International Journal of Agricultural Science and Food Technology

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Dates: Received: 01 September, 2015; **Accepted:** 25 September, 2015; **Published:** 28 September, 2015

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www.peertechz.com

ISSN: 2455-815X

Keywords: Antioxidants; Polyphenols; White/red organic wine preservation

Short Communication

Botanical Extracts Used as Wine Preservatives

Abstract

The aim of the study was to eliminate the potentially harmful sulfite salts normally added to wine – based products for preservation purposes with the introduction into the wine natural products, with pronounced antioxidant activity. Frieze – dried samples taken from the plants *Hippophaes* and *Goji Berry* were added to dry white and red wines after their fermentation phase. In all sample tests the sensory and oenological characteristics remained almost unchanged compared to original samples (without the addition of natural products), although their antioxidant activity was significantly increased when leaves of the plant *Hippophaes* were added. *Goji berry* fruits and fruit of *Hippophaes* plant increased antioxidant activity compared to original samples.

Introduction

The last decade of the past century was characterized by systematic procedures applied in the food and beverage industry, leading to safer products. For the food and beverage industry, special efforts have been made, in the area of preservation (techniques and materials) Specific efforts are based on the natural antioxidants and their activity, in order to provide to the consumers higher quality and occasionally enhanced functionality products [1]. A substantial amount of research has been devoted to the replacement of the sulfite salts. To some extent, sulfites occur naturally in all wines. They are commonly introduced to arrest fermentation at a desired time, and may also be added to wine products as preservatives to prevent spoilage and oxidation, at several stages of the winemaking. They are added therefore at two stages of the wine production: prior to fermentation (for selectivity) and before bottling (for preservation) According to the reaction: $K_2S_2O_5(s) \rightarrow K_2SO_3(s) + SO_2(g)$ the easily formed from the parent molecules (potassium & sodium metabisulfite, E224 & E223) Sulfur dioxide, prevents most wild microorganisms from growing, and protects both the color and delicate flavors of wine. Organic wines are not necessarily sulfite-free, but generally have the lowest amount because no additional (for the selected fermentation purposes) sulfites are permitted to be added as with most wines. In general, white wines contain more sulfites than red wines, and sweeter wines contain more sulfites than dryer ones. In the United States, wines bottled after 1987 must have a label stating that they contain sulfites if they contain more than 10 ppm. In the European Union an equivalent regulation applies since 2005. The reason is that several reports are linking the presence of relatively elevated amounts of sulfite salts in the human organism with many adverse reactions. These range from hives, sneezing, swelling of the throat and breathing difficulty and extend to anaphylaxis and other life threatening allergenic reactions in sulfite sensitive individuals and asthmatics. Sulfites are also known to destroy vitamin B_1 (thiamin).

It is for these reasons that many research projects and regulations are aiming at substituting this efficient chemical, especially for the organically labeled products. Successful results have been achieved with the use of certain edible herbal plants and their fruits and berries where, characterized by remarkable antioxidant capacities (oxygen radical absorbance, ORAC) and total phenolic and flavonoid content. Their increased functionality in the human diet is also linked with diverse biological effects, such as anticancer and antimicrobial activities [2,3] . From these fruits and berries, *Hippophaes rhamnoides* and *Lucium barbarum* (Goji berry) are widely found in nature/ mountains and cultivated in many countries from South-East Europe to South- East Asia. These small red fruits are dried and used in many food recipes and functional products. Their bitter taste, due to the tannins and mild flavors are considered as appropriate for their addition to wine and wine based beverages. The aim of the study was to eliminate the potentially harmful sulfite salts normally added to wine – based products for preservation purposes with the introduction into the wine of natural plant products (plants *Hippophaes* and *Goji – Berry*), with pronounced antioxidant activity.

Experimental Section

Wine samples and plants

Wine samples used for the study were representative of Greek wine varieties. One white (Savatiano, Attica region) and one red (Agiorgitico, nemea region, Peloponnese). Leaves and fruits of the Plant *Hippophaes rhamnoides* (common sea-buckthorn) and fruits of *Goji – Berry* were used to possibly replace sulfite salts in wines, after their fermentation phase, due to their known antioxidant activity at concentration 0.3g/L.

Folin-Ciocalteau method for total phenolics in wine

Folin Ciocalteu Reagent. This is usually purchased as the 2N reagent available from Sigma (F9252) or from Fisher Scientific (ICN19518690), and presumably others.

Gallic Acid Stock Solution. In a 100-mL volumetric flask, dissolve 0.500 g of dry Gallic acid in10 mL of ethanol and dilute to volume with water. Can be opened daily, but to store, keep closed in a refrigerator up to two weeks.

Sodium Carbonate Solution. Dissolve 200 g of anhydrous sodium carbonate in 800 mL of water and bring to a boil. After cooling, add a few crystals of sodium carbonate, and after 24 hr., filter and add water to 1 L.

To prepare a calibration curve, add 0, 1, 2, 3, 5, and 10 mL of the above Gallic acid stock solution into 100 mL volumetric flasks, and then dilute to volume with water. These solutions will have phenol concentrations of 0, 50, 100, 150, 250, and 500 mg/L Gallic acid, the effective range of the assay. Left over Gallic acid solutions can be poured down the drain.

From each calibration solution, sample, or blank, pipet 20 µL into separate cuvettes, and to each add 1.58 mL water, and then add 100 µL of the Folin-Ciocalteu reagent, and mix well. Wait for between 30 sec and 8 min, and then add 300 µL of the sodium carbonate solution, and shake to mix. Leave the solutions at 20°C for 2 hr., and determine the absorbance of each solution at 765 nm against the blank (the "0 mL" solution) and plot absorbance vs. concentration. Alternatively, they can be left at 40°C for 30 min before reading the absorbance.

For white wines, add 20 µL as for the calibration solutions, but in the case of red wines, dilute the wines by 10 first, then add 20 µL (or skip the dilution and add 2 µL if you have precise micro pipettors).

Create a calibration curve with the standards and determine the levels in the samples. Do not neglect to multiply the observed concentrations by any dilution factor of the original sample. Results are reported as mg/L Gallic Acid Equivalent, GAE, because the phenols in wine contain mostly other phenols, and only small amounts of Gallic acid [4,5].

DPPH Assay for the determination of the antioxidant capacity of wines

The methanol organic solvent used was of analytical grade. The reagents DPPH• and galic acid were purchased from Aldrich (Sigma-Aldrich Chemie, Steinheim, Germany), and reagent 6-hydroxy-2,5,7,8-tetramethylchloroman-2-carboxylic acid (Trolox) was obtained from Merck (Darmstadt, Germany).

The antioxidant capacity of wines by the DPPH free radical scavenging method was determined following Nixdorf and Gutierrez [6]. Aliquots of 100 µL of wine samples previously diluted in methanol (1:25) were added to 2.9 mL of a DPPH methanolic solution (60 mM), homogenized, and left to rest for 30 min in the dark. The absorbance of the solutions was measured at 515 nm using a spectrophotometer (model Cary 50, Varian). The analytical curve was plotted for methanol solutions of Trolox (Merck) in concentrations from 8.0 to 60.0 mM. The response was given in millimoles of Trolox equivalent [7].

TEAC method (Trolox Equivalent Antioxidant Capacity or Total Antioxidant Activity – TAA)

The total volume used in the original procedure [8,9,12,13] was reduced to 1 ml. The stock solution, a 1:1 (v/v) mixture of ABTS (7 mmol/l) and potassium persulfate (4.95 mmol/l), was left to stand for 12 h at laboratory temperature in dark to form radical-cation ABTS•+. The final solution was stable for at least one week at 4°C in dark. The stock solution was diluted with phosphate buffer solution to give the absorbance values between 1.0 and 1.5 AU at 734 nm (the same absorbance value must be used for the standard and samples). The standard or sample (20 µl) of 4-fold and 20- or 40-fold diluted white and red wines (according to the reaction intensity), respectively, were mixed with the working solution (975 µl) and adjusted to 1000 µl with deionised water. The decrease of the absorbance at 734 nm was measured after 30 min (after reaching plateau). Aqueous phosphate buffer solution (1 mmol/l, without ABTS•+) and Trolox (0.5 mmol/l) were used as a control and a calibrating standard, respectively.

Ferric reducing antioxidant power (FRAP) method for wines

It was modified to a semi micro scale using the total volume of 1 ml. A portion of an aqueous 10 mmol/l solution of TPTZ reagent in 40 mmol/l HCl was mixed with the same volume of 20 mmol/l FeCl3·6 H2O and ten times higher volume of acetate buffer of pH 3.6 (3.1 g sodium acetate and 16 ml acetic acid per liter). The mixture was incubated at 37°C for 5 minutes. A portion (900 µl) of the Fe3+-TPTZ mixture and the sample of wine (25 µl; red wines diluted 5 to 10-fold) or the standard or water (for blank) were adjusted to 1000 µl with deionised water, incubated for 30 min (after reaching plateau), and the absorbance at 593 nm was read. Trolox (0.5 mmol/l) was used for calibration [10,11].

Chemical and sensory characteristics of wines

Major characteristics of wines used are presented in Table 1 and were determined according to Jung et al. [14]. Sensory characteristics

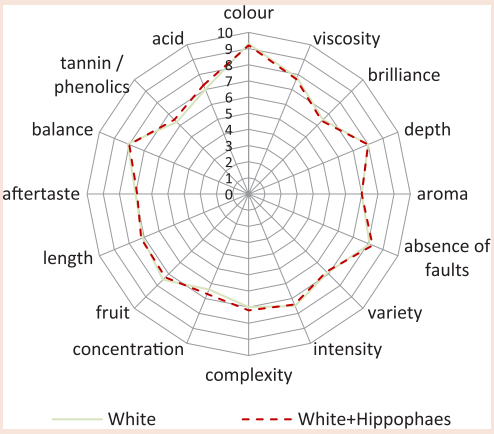

Figure 1: Sensory profile of white wine used before and after the addition of *Hippophaes* leaves.

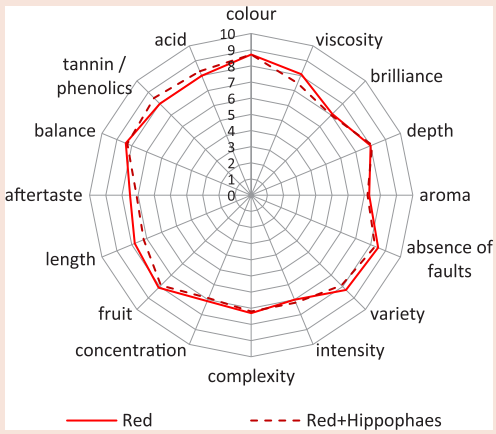

Figure 2: Sensory profile of red wine before and after the addition of *Hippophaes* leaves.

of wines before and after the addition of natural plant products were determined according to Sonia García-Muñoz et al. [15] (Figures 1,2).

Results and Discussion

The determined contents of total phenolic compounds and antioxidant activity values are presented in Table 1. The great differences in the contents of phenolic compounds in white and red wines indicate that anthocyanin's form the most important part of the phenolic compounds in red wines. The average values of phenolic compounds content in white wines determined by FCM were in the interval of 150–210 mg/L GAE. The values of phenolic compounds content in red wines were approximately 10- to 15-times higher, namely from 1350 to 2160 mg/L GAE. Addition of leaves of the plant *Hippophaes* significantly increased total phenolic. *Goji berry* fruits moderate increased the amount of total phenolic while the addition of fruit of *Hippophaes* slightly increased total phenolic content. The great differences in the contents of phenolic compounds in white and red wines indicate that anthocyanin's form the most important part of the phenolic compounds in red wines. Beside other factors, the grape variety, intensity of solar irradiation at the ripening time of grapes, and winemaking procedure mostly influence the phenolic compounds content. Therefore, much higher contents of anthocyanins and total contents of phenolic compounds are present especially in red wines from sunny regions (Italy, Spain, Greece, California etc.). Trolox was used as a common standard for the calibration of all the methods used for the assessment of the total antioxidant activity. Millimolar absorption of Trolox for TEAC and DPPH methods are almost the same, and they are approximately about 56% higher than for FRAP method. TEAC method is the most reactive one in the reaction with phenolic compounds, yielding approximately 2.8- and 4.8-times higher values than FRAP and DPPH methods, respectively. The higher reactivity of ABTS reagent with phenolic compounds is the most important factor. It is difficult to confront our values of antioxidant activity with the literature data since similar experiments are very scarce in the literature. However the majority of authors used various methods for determining antioxidant activity in wines such as the inhibition of lipid oxidation, DPPH method with the evaluation of EC50 (the sample concentration necessary to reduce the remaining DPPH by 50%), and ORAC method (Oxygen Radical Absorbance Capacity) [7-9]. It is possible to compare, in part, some of the values determined by the method TEAC using ABTS radical. In all sample tests the sensory and oenological characteristics remained almost unchanged, although their antioxidant activity was significantly increased when leaves of the plant *Hippophaes* were added. *Goji berry* fruits and fruit of *Hippophaes* plant increased antioxidant activity compared to original samples.

Table 2 shows the chemical characteristics of wines (white and red cultivars) used and the same wines products without the addition of sulfite salts but with leaves of *Hippophaes* added to both wines right after their fermentation phase. At Figures 1, 2 the sensory profile of wines before and after the addition of *Hippophaes* leaves is presented emphasizing on characteristics of sight, nose, palate and finish. Results of addition of *Hippophaes* leaves are shown since this product had shown better antioxidant activity and Higher amounts of Total phenols (Table 1). As it can be seen in all sample tests the sensory and oenological-chemical characteristics remained almost unchanged compared to original samples (without the addition of natural products).

Table 1: Content of total phenolic compounds and antioxidant capacity in wines.

No.[1]	FCM[2]	TEAC[3]	FRAP[3]	DPPH[3]
White wines				
1	150 ± 2.9	4.30 ± 0.19	1.79 ± 0.11	0.42 ± 0.02
2	155 ± 2.5	4.35 ± 0.20	1.85 ± 0.10	0.43 ± 0.04
3	210 ± 2.8	6.40 ± 0.22	2.24 ± 0.14	1.82 ± 0.03
4	171 ± 2.6	5.29 ± 0.19	1.91 ± 0.12	0.71 ± 0.03
Red wines				
1	1350 ± 5.1	17.84 ± 0.01	6.34 ± 0.01	3.70 ± 0.01
2	1370 ± 5.4	18.01 ± 0.05	6.44 ± 0.01	3.72 ± 0.02
3	2160 ± 5.6	18.89 ± 0.07	11.34 ± 0.05	5.70 ± 0.04
4	1396 ± 4.9	17.99 ± 0.05	7.94 ± 0.04	4.25 ± 0.03

[1]samples: 1= control, 2= wine + Hippophae L. fruit, 3= wine + Hippophae L. leaves and 4= wine + goji berry fruit.
[2]mg/L gallic acid equivalents (GAE), 3 mM Trolox equivalents (TE), All results are expressed as mean ± SD, n = 3.

Citation: Proestos C, Sflomos K, Zoumpoulakis P, Tatarides P, Sinanoglou VJ (2015) Botanical Extracts Used as Wine Preservatives. Int J Agricultural Sci Food Technology 1(1): 007-011. DOI: 10.17352/2455-815X.000003

Table 2: Chemical characteristics of wines (white and red cultivars) used and the same wines products without the addition of sulfite salts but with leaves of *Hippophaes*.

Samples				
Chemical /Enological Characteristics	**White wine**	**Red wine**	**White wine + *Hippophaes* leaves**	**Red wine + *Hippophaes* leaves**
% Vol.	13.25	12.4	13.23	12.5
Total Acidity (tartaric acid)	4.25	4.98	4.46	5.13
pH	3.31	3.36	3.31	3.41
Reducing sugars (g/L)	2.1	2.2	2.2	2.2
Volumetric ac. (g/L)	0.21	0.24	0.32	0.38
Ethyl acetate (mg/L)	33	22	33	41
Malic acid (g/L)	0.78	1.14	0.82	1.06
Lactic acid (g/L)	0.8	0.6	0.7	0.7
Density	0.9843	0.9904	0.9843	0.9898
A420 (nm)	0.11	3.08	0.13	3.23
A520 (nm)	-	4.51	-	4.85
A620 (nm)	-	1.06	-	1.25
Color Intensity	-	8.65	-	9.33

Conclusion

The addition of parts of plants to wines for replacing sulfites are very scarce in the literature (Gonzalez-Rompinelli et al. [16]. The tradition is to classify the quality of wine based on its colour, smell, and taste rather than on its content of compounds beneficial to health. The determinable laboratory characteristics so far used like alcohol, organic acids, saccharides, and sulfites concentration relate more to the taste quality of wine as well. The determinations of total phenolic compounds and the total antioxidant activity of wine tell more about the health effect of a particular wine and can be used as criteria of quality and beneficial health effect. Several different methods for the evaluation of phenolic compounds and antioxidant activity of wines are used and little is known about the possibility of the comparison of the values obtained by different methods. Through the analyses of the same sample by different methods we investigated the correlations of values determined by the used methods. The values found with the methods for total phenolics FCM and total antioxidant activity (TEAC, FRAP, and DPPH) determination correlated with one another and also with the colour of wines very significantly with the exception of FRAP method for white wines (so far for unexplained reasons). The insignificant correlation between the contents of total phenolics or antioxidant activity and the intensity of red wines colour might indicate artificial colouring of red wine or mixing white and red wine. FCM for the determination of total phenolics and TEAC method for the determination of total antioxidant activity can be particularly easy to use and may be the best of the tested methods for rapid objective evaluation of a large number of wine samples. The assessment of phenolics content and total antioxidant activity in white and red wines and the introduction of these values on the bottle label would better inform the consumer about the quality and health benefit of the purchased wine. Frieze – dried samples taken from the plants *Hippophaes and Goji - Berry* were added to dry white and red wines after their fermentation phase. In all sample tests the sensory and oenological characteristics remained almost unchanged, although their antioxidant activity was significantly increased when leaves of the plant *Hippophaes* were added.

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Citation: *Proestos C, Sflomos K, Zoumpoulakis P, Tatarides P, Sinanoglou VJ (2015) Botanical Extracts Used as Wine Preservatives. Int J Agricultural Sci Food Technology 1(1): 007-011. DOI: 10.17352/2455-815X.000003*