the extract that determines the inhibition of 50% of free radicals) expressed in  $\mu$ g/mL.

## **2.7. Statistical processing**

Results are shown as mean  $\pm$  SD of data (n=3). The data were processed statistically with the software IBM SPSS Statistics 28.0.1.0., and the comparison of means was made with the student T-test.4.

### 3. Results and discussion

#### 3.1. Evaluation of nutritional declaration and quality of additives

From the analysis of the products label, it was found that the nutritional information is presented regarding the energy value and the chemical composition of the nutrients, expressed in appropriate mass units and in percentages of the recommended dietary allowance (RDA) per 100 mL of the product, respectively on the portion recommended by the manufacturer and the ingredients in the product, according to the legislation in force (Table 1) [22, 23]. Thus, functional waters (category A products) have a low energy value and a low sugar content (they contain 3.2 g sugars/100 mL, equivalent to 12.8-19.2 g sugars/portion and have 13-18 kcal/100 mL). The products in category B (fruit juices) have a higher energy value (42-48 kcal/100 mL), respectively a higher sugar content, compared to the previous ones (sugars 10-11 g/100 mL and 25-27.5 g /portion). If we take into account the portion of the product recommended by the manufacturer and the permissible limit of sugars by the WHO (for a requirement of 2000 kcal/day, sugars must cover 10% and be 50 g/day), both contribute with a sufficient intake of big.

Thus, category A products, from one portion, cover an intake between 25% - 38.4%, and category B products, cover an intake of 50-55%, which increases the danger of increasing blood sugar. Nutrивigilance involves a greater awareness of the adverse effects that can affect the nutritional and metabolic balance of the consumer.

The nature of the ingredients is presented, specifying the role of each additive. Natural dyes are used - carotenes, safflower and anthocyanins from added fruit extracts (currants, pomegranate, acai, pineapple, coconut, apple, lemon). The antioxidant potential of these fruits has been studied a lot [16], which explains why manufacturers extend the enrichment of the nutritional mineral and vitamin content of drinks, with the addition of a natural source of antioxidants.

The pasteurization method is noted as the predominant preservation method for most products, with the exception of 3 functional waters, which contain chemical preservatives (benzoate and sorbate), which will reduce their quality, considering that benzoate, by accumulating in the human body, can have toxic potential or in association with some dyes, potentiates ADHD syndrome [13].

Table 1

Energy profile, mineral and total polyphenols content of the evaluated soft drinks					
Sample*	Caloric value**	Mineral elements content		Total polyphenols****	
	kcal/100	(mg/	%	mg GAE/100mL	mg GAE

	<i>mL</i>	<i>portion)*</i>	<i>RDA/portion***</i>		<i>/portion*</i>
<i>Category A</i>					
VW1.Vitamin aqua+Mg	14	Mg 12.70	18	13.90±0.1000	83.53±0.0763
VW2.Vitamin aqua+Zn	18	Zn 0.28	18	11.05±0.0152	66.30±0.0500
VW3.Vitamin aqua+D+Ca	14	Ca 24.60	18	14.63±0.0763	87.70±0.0500
VW4.Vitamin aqua+B	14	-	-	10.55±0.0500	63.08±0.0288
VW5.Vitamin aqua+C	18	-	-	15.94±0.0404	39.88±0.0289
<b>mean</b>				<b>13.21±2.3291</b>	<b>68.18±19.0193</b>
TW6.+Mg	13	Mg 11.00	18	0	0
TW7.+Zn	13	Zn 0.36	22	0	0
TW8.+Vitamine B	13	-	-	0	0
<b>mean</b>					
AW9.+Mg	14	Mg 30.00	32	0	0
AW10.+Zn	14	Zn 0.40	16	0	0
<i>Category B</i>					
SJ1 (cherries 9% +1% grapes)	42	0.67	-	69.10±0.2645	172.46±0.4509
SJ2 (10% peaches)	48	0.47	-	58.93±0.4041	147.61±0.1053
SJ3 (12% orange)	48	0.58	-	62.67±0.0721	156.75±0.2251
SJ4 (8% cherries)	45	0.62	-	65.11±0.2443	162.64±0.2926
SJ5 (8% apple)	45	0.35	-	35.85±0.1101	89.54±0.4413
SJ6 (10% orange)	46	0.42	-	50.85±0.1322	127.11±0.1040
<b>mean</b>				<b>57.86±12.1080</b>	<b>142.68±30.2460</b>
SJ7 (30% aloe vera)	44	0.71	-	136.07±0.1967	340.37±1.6075
SJ8 (30% aloe vera+0.1% pomegranate)	39	0.69	-	129.58±0.5965	322.47±2.4094
SJ 9 (20% aloe vera)	45	0.54	-	91.51±0.2498	228.59±0.4506
SJ10 (20% aloe vera+0.5% vitamin C)	46	0.62	-	89.33±0.1769	223.27±1.6100
<b>mean</b>				<b>111.62±24.7432</b>	<b>278.67±61.3817</b>

\* the portions are: 600 mL for category A 1-7 I; 400 mL for category A8-10; 250 mL for category B 1-10; Samples A1-5 also contain 1-3% fruit extract

\*\*low energy value = category A 1-10; in the case of liquids with less than 20 kcal/100 mL [23]

\*\*\* source of minerals = category A 1-10; in the case of products covering at least 15% of the mineral element RDA; category B 1-10 negligible [23]

\*\*\*\*Results are indicated as mean ± SD of data (n=3)

### 3.2. Minerals content

For the analyzed products—category A, the added minerals content/product portion (Table 1), determined on the basis of the standard curves recorded at the specific

wavelengths of the primary line for the elements, Ca (422.6728 nm), Mg (285.2125 nm) and Zn (213,857 nm), covers the daily requirement in a percentage of 18-30%, which falls within the minimum limits allowed by EU Regulation 1169/2011 (15% RDA/portion). The obtained values, compared to the admitted ones, according to the legal requirements [22, 23], place the products in the "Food sources for minerals" category. However, there is a disadvantage for the consumer, because inorganic combinations of these minerals are also used (carbonate, chloride, sulfate), which implies low bioavailability, compared to organic ones (gluconate, lactate, citrate). Compared to their content in drinking water, the values are much higher, which supports their previous classification as "Food sources for minerals", considering that in drinking water, according to the legislation of the country, the values of mineral elements are: calcium  $\leq$ 100 mg/L, magnesium  $\leq$ 50mg/L, zinc  $\leq$ 5mg/L [29].

For the products in category B, the total of the three mineral elements (Ca, Mg and Zn) was determined, obtaining cumulative values, between 0.32-0.71 mg/100 mL, subunit values, which does not allow the inclusion in "Food sources for minerals". Although they have a lower content of mineral elements, these products stand out for their polyphenols content and antioxidant capacity.

### 3.3. Total polyphenols content

Polyphenols and other natural antioxidant compounds from fresh fruit juices have been proposed to replace synthetic antioxidants that can cause genotoxicity and carcinogenicity [17], so that their presence in products provides a benefit to the body.

In most of samples, the total phenolic (TP) content (Table 1), was considered low (10.55-69.10 mg/100 mL), with the exception of products B 7-10, in which the polyphenolic content is between 89.33-136.07 mg/100 mL. The higher values of polyphenols are explained on the basis of the percentage of plant extract (20-30%) of aloe vera, one of the natural sources with a composition of active chemical compounds with beneficial effects for the body [30]. For samples B 1-7, a content of 35.85-69.10 mg/100 mL was obtained, and for all samples A, the content is negligible ( $\leq$ 15.94 mg/100 mL).

There are some authors who estimate that the daily nutritional intake of polyphenols is between 150 mg - 1 g [15], and by consuming 100 mL of commercial fruits juice with 5% fruit concentrate, only a maximum of 65 mg of phenols is provided [16]. Thus, among all the analyzed B category products, the B7-10 having more than 65 mg of phenols/100 mL, ensure the minimum polyphenolic nutritional intake, which strengthens the quality of functional products.

To highlight the analyzed products which contain a valuable polyphenols level, but also with a healthy nutritional profile, the polyphenol content can be expressed per portion, as shown by recent studies [31]. Thus, Table 1 also, shows the content of total polyphenols/portion. Among all the samples, the majority of

category B, even if they have a higher sugar content, compared to the samples of category A (with a negligible content of polyphenols), were found to have a high content of polyphenols (58.93-136.07 mg/100 mL), which meet the daily requirement.

The results obtained emphasize a low nutritional profile for functional waters, despite the vitamins and minerals content. Recent studies confirm the phenomenon of commercial promotion of some nutritionally unbalanced food products, from all categories, on a national and international scale, including for antioxidant functional drinks [9, 32, 33]. It was found that some of functional antioxidant drinks, the more they are processed, the more the nutrients are degraded, thus, the antioxidant activity can be diminished as a result of the degradation of present polyphenols.

### 3.4. Antioxidant capacity by photochemiluminescence method

The highest values of the total antioxidant capacity (TEAC) were obtained in the case of samples containing 30% fruit (B7-10), followed by those containing 10-12% (B1-6), and in the case of samples with 1-3% added fruit (A1-5), the values obtained have been the lowest (Table 2). Antioxidant activities are higher in samples with aloe vera content (5.256-7.600 µg Trolox/mL), compared to those based on fruits (4.189-4.902 µg Trolox/mL), and within them, cherries show a higher activity (4.902 µg Trolox/mL). The antioxidant activity is significantly higher ( $p<0.05$ ) in samples from category B7-10 (6.457 µg Trolox/mL) compared to those from category B1-6 (4.598 µg Trolox/mL).

For the functional water samples analyzed, a higher antioxidant capacity was found in those containing vitamin C and magnesium (4.323-5.692 µg Trolox/mL) compared to those containing zinc (2.483-3.514 µg Trolox/mL).

Table 2  
Antioxidant capacity results determined through TEAC and DPPH methods

Sample*	Total antioxidant capacity (TEAC) ** (µg Trolox/mL)	% Inhibition DPPH***	IC <sub>50</sub> (µg/mL)
<i>Category A</i>			
VW1.Vitamin aqua+Mg	5.267±0.0096	21.547±0.0015	158
VW2.Vitamin aqua+Zn	2.483±0.0017	12.862±0.0052	310
VW3.Vitamin aqua+D+Ca	3.975±0.0045	7.015±0.0015	532
VW4.Vitamin aqua+B	3.331±0.0015	6.327±0.0015	640
VW5.Vitamin aqua+C	5.692±0.0046	28.364±0.0011	122
<b>mean</b>	<b>4.15<sup>a</sup>±1.3329</b>	<b>15.223<sup>ab</sup>±8.8411</b>	
TW6.+Mg	4.323±0.0011	15.401±0.0031	427
TW7.+Zn	2.959±0.0040	7.825±0.0040	505
TW8.+Vitamine B	1.875±0.0030	3.583±0.0035	893
<b>mean</b>	<b>3.052<sup>b</sup>±1.2266</b>	<b>8.937<sup>a</sup>±5.1841</b>	
AW9.+Mg	4.405±0.0040	14.184±0.0031	496
AW10.+Zn	3.514±0.0040	7.762±0.0015	504

<b>mean</b>	<b>3.959±0.6300</b>	<b>10.973<sup>bc</sup>±3.5172</b>	
<i>Category B</i>			
SJ1 (cherries 9% +1% grapes)	4.902±0.0056	23.908±0.0026	108
SJ2 (10% pears)	4.586±0.0052	20.392±0.0025	178
SJ3 (12% orange)	4.731±0.0047	18.403±0.0015	239
SJ4 (8% cherries)	4.872±0.0073	19.035±0.0025	302
SJ5 (8% apples)	4.189±0.0025	18.740±0.0015	242
SJ6 (10% orange)	4.310±0.0045	17.289±0.0045	226
<b>mean</b>	<b>4.598±0.2951</b>	<b>19.628±2.1832</b>	
SJ7 (30% aloe vera+0.1% pomegranate)	7.600±0.0200	31.467±0.0015	93
SJ8 (30% aloe vera)	7.553±0.0603	30.290±0.0025	125
SJ9 (20% aloe vera+0.5% vitamin C)	5.420±0.0436	29.303±0.0026	123
SJ10 20% aloe vera	5.256±0.0416	27.606±0.0251	205
<b>mean</b>	<b>6.457<sup>ab</sup>±1.2942</b>	<b>29.666<sup>c</sup>±1.4779</b>	
Acid galic	-	94.09±0.1000	IC 50 = 5.290

\*the portions are: 600 mL for category A1-8; 400 mL for category A9-10; 250 mL for category B 1-10

\*\*Results are indicated as mean ± SD of data (n=3)

The various superscript letters (a, b) in the columns show significant differences (p<0.05).

\*\*\*Results are indicated as mean ± SD of data (n=3). The various superscript letters (a, b, c) in the columns show significant differences (p<0.001)

### 3.5. Antioxidant capacity by DPPH method

Through the DPPH free radical method it is proven that waters containing vitamin B have the lowest percentage of inhibition (3,582-6,327%), respectively an IC<sub>50</sub> between 640 -893 µg/mL sample. Conversely, waters containing magnesium have the highest antioxidant potential (15,401-21,548%), respectively an IC<sub>50</sub> between 158-496 µg/mL sample. Vitamin C from vitaminizing waters and aloe juices considerably increases the antioxidant capacity, the water sample corresponds to IC<sub>50</sub> = 122 µg/ mL, and the aloe juice sample (IC<sub>50</sub> = 123 µg/ mL). Compared to gallic acid, the analyzed samples emphasized a lower antioxidant capacity, the standard having a much lower IC<sub>50</sub> (IC<sub>50</sub> = 5.290 µg/mL; R<sup>2</sup>=0.9997).

The DPPH method confirms the same dynamics of the antioxidant activity as the photochemiluminescence method. Thus, all juice samples (B1-10) have a higher DPPH inhibition compared to that of the functional waters (A1-10). There are significant differences (p<0.001) between the average values for category A1-5 and A6-8, as well as between category A1-5 and A9-10. Samples from category B7-10 (samples with the highest content of fruit extract) have the highest mean values, insignificant compared to samples B1-6 (which have a lower percentage of fruits than samples B7-10), but significant compared to samples from category A9-10 (p<0.001).

The results obtained for  $IC_{50}$  are corroborated with the increase in the DPPH radical capture percentage by the analyzed samples. The lower the  $IC_{50}$ , the more the sample has a higher antioxidant capacity (Table 2). The highest antioxidant capacity is represented by the sample containing aloe and pomegranate (% I =  $31.467 \pm 0.0015$  and  $IC_{50} = 93 \mu\text{g}/\text{mL}$ ), while the reference antioxidant is stronger (% I =  $86.50 \pm 0.1$  and  $IC_{50} = 5,290 \mu\text{g}/\text{mL}$ ).

Regarding the correlation of the antioxidant capacity determined by the two comparative methods, with the content of polyphenols, a proportionality between the values is found, valid for samples B7-10 (where the values are the highest), respectively B1-6 (average values). The lowest values, however, correlated between antioxidant capacity and polyphenols content, are found in functional water samples A1-10, some of them even 0 polyphenols content (A6-10), Fig. 1 and Fig. 2.

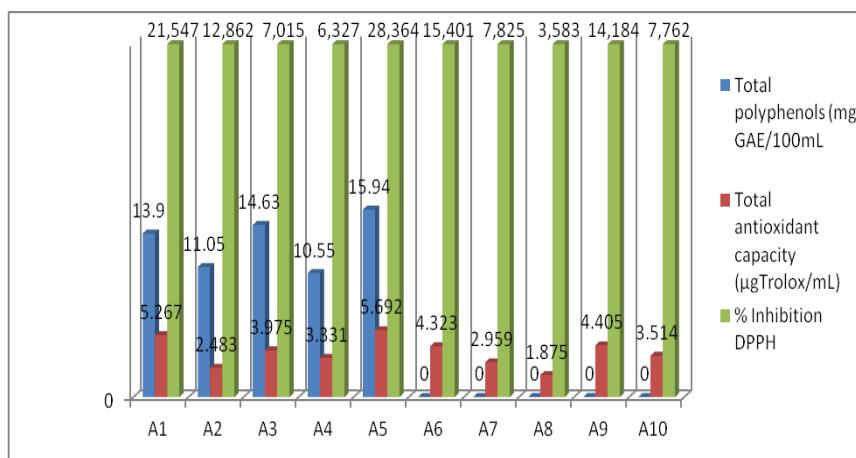


Fig. 1. The values of antioxidant capacity and total polyphenols (TP) content in functional water samples (A1-10)

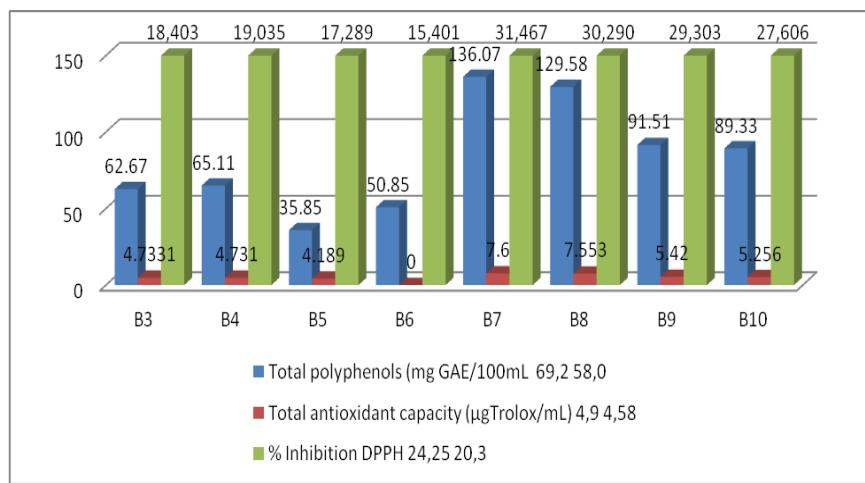


Fig. 2. The values of antioxidant capacity and total polyphenols (TP) content in juice samples (B 1-10)

The Pearson correlation coefficient applied to all the data representing the antioxidant activity measured by the two different methods, shows a high positive correlation ( $R^2=0.8860$ ) between the measurement of TEAC and DPPH methods, Fig. 3 and Table 3. Regarding the content of total polyphenols, according to the correlation coefficient, the results show a sufficiently high positive correlation ( $R^2=0.7990$ ) between TPC and DPPH measurement of antioxidant activity, respectively between TPC and TEAC of the total antioxidant capacity ( $R^2=0.8050$ ), Table 4.

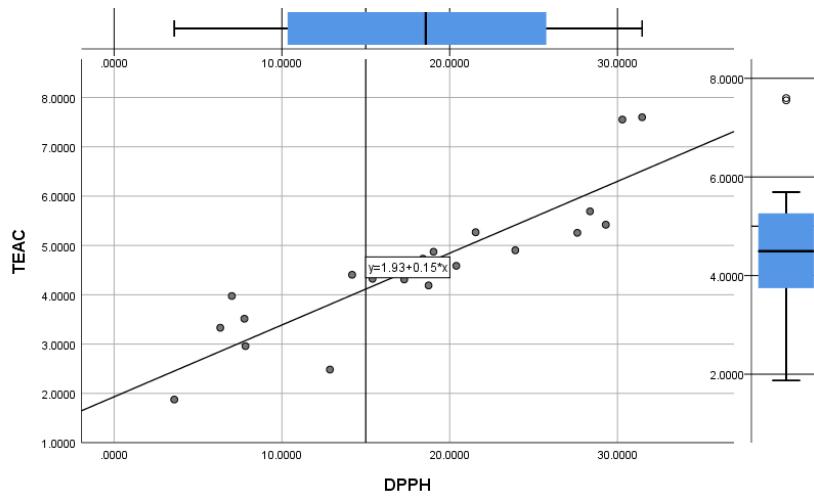


Fig. 3. Values correlation of the antioxidant activity by TEAC and DPPH methods for the analyzed samples

Tabel 3

**The Pearson correlation coefficient for the antioxidant activity of the samples measured by the TEAC and DPPH methods**

	TEAC	DPPH
Pearson Correlation	1	0.8860
Bayes factor		0.000
N	20	20
Pearson Correlation	0.8860	1
Bayes factor	0.000	
N	20	20

a. Bayes factor: Null versus alternative hypothesis

Tabel 4

**The Pearson correlation coefficient for the TP content and the antioxidant activity of the samples measured by the TEAC and DPPH methods**

	TP	DPPH	TP	TEAC
Pearson Correlation	1	0.7990	1	0.8050
Bayes factor		0.001		0.001
N	20	20	20	20
Pearson Correlation	0.7990	1	0.8050	1
Bayes factor	0.001		0.001	
N	20	20	20	20

a. Bayes factor: Null versus alternative hypothesis

The variation in polyphenols content and antioxidant activity in the analyzed products is supported and confirmed by the results obtained by other authors who studied various fruits extracts, compared to commercial or fresh juices. Thus, the higher the percentage of the fruits extract, the higher the values, found in the extracts of mango, guava, pomegranate, orange, aloe, lime [17, 18, 34], red grapes, haskap or blue honeysuckle berries, bitter cherry, cornelian cherry, or red grape pomace [35 - 41].

#### 4. Conclusions

The samples of functional waters with the addition of minerals and vitamins (A1-10) belong to "products with low energy value and "significant sources of minerals", but with an insignificant content of polyphenols (<87.70 mg/portion) or even zero to some of them, with an impact on the antioxidant activity.

Antioxidant fruit juices with 8-30% fruit extract (B1-10), have a content in polyphenols, correlated with antioxidant activity, which satisfies the daily requirement (contained between 142.68- 278.67 mg/portion), but present a lower content of mineral elements. The antioxidant activity is significantly higher ( $p<0.001$ ) in samples from category B 7-10 (with 30% aloe vera concentrate) compared to those from category B 1-6 (with 8-12% fruits concentrate).

Compared to fruits, in which nutrients content, including polyphenols correlated with antioxidant activity, is much higher, commercial fruit juice could be the secondary choice, if the fresh fruits are not available, and the consumption of water with addition of minerals and vitamins does not contribute significantly to the daily intake of phenolic compounds.

The globalization of the modern food industry has as a consequence the obtaining of products, sometimes with diminishing quality in favor of quantity, therefore we recommend to consumers to a careful evaluation of the labels and the consumption of these new products according to the specific body's needs. The work contributes to estimation of the nutrients' intake from vitaminizing and mineralizing waters, compared to fruit juices, as well as informing consumers about paying more attention to the labeling of these products, to increase their safety and health.

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