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# COMPARATIVE STUDY BETWEEN SINGLE STRENGTH JUICES AND COMMERCIAL NATURAL JUICES BY IRMS AND EPR

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**ABSTRACT.** Authenticity has probably always been a major concern of many consumers and it is still gaining more and more importance. In our study we used two analytical methods to characterize antioxidant activity and to evaluate the authenticity of juices; isotope ratio mass spectrometer (IRMS) and Electron Paramagnetic resonance (EPR). Isotope ratio mass spectrometry is a promising tool for origin assignation of food, thus $^{13}C$, $^{18}O$ and $^{2}H$ measurements are intensively used in forensic study to prove product authenticity. This application has been particularly useful in food quality control, because it allows the detection of added sugar and water in fruit juices and in tracing the geographical origin of food. The obtained results have shown that the both commercial juices are authentic fruit juices with no exogenous water and no addition of $C_4$ sugar, according to those labels. Antioxidant properties of commercially-available ground and instant coffees were investigated by means of electron paramagnetic resonance (EPR) using as oxidants, 4-hydroxy-2,2,6,6-tetramethylpiperidine N-oxyl (TEMPOL)

***Keywords:*** *Fruit juice, antioxidants, Stable isotopes, EPR spectroscopy, Tempol*

## INTRODUCTION

The study of antioxidant capacity of different fruits, juice and vegetables has prompted research in the fields of food science and human health, paying an important role in reducing the risk of degenerative diseases such as cardiovascular disease, various cancers and neurological diseases. In this context, the spectroscopic and spectrometric methods for the determination of the antioxidants activity of natural sources for different types of foods and extracts are important tools in development of efficient extraction procedures.

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At the same time, the antioxidant activity of natural source has a great importance in selecting effective antioxidant extracts to be used in foods and nutritional supplements.

On the other hand, fruit juice quality and authenticity determination is an important research subject with significant relevance for industry, food safety and consumer protection. Unscrupulous companies and traders seek substantial benefits using adulterated juices to gain market advantage by using sugars, syrups, raw materials or false label indications for consumers. Adulteration of a product consists in making it impure by fraudulent addition of a foreign or inferior substance. The result is either an alteration of the product and of its quality or a falsification. The falsification is a voluntary act with the intention of abuse. The falsification may be more or less sophisticated and its sophistication as well as its costs increases with the improvement of analytical methods [1].

In our study we used two analytical methods to characterize antioxidant activity and to evaluate the authenticity of juices; isotope ratio mass spectrometer (IRMS) and Electron Paramagnetic resonance (EPR).

The isotope ratio mass spectrometer (IRMS) allows the precise measurement of mixtures of stable isotopes, measuring isotopic variations arising from mass-dependent isotopic fractionation in natural systems

Each plant has its own unique pattern of naturally occurring stable isotopes of carbon ($^{12}C$, $^{13}C$), hydrogen ($^{1}H$, $^{2}H$) and oxygen ($^{16}O$, $^{18}O$), whose distribution has been influenced by a number of physical and/or biochemical properties and geoclimatic conditions.

Determination of $\delta^{18}O$ and $\delta^{2}H$ values of water from fruit juices is today applied in routine analysis as an automated and acknowledged method in order to differentiate between directly pressed and rediluted single strength juices. The principle consists in the fact that authentic juices have elevated $\delta^{18}O$ and $\delta^{2}H$ content of water as compared to water from rediluted products made using tap water which is relatively depleted in heavy oxygen and hydrogen isotopes [2].

Photosynthetic $CO_2$ assimilation via the $C_3$, $C_4$ and CAM pathways is of major importance in the use of carbon stable isotope ratio analysis in food authenticity control. The detection of commercial $C_4$ cane or corn derived sugar syrups in $C_3$ agricultural products is thus facilitated by characteristic differences in $\delta^{13}C‰$ values. The technique is necessarily comparative, as it must take into account the natural variation of $\delta^{13}C$ values in authentic products due to environmental factors such as water availability and light intensity [3].

The antioxidant activities of studied juice samples were followed by means of EPR spectrometer EMX monitoring of the radical concentrations of the oxidant 4-hydroxy-2,2,6,6-tetramethylpiperidine N-oxyl (TEMPOL) after the addition of the juice drinks.

In this study two single strength juices (one apple juice and one grape juice) were compared with other two commercial fruit juices (one apple juice and one grape juice), in order to check the correct labeling of present on Romanian market, using stable isotope measurements (oxygen, hydrogen and carbon) and EPR spectroscopy.

## RESULTS AND DISCUSSION

*Isotope ratio mass spectrometer (IRMS)*

Vacuum concentration with aroma does not affect the chemical composition of fruit juices and therefore the determination of deuterium (D) and oxygen-18 ($^{18}O$) content in waters is the most reliable procedure for differentiating between a natural single strength juice and a juice rediluted from concentrate. The (IRMS) technique is based on the fact that when absorbed by a plant, the rainwater or the irrigation water is fractionated by evapotranspiration [4,5], and enriched in the heavy isotopes (deuterium and oxygen-18) with respect to the light isotope (hydrogen and oxygen-16). It is known that climatic conditions affect the isotope content of rainwaters and therefore that of fruit juices waters: the warmer climate, the higher the deuterium and oxygen-18 contents of water [2]. Rainwater and tap water have nearly the same isotopic content and the water of fruit juices derived from concentrate by dilution with tap water has an isotopic content close to that of tap water. This makes it easy to distinguish diluted concentrates from the isotopically more enriched water of authentic single strength juice [2].

The variation of $\delta^{18}O$ values in water of authentic apple single strength juice from central Europe (Germany, Italy, Belgium, Poland, Austria, Hungary and the Czech Republic) for the seasons 1991-1994 obtained by Rossmann [6], have featured a relatively large range of variation, with a mean value of about -4‰. In our study we obtained for the single strength apple juice, made from apple from Transylvania, values of $\delta^{18}O$ =-5.52‰ and δD=-51.7 ‰, very close to those obtained for commercial apple juice ($\delta^{18}O$ =-5.24‰ and δD=-49.3‰), meanwhile the isotopic values obtained for lab water (tap water) were $\delta^{18}O$ = -10.78‰ and δD = -76.5‰, respectively (Table 1). From these results we can conclude that the commercial apple juice is authentic apple juice with no exogenous water, according to its label. Also, for the grape commercial juice the isotopic analysis we obtained $\delta^{18}O$ = 0.95‰ and δD = -16.5‰, have shown that this juice does not contain exogenous water. Its isotopic values are even more positive than the isotopic values that we obtained for grape single strength juice. The explanation consists in the fact that the grapes that were used in preparation of investigated juices come from different geographic regions.

Depending on their origin, sugars added are divided into two types: $C_3$ and $C_4$. Sugars naturally occurring in apples and grapes juices belong to the $C_3$ type, while cane sugar and sugars produced from the hydrolysis of maize starch are of the $C_4$ type.

**Table 1.** Oxygen, hydrogen and carbon stable isotope data for investigated samples

| Sample number | Type of sample | $\delta^{18}O_{vs\ SMOW}$ (‰) | $\delta D_{vs\ SMOW}$ (‰) | $\delta^{13}C_{vs\ PDB}$ (‰) |
|---|---|---|---|---|
| 1. | Apple single strength juice | -5.52 | -51.7 | -30.2 |
| 2. | Grape single strength juice | -0.99 | -35.4 | -28.8 |
| 3. | Commercial apple juice | -5.24 | -49.3 | -27.4 |
| 4. | Commercial grape juice | 0.95 | -16.5 | -25.9 |
| 5. | Tap water | -10.78 | -76.5 | - |

Generally, $C_4$ plants, have $^{13}C/^{12}C$ isotopic ratios, referred to as $\delta^{13}C$ values, ranging between -8 and -13‰, whereas $C_3$ plants, have values between -22 and -30‰. An addition of $C_4$ sugars in apple and grape juices would result in a higher value of $\delta^{13}C$. The results obtained by Rumel et al. [7] for approximately 150 authentic orange juices shown that the mean value $\delta^{13}C$ of investigated samples are varying between (-29‰ to -23‰). Such differences in $\delta^{13}C$ values due to environmental factors were reported by different authors for orange juices. For apple juices Doner et. al. [8] observed that the mean $\delta^{13}C$ value of juices from different varieties of apples and cultivated in different geographical locations was around -25.4‰, with no significant correlation between the variety of apple or geographical origin.

For our investigated samples, the obtained $\delta^{13}C$ value is typical for $C_3$ plants, with no evidence about a possible adulteration with $C_4$ sugars. The differences in $\delta^{13}C$ values that appear here are more probably due to environmental factors than to the apple or grape variety.

*EPR spectroscopy*

The nitoxide radicals are relatively stable towards oxidation but they can be easily reduced to the corresponding hydroxylamines. The standard redox-potential of piperidine nitroxide derivatives ($E_0$=0.2eV is high enough to oxidise biological compounds such as polyphenolics, ascorbic acid, semiquinones and superoxide radicals [9-12]. Thus, when mixing a nitroxide radical with an antioxidant-containing juice, the number of paramagnetic species decreases in time with different rates, depending on the sort of juice. Recording the EPR signal decay caused by the reaction with natural or artificial reductants

or with products of metabolic reactions, it is possible to draw conclusions about antioxidant capability. An important constituent of the antioxidant complex of apples and grapes is the ascorbic acid. This vitamin is a versatile, water-soluble, donor, antioxidant. The chemical instability of vitamin C is due to the fact that it is a strong reducing agent and can be deactivated by a wide range of oxidizing agents [13].

The intensity of the EPR signal in experimental samples was found to decrease in time and was correlated to the total content of polyphenols and vitamins (Figures 1 and 2)

The rate of reaction between antioxidant compounds and Tempol was monitored based on the normalized double integrated residual EPR signal, which is in turn correlated with number of paramagnetic species in time (Figures 3 and 4). The decrease of relative concentration of the paramagnetic species in time was fitted to an exponential function [10]: $I(t) = I_0 + a e^{(-kt)}$ where $I_0$ and a are parameters depending on the experimental conditions [14,15]. The antioxidant nature, expressed by kinetic reduction constant (k) is a fingerprint of authenticity of juices or antioxidant compounds.

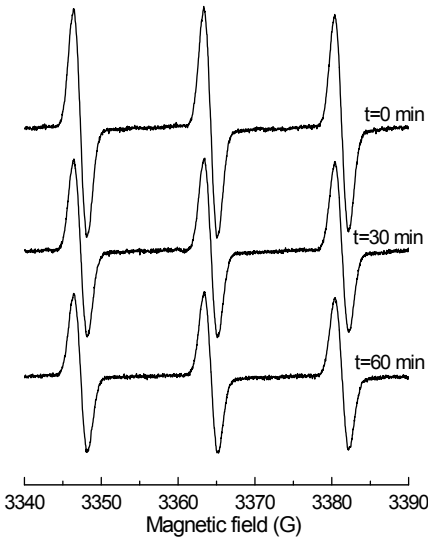



**Figure 1.** EPR spectra of Tempol at different time of incubation in fresh apple juice

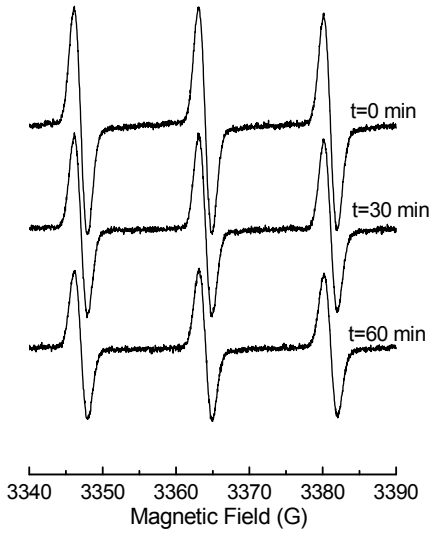
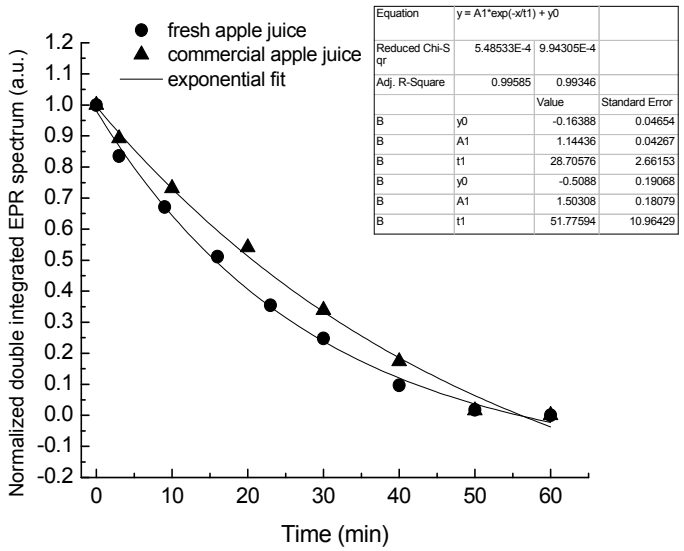
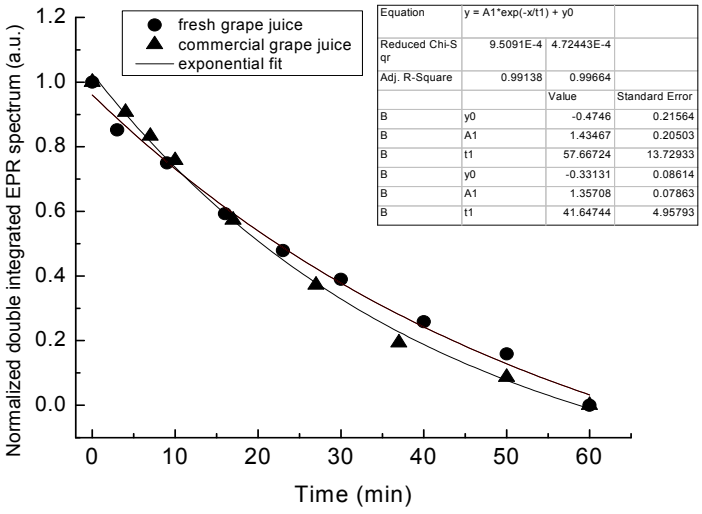



**Figure 2.** EPR spectra of Tempol at different time of incubation in fresh grape juice




| Equation | y = A1*exp(-x/t1) + y0 | | |
|---|---|---|---|
| Reduced Chi-Sqr | 5.48533E-4 | 9.94305E-4 | |
| Adj. R-Square | 0.99585 | 0.99346 | |
| | | Value | Standard Error |
| B | y0 | -0.16388 | 0.04654 |
| B | A1 | 1.14436 | 0.04267 |
| B | t1 | 28.70576 | 2.66153 |
| B | y0 | -0.5088 | 0.19068 |
| B | A1 | 1.50308 | 0.18079 |
| B | t1 | 51.77594 | 10.96429 |

**Figure 3.** Normalized double integrated EPR spectra for fresh and commercial apple juice in function of time




| Equation | y = A1*exp(-x/t1) + y0 | | |
|---|---|---|---|
| Reduced Chi-Sqr | 9.5091E-4 | 4.72443E-4 | |
| Adj. R-Square | 0.99138 | 0.99664 | |
| | | Value | Standard Error |
| B | y0 | -0.4746 | 0.21564 |
| B | A1 | 1.43467 | 0.20503 |
| B | t1 | 57.66724 | 13.72933 |
| B | y0 | -0.33131 | 0.08614 |
| B | A1 | 1.35708 | 0.07863 |
| B | t1 | 41.64744 | 4.95793 |

**Figure 4.** Normalized double integrated EPR spectra for fresh and commercial apple juice in function of time

Comparing the antioxidant characteristics of fresh apple juice with the commercial apple juice we may say that fresh apple juice has the most significant antioxidant character ($k_{fresh}$=0.02, $k_{commercial}$=0.035). A similar situation was found in the case of grape juice;($k_{fresh}$=0.017, $k_{commercial}$=0.024). In terms of antioxidant activity, it can be concluded that the studied juices have similar quality fresh juices.

Although there is a small difference in the antioxidant activity of fresh apple juice and commercial apple juice and between fresh grape juice and fresh grape juice, respectively, it can be said that EPR method together with MS suggests that the two commercial juices are authentic fruit juices.

## CONCLUSIONS

In this study two single strength juices (one apple juice and one grape juice) were compared with other two juices commercial fruit juices (one apple juice and one grape juice), in order to check the correct labeling of present on Romanian market were investigated by the mean of stable isotope measurements (oxygen, hydrogen and carbon).

The obtained results have shown that the both commercial juices are authentic fruit juices with no exogenous water and no addition of C4 sugar, according to those labels.

Under antioxidant activity studied by EPR spectroscopy, it can be concluded that the studied juices have similar quality fresh juices.

## EXPERIMENTAL SECTION

### *1. Sample preparation*

For oxygen-18 determination 5 ml of raw juice (neither centrifuged nor filtered) was equilibrated with $CO_2$ for 15 hours according to the CEN:ENV 13141:1997 method at 25±0.1$^0$C [2]. The carbon dioxide was then extracted and purified. For the hidrogen analysis a distillation under static vacum was performed by using Rittenberg „trausers” on 2-3 ml of fruit juice, always with the quantitative recovery of the water [16].

For $\delta^{13}C$ analysis, the separation and purification of the pulp was made according to [17-18] by the separation of a sample of about 50ml of fruit juice by centrifugation (10 min at 1400 times g) from the pulp. The pulp was then re-suspended in water (50 ml), mixed thoroughly, centrifuged (10 min at 1400 times g) and the supernatant was discarded. Then, the washing process was repeated once with water and twice more with acetone and the resulted precipitate was dried under vacuum. The obtained dried solid was homogenized by mixing it with a spatula.

*2. Isotope measurements*

The procedure of IRMS consists in measuring the isotope ratio of an analyte converted into a simple gas, isotopically representative of the original sample, before entering the ion source of an IRMS [4]. The $^{18}O$ isotopic contents of the water samples were then analyzed using a stable isotope ratio mass spectrometer IRMS (Delta V Advantage, Thermo Scientific). For $\delta^2H$ the equipment used was a Liquid-Water Isotope Analyzer (DLT-100, Los Gatos Research).The results of our $^{18}O$ and $^{2}H$ analyses of the fruit juices are reported using conventional δ notation relative to the Vienna-Standard mean Ocean Water (V-SMOW) standard (i.e. δ (‰)= $[(R_x/R_S)-1]x1000$, where $R_x$ is the $^{18}O/^{16}O$ or $^{2}H/^{1}H$ isotopic ratio of the water sample and $R_S$ is the $^{18}O/^{16}O$ or $^{2}H/^{1}H$ isotopic ratio of the V-SMOW standard.

The measurements of $\delta^{13}C$ from pulp fruit were carried out on an Elemental Analyser (Flash EA1112 HT, Thermo Scientific), coupled with an isotope ratio mass-spectrometer IRMS (Delta V Advantage, Thermo Scientific). NBS-22 oil with a certified value of -30.03‰ vs. PDB (Pee Dee Belemnite) was used as standard. The analytical reproducibility was ±0.1‰ for $\delta^{13}C$, ±0.1‰ for $\delta^{18}O$ values and ±0.6‰ for $\delta^{2}H$ values.

*3. EPR measurement*

1mM water TEMPOL solution was added to 2 ml of juices. The homogenized solution was injected with a Hamilton microsyringe into a quartz capillary of about 10 cm length and an interior diameter about 1 mm. EPR measurements were performed on a Bruker EMX spectrometer operating at 9.4548 GHz with 100 kHz modulation frequency, at room temperature. The reaction mixture-containing 20 µl capillary was then positioned in the standard cavity TE102 (Bruker Instruments, ER 4102ST). The sample was scanned using the following parameters: center field, 3510 G; sweep width, 60 G; power, 2 mW; receiver gain, $1\times10^3$; modulation amplitude, 2 G; time of conversion, 30 ms; time constant, 61.4 ms; number of scans, 1. The spectra were recorded at different time intervals. The integral intensities of EPR spectra were obtained by evaluating their double integrals (DIEPR) using the WIN EPR program (Bruker).

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