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Antioxidant activity of certain vegetal active principles compared to that of citrus juices and wines

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Abstract

Extensive research has demonstrated the protective properties of antioxidants which scavenge reactive oxygen species. The aim is to provide a comparative study regarding antioxidant activity of two vegetal active principles in relation to that of certain citrus juices and two types of red wine. The total two vegetal active principles of polyuronide and flavonoid type were obtained from dried leaves of plants from the Crassulaceae family. Active principles were selected for their pharmacological action: immunomodulatory and anti-inflammatory; this action may result from their antioxidant potential. Lemons, oranges, and grapefruits were used from among citrus fruits. Cabernet Sauvignon and Pinot Noir were the two types of red wines. Commercially available citrus fruits were imported from Greece. Commercially available Cabernet Sauvignon (2007) and Pinot Noir (2007) wine samples were from the Murfatlar, Romania. Antioxidant action of samples was evaluated by means of Total Antioxidant Status (TAS) determination. As anticipated, the total flavonoids had the highest antioxidant action. Among the citrus juices, the lemon juice had a greater antioxidant action. The two red wines had a comparable antioxidant action. The polyuronides had a greater antioxidant action than the citrus fruit juices and the two red wines. After 48 hours, the antioxidant action of the polyuronides and total flavonoids was less diminished than the citrus fruit juices and two red wines. Considering the preliminary results obtained, the two vegetal active principles could be introduced as food supplements.

Keywords: antioxidant action, polyuronides, flavonoids, *Crassulaceae* family

Introduction

Many human diseases have been recognized as being a consequence of free radicals damage. For this reason, the role of nutrition in health has captured the interest of researchers in antioxidants and their capacity to protect the body from damage induced by oxidative stress [1]. Extensive research has demonstrated the protective properties of antioxidants, which scavenge reactive oxygen species and their precursors [2]. The consumption of fruits and vegetable has been associated with lower incidence and lower mortality rates of cancer in several human cohort studies [3]. Due to the chemical diversity of antioxidant compounds present in foods, complete databases on food antioxidant content are not yet available [4]. The levels of single antioxidant in food do not necessarily reflect their total antioxidant action because this also depends on the synergic and redox interactions among the different molecules present in food. For this reason, in the last few years, scientific interest has increasingly focused on dietary phytochemicals. Vegetable flavonoid substances are the most common phytochemicals in fruits and vegetables. The antioxidant activity of many flavonoids is higher than that of the antioxidant vitamins E and C [5]. Part of the antioxidant action of

wines and citrus fruits may derive from flavonoids with protective redox potential. Therefore, the aim for this paper is to provide a comparative study regarding the antioxidant activity of two vegetal active principles in relation to that of certain citrus juices and two types of red wines, which was determined in the following time intervals: zero hours, 24 and 48 hours. The two vegetal active principles, polyuronide type and total flavonoids, were selected for their pharmacological action: immunomodulatory and anti-inflammatory [6, 7]. The citrus fruit and red wines were chosen for their polyphenols content [8].

Materials and Methods

The two vegetal active principles were separated from a plant that belongs to genus Sedum of the *Crassulaceae* family.

Materials

Phosphate buffered saline 80 mM and pH 7.4, metmyoglobin 6.1 μM, ABTS^R (2.2$^'$ – Azino-di-3ethylbenzthiazoline sulphonate) 610 μM, hydrogen peroxide (in stabilized form) 250 μM, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid 2 Mm (Trolox as standard), MeOH 50%, deionized water. Vegetal active principles were in powder form. Commercially available citrus fruits were imported from Greece. Commercially available *Cabernet Sauvignon* (2007) and *Pinot Noir* (2007) wine samples were from the Murfatlar Brand, Romania.

Methods

Extraction The polyuronides were obtained according to a patent [9]. The total flavonoids (TF) was obtained as follows: about 20g of powdered leaves of the plant was extracted with 100mL methanol 50% [10] using Soxhlet apparatus for 48 h. The extract was concentrated under reduced pressure using Turbovap 500. Citrus juices were obtained by squeezing and filtering.

Evaluation of antioxidant action

The antioxidant action of vegetal active principles, in citrus fruit juices and red wines was determined using the Total Antioxidant Status (TAS) assay. TAS was evaluated with a kit supplied by Randox Laboratories Ltd. using a colorimetric method based on the reduction of ABTS radical cation generation by antioxidants in the samples. The antioxidative potential was examined by spectrophotometric evaluation on a Beckman Synchron CX7 coulter of the intensity of staining after the addition of ABTS cation radical. TAS in the samples of the vegetal active principles, citrus juices and two wines was presented in mM as an equivalent of Trolox(standard). The calculation was performed relative to the factor F, which was determined earlier: F= concentration of standard (Trolox)/ΔAblank – ΔAstandard(Trolox), where A_2-A_1 = ΔA of sample/standard/blank, A_1 being the initial absorbance and A_2 the absorbance after exactly 3 min of reaction. F was used to calculate TAS: mM = F x (ΔA blank – ΔA sample). TAS determination producing company indicates use of kit for serum, plasma, juice, and wine. TAS determination of vegetal active principles was made using 0.2 mg mL^{-1} water solutions. Flavonoid solutions were dilluted with 1:10 distilled water and wine solutions with 1:3 distilled water. Water solutions of vegetal active principles, citrus juices, and wines were kept in brown bottle containers. TAS was measured at the following time intervals: 0, 24, and 48 hours. These time intervals were considered sufficient for comparative TAS evaluation over time.

Statistical Analysis

All data were expressed as mean ± standard deviation (SD) (n=10). Analysis of variance was performed by means of ANOVA (One–Way) procedures and $p < 0.001$ was considered to be statistically significant. F-statistic is a ratio of the between group variation divided by the within group variation.

Results and Discussions

Total Antioxidant Status of two vegetal active principles, orange juice, lemon juice, grapefruit juice and red wines was evaluated. The results were presented in Table 1.

Table 1. TAS of two active principles, citrus fruits juices, red wines in time intervals

	Sample (n=10)	**TAS (mM) zero hours**	**TAS (mM) 24 hours**	**TAS (mM) 48 hours**
1	Polyuronides	2.7350±0.02321	2.6890±0.02079	2.6440±0.01265
2	Total flavonoids	19.1090±0.07385	18.3850±0.04696	15.3280±0.01317
3	Orange juice	1.4190±0.01370	1.2020±0.01135	0.7700±0.00943
4	Lemon juice	2.1450±0.03536	1.9180±0.01229	1.3430±0.01767
5	Grapefruit juice	1.3230±0.01494	0.9910±0.03247	0.7350±0.01716
6	Cabernet Sauvignon	1.6750±0.01434	1.2090±0.00876	0.6390±0.01595
7	Pinot Noir	1.7150±0.01080	1.1950±0.01434	0.6020±0.01687

The results for F and p obtained by means of ANOVA procedure were shown in Table 2.

Table 2. The results for F and p obtained by means of ANOVA procedure

Sample	F	(p)
Polyuronides	54.91	< 0.001
Total flavonoids	15424.71	< 0.001
Orange juice	8074.28	< 0.001
Lemon juice	2992.29	< 0.001
Grapefruit juice	1658.49	< 0.001
Cabernet Sauvignon	15049.86	< 0.001
Pinot Noir	15336.41	< 0.001

According to ANOVA procedure, the modifications of TAS for the samples in time intervals: zero hours, 24 and 48 hours, were given in figures 1, 2, 3, 4, 5, 6, 7.

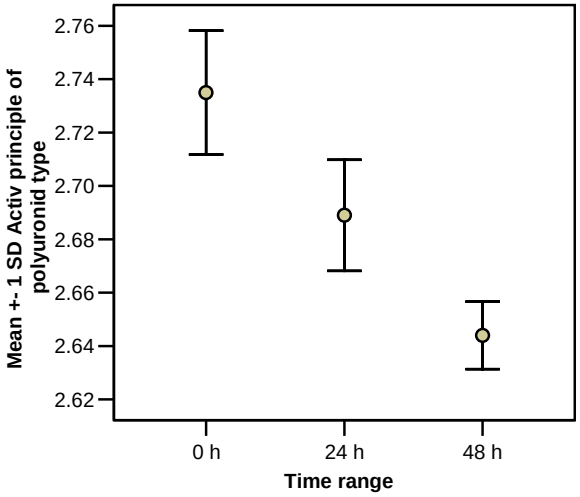

Figure 1. Modification of TAS in time for polyoronides

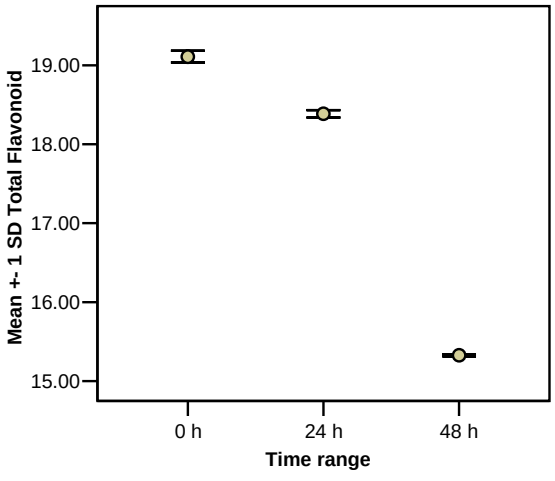

Figure 2. Modification of TAS in time for total flavonoids

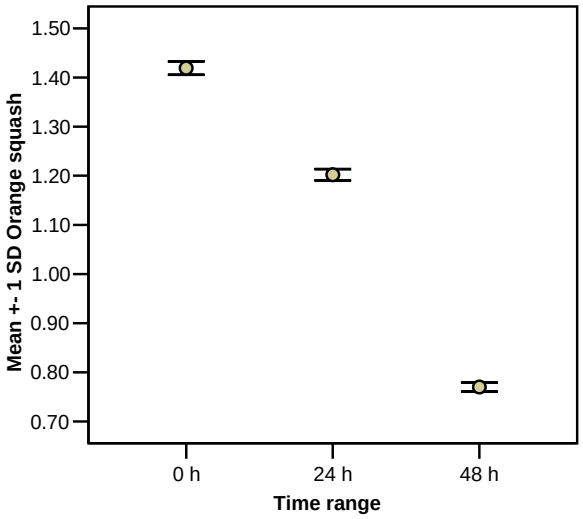

Figure 3. Modification of TAS in time for orange juice

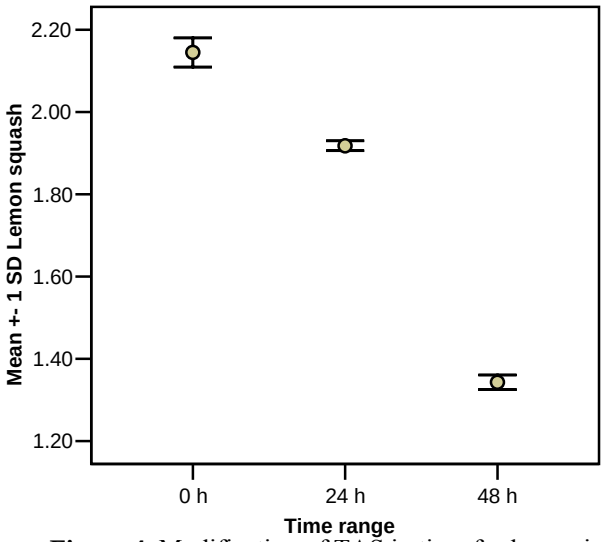

Figure 4. Modification of TAS in time for lemon juice

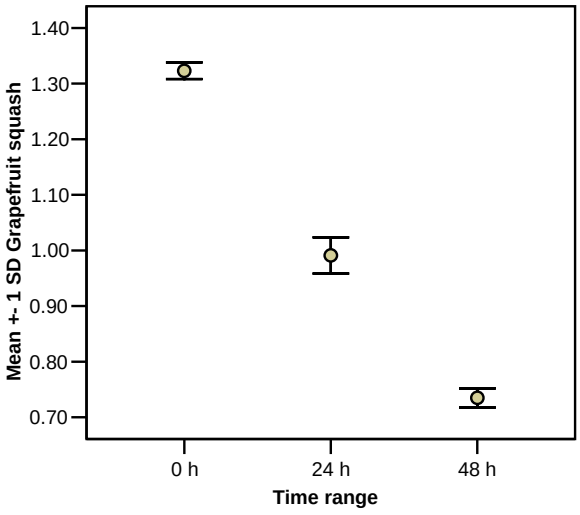

Figure 5. Modification of TAS in time for grapefruit juice

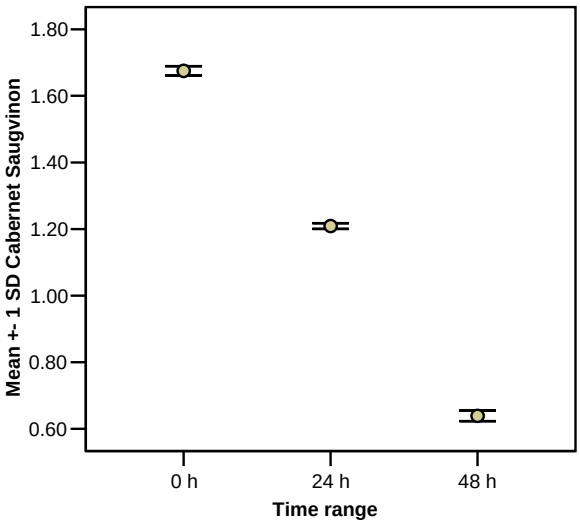

Figure 6. Modification of TAS in time for Cabernet Sauvignon

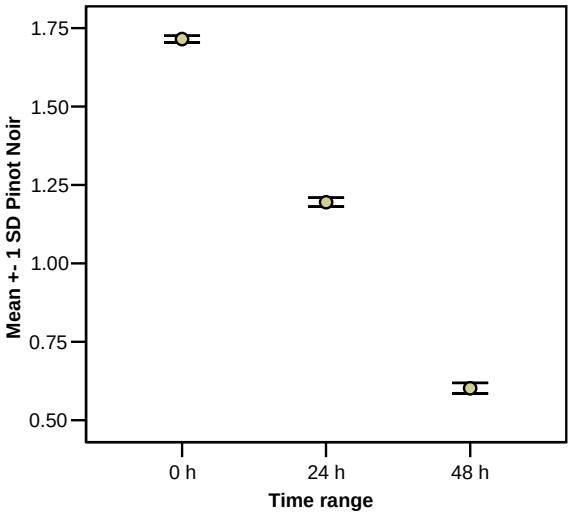

Figure7. Modification of TAS in time for Pinot Noir

A comparative comment regarding antioxidant action of samples can be made on the basis of the fact that TAS in all samples was presented in mM as an equivalent of Trolox. The results of these experiments were shown that the total flavonoids had the highest antioxidant action for all time intervals (Table 1).
The ANOVA procedure has shown that, for all samples, significant differences between the means of data were detected in the following time intervals: zero hours, 24 and 48 hours ($F > F_{critic,}$ $p < 0.001$) (Table 2). The polyuronides had a greater antioxidant action than citrus fruit juices and red wines at zero time, after 24 and 48 hours. Among the citrus fruits juices, the lemon juice had the highest antioxidant action followed by orange and grapefruit juices at all time intervals. The two red wines had a comparable antioxidant action. ANOVA procedure has shown that, for all samples, the antioxidant action was diminished after 24 and 48 hours.
As regards the polyuronides, its antioxidant action were the least diminished in time, as a result, its water solution is the most stable (Figure 1). Stability in time of polyuronides water solution may be caused by the packed polyuronide type structure, which protects the bonds responsible with the antioxidant action. The water solution of the total flavonoids was less stable in time as compared to the polyuronides (Figure 2). The lesser stability in time of total flavonoids may be caused by its phenol type structure which, in the presence of oxygen in the air, may alter at the antioxidant action level. The antioxidant action of citrus fruit juices was less stable in time as compared to the polyuronides and total flavonoids (Figure 3, 4, 5). Red wine is known to contain an average of 1.5 mg mL^{-1} polyphenols [8].
TAS results of total flavonoids and polyuronides showed that, for a concentration of 0.2mg mL^{-1}, TAS measurements were approximately 11 and respectively 1.6 higher than for the Pinot Noir red wine at zero time (Table 1). Among the samples, the red wines had the smallest antioxidant action in time intervals (Figure 6, 7).

Conclusions

1. Total flavonoids measurements showed the highest antioxidant action for all time intervals.
2. Polyuronides showed the most time-stable antioxidant action, considering that TAS went through the lowest-level changes for all time intervals.
3. Considering the preliminary results obtained, the two vegetal active principles could be introduced as food supplements, because of their antioxidant action.

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