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Incorporating Vitamins and Resveratrol into Wines and Tsipouro to Enhance their Antioxidant Capacity

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Abstract: The subject of this study is the incorporation of natural products such as resveratrol (RVT), retinol (vitamin A), tocopherol (vitamin E), and ascorbic acid (vitamin C) into alcoholic beverages and the enrichment of alcoholic beverages such as red, white, and rosé wine, and spirits such as tsipouro with the vitamins mentioned above in order to increase the added nutritional value of the final product. Enrichment was achieved with substances, such as resveratrol and the vitamins mentioned above, encapsulated in suitable matrices (microcarriers) compatible with food and beverages, such as cyclodextrin. This incorporation is expected to increase the antioxidant properties of alcoholic beverages. The substances used were placed in wines and spirits (tsipouro) at a specific concentration (300mg /1000ml), and the sampling was done over a fixed period of time. The final maximum values were evaluated, and thus, the antioxidant activity was measured by DPPH, ABTS, and the total content of phenolic compounds was measured by the Folin-Ciocalteau method. These analyses showed that the substances we placed in the alcoholic beverages positively affected them and increased their antioxidants. The purpose of this work was achieved, and the drinks increased their nutritional value, and their economic value will undoubtedly increase.

Keywords: wine; tsipouro; retinol (vitamin A); tocopherol (vitamin E); ascorbic acid (vitamin C); resveratrol (RVT); DPPH; ABTS; Folin-Ciocalteu.

1. Introduction

Recent research shows France has lower cardiovascular case rates than other European countries. This is due to the regular but moderate consumption of wine, the so-called French paradox, which helps us to understand how these low rates are explained [1,2]. It has been suggested that the abundant phenolic compounds in red wine may be responsible for improving public health in France. Although the mechanisms of action are not yet fully understood, it is known that these molecules behave as free radical scavengers and powerful antioxidants. They have also been proven to protect the human body from lousy cholesterol [3,4].

Substances, such as resveratrol and vitamins that have high antioxidant, antimicrobial, and other properties, are suitable to be taken daily in specific amounts to strengthen the human body. Alcoholic beverages, such as wine, contain certain amounts of these substances, such as resveratrol, but for the average person to get the specific amounts needed from these substances, they would have to consume large amounts of drink, i.e., alcohol, which we don't want. So the uniqueness of this research is to try to strengthen this product by adding these substances to it so that with the consumption of one to two glasses approximately, as stated as the daily consumption of wine according to the World Health Organization (WHO), the average person takes, if not the whole, at least half the amount needed of these substances.

The substances used are resveratrol, a natural substance of the class of phytoalexins with the basic structure of stilbenes, and vitamins such as vitamin E (tocopherol), vitamin C (ascorbic acid), and vitamin A (retinol).

1.1. Wine.

The Esters are qualitatively the major constituents of wines and, in addition to water, ethanol, and fusel alcohols, major quantitative constituents. Epidemiologic studies from numerous disparate populations reveal that individuals with the habit of daily moderate wine consumption enjoy significant reductions in all causes and particularly cardiovascular mortality when compared with individuals who abstain or who drink alcohol to excess. Researchers are working to explain this observation in molecular and nutritional terms. Moderate ethanol intake from any type of beverage improves lipoprotein metabolism and lowers cardiovascular mortality risk. The question is whether wine, particularly red wine, with its abundant content of phenolic acids and polyphenols, confers additional health benefits. Discovering the nutritional properties of wine is challenging, requiring the biological actions and bioavailability of the >200 individual phenolic compounds to be documented and interpreted within the societal factors that stratify wine consumption and the myriad effects of alcohol alone. A further challenge arises because the health benefits of wine address the prevention of slowly developing diseases for which validated biomarkers are rare. Thus, although the benefits of the polyphenols from fruits and vegetables are increasingly accepted, the consensus on wine is developing more slowly. Scientific research has demonstrated that the molecules present in grapes and wine alter cellular metabolism and signaling, which is consistent mechanistically with reducing arterial disease [5].

Today's wine industry is characterized by regional differences in the wines themselves and the business models by which these wines are produced, marketed, and distributed. Small family vineyards and cooperative wineries abound in Old World countries such as France, Spain, and Italy. In New World regions like the United States and Australia, the industry is dominated by a handful of substantial producers [6].

1.2. Tsipouro.

Tsipouro has 36 to 45 alcoholic degrees often. Its main difference from raki-chicken is that tsipouro is commonly used for dual distillation, and often, a difference is added in some areas. It should not be confused with ouzo, a widespread Greek drink prepared differently. The production of tsipouro is lost in the depths of time, but it is said that it began in the 14th century on Mount Athos by monks who lived there. Over the years, it has spread to various parts of Greece, mainly in Macedonia, Epirus, Thessaly, the Peloponnese, and Crete.

The raw material for the production of distillates is the marcs, that is, the mass left after the grape paste, in order to produce wine.

The tsipouro can be produced by mark beads derived from red winemaking with a smaller or more significant amount of wine. In addition, they can also be used separately from the bulk of the must, which comes from white grapes, but also from red grapes, which have been used to produce rosé or white wine with direct compression.

Often, the tsipouro is distilled a second time, as it improves its quality.

1.3. Vitamin C.

Vitamin C is a water-soluble vitamin, a white to off-white "powder", which has a carbohydrate chemical structure and takes part in metabolic processes mainly of animal organisms (Figure 1a) [7-10].

1.4. Vitamin E.

Vitamin E is a fat-soluble vitamin of a light yellow color and an oil-like form with a very slight characteristic odor. A substance practically insoluble in water and miscible in any proportion with oils, acetone, alcohol, chloroform, ether, and other fatty solvents, it is the primary fat-soluble antioxidant agent of the antioxidant defense system of cells (Figure 1b) [11].

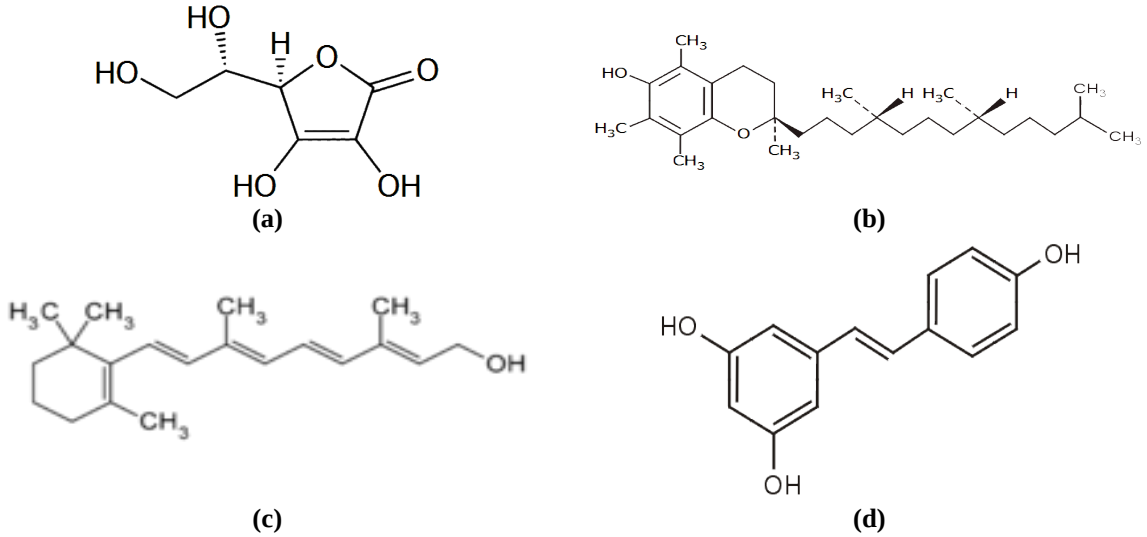

Figure 1. Chemical structures: **(a)** Vitamin C [7]; **(b)** Vitamin E [11]; **(c)** Vitamin A [12]; **(d)** Resveratrol [22].

1.5. Vitamin A.

Vitamin A is a fat-soluble vitamin, yellow-orange in color and of a "sticky" texture. It is necessary for the proper functioning of the retina of the eye (Figure 1c) [12-13].

1.6. Resveratrol.

The compound resveratrol (RVT) is a natural substance of the phytoalexins class with a basic stilbenes structure. Resveratrol (RVT) is mainly found in high concentrations in the skin of grapes, especially red grapes, nuts, and berries (mulberries, blueberries, cranberries, bilberries), and in smaller quantities, it has been found in 70 more plant products. The resveratrol (RVT) content in the skin of fresh red grapes is 50-100 μg/g, while in red wine, it can be found in concentrations of 1.5-3 mg/L [14-20]. White wine can be found in smaller

quantities because the fermentation of the wine is carried out after removing the skins of the grapes. Resveratrol (RVT), like other phytoantioxidants, is a natural component of plants with antibiotic activity to protect against fungi and oxidative damage. It is a colorless to pale yellow crystalline substance (depending on its purity). It is a natural substance of the class of phytoalexins and has the basic structure of stilbenes. With a strong antioxidant effect (Figure 1d) [21-27].

2. Materials and Methods

2.1. Materials.

Specific methods were used to conduct the experiments, and all the machines and reagents that were used to conduct the experiments are mentioned below. The machines and reagents used for the analyses are:

- ASCORBIC ACID A.G. (vitamin C), 99, 5%, MW=176, 13 gr/mol, PENTA;
- DL-a-Tocopherol (vitamin E), 96%, MW=430, 72 gr/mol, TCI;
- all-trans-Retinol (vitamin A), 95%, MW=286, 4516 gr/mol, ACROS ORGANICS;
- Resveratrol (RVT), 99%, MW=228, 25 gr/mol, TCI;
- 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 95%, MW=394, 32 gr/mol, Alfa Aesar GmbH Co KG;
- Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylicacid), 97%, $C_{14}H_{18}O_4$, MW=250,29 g/mol CAS: 53188-07-01, Sigma-Aldrich, Germany.
- Sodium Carbonate anhydrous(Na_2CO_3): Assay 99,5-100,5%, MW=105,99 g/mol, CAS: 497-19-8, Carlo EΓ reagents, Italy
- Gallic Acid: 3,4,5-Trihydroxybenzoic acid anhydrous 99%, $C_7H_6O_5$, MW=170,12 g/mol, CAS: 149-91-7, Alfa Aesar GmbH&Co KG, Germany
- Potassium Persulfate ($K_2S_2O_8$): 99,0%, MW=270,32gr/mol, ACS Reagent
- Methanol for HPLC: 99,9%,CH_3OH, MW=32,04 gr/mol, Sigma-Aldrich(4)
- Lab spatula: Cole-Parmer Essentials Laboratory(7)
- UV-Visible spectrophotometer, Novaspec III;
- Analytical balance: KERN ADJ, TechnoLab;
- Water bath, Heraus

2.2. Methods.

2.2.1. Measurement of antioxidant activity with the DPPH method.

2.2.1.1. DPPH mother solution.

The balance is set to zero with the beaker to be used, and an amount of 0.0060 g of DPPH is added to it with the help of a - lab spatula. Then, a small amount of methanol is added to the beaker at a time while stirring until the DPPH granules dissolve. Finally, place the beaker's contents in a 250 ml volumetric flask and add methanol to the mark. The solution should not be used immediately after preparation. Leave the solution in the dark for 30 minutes. It can be kept in the fridge at 3°C (5) for up to one day [28-30].

2.2.1.2. Trolox solution.

The balance is zeroed with the beaker used, and 0.0250 g Trolox is added to it with the help of a special spatula. These are dissolved in 90 ml of methanol, and the solution is transferred to a 100 ml volumetric flask made up of a volume of deionized water (10 ml).

2.2.1.3. Dilution of samples.

The initial concentration of substances incorporated into alcoholic beverages is 300 mg of substances in 1000 ml of alcoholic beverage. Samples were taken, diluted, and then measured at a specified time. Specifically, for red wine, 100 µl were diluted with 99.9 ml of methanol, and for rose wine, 100 µl were diluted with 49.9 ml of methanol. The white wine and tsipouro were not diluted (6).

2.2.1.4. Experiment procedure.

50µl of the sample (standard or sample) and 1450µl of DPPH mother solution are added to an Eppendorf with the help of pipettes.

After vortexing for 30 sec, absorbance at a wavelength of 517 nm (t=0) is measured in wells. After 30 minutes, the samples remain in the dark, a vortex is performed, and the absorbance is measured again (t=30 sec). The spectrophotometer is zeroed with methanol.

From the two absorbances, the % difference in absorbance is calculated according to the formula:

$$\%\Delta A\ (517nm) = [A(0) - A(30)/\ A(0)] \times 100. \quad (1)$$

The Trolox reference curve expresses the antioxidant capacity in Trolox equivalents.

Trolox solution: 0.0250 g of Trolox is dissolved in 90 ml of methanol and 10 ml of deionized water.

A Trolox solution with a concentration of 2 mM (solution A) is prepared for the standard reference curve.

In 10 ml volumetric flasks, the corresponding amounts of 8, 6, 4, and 2 ml of solution A are added, and the volume is made up of pure methanol.

2.2.2. Measurement of antioxidant activity with the ABTS method.

2.2.2.1. ABTS mother solution.

The balance is set to zero with the beaker to be used, and with the help of a special spatula, the amount of 0.1801 g ABTS and 0.0331g $K_2S_2O_8$ is added to it, and we first dilute them with 20 ml of deionized water. The solution is then added to a 50 ml volumetric flask, and deionized water is added up to the mark. Leave the solution for 16 hours in the dark at room temperature to acquire an intense deep blue color. Finally, we take 3.6 ml of the initial solution prepared above, place it in a 250 ml volumetric flask, and dissolve it with 246.4 ml of methanol. Measure the absorbance, which should be close to 0.700 when the spectrophotometer is at 734 nm. The solution can be used immediately after its preparation. Keeps in the fridge for many days [31-33].

2.2.2.2. Trolox solution.

The scale is zeroed with the beaker to be used, and 0.0250 g of Trolox is added to it with the help of a special spatula. These are dissolved in 90 ml of methanol, and the solution

is transferred to a 100 ml volumetric flask and made up to the mark with deionized water (10 ml).

2.2.2.3. Dilution of samples.

The initial concentration of the substances is 300 mg in 1000 ml of alcoholic beverage. At a specified time, a number of samples were taken, diluted, and measured. Red wine was diluted with 100 µl of red wine in 99.9 ml of methanol, rosé wine was diluted with 100 µl of rosé wine in 49.9 ml of methanol. The white wine was diluted with 100 µl of white wine in 49.9 ml of methanol, and the tsipouro was not diluted.

2.2.2.4. Experiment procedure.

50µl of the sample (standard or sample) and 1450 µl of ABTS mother solution are added to an Eppendorf with the help of pipettes.

Strong vortexing is carried out for 30 sec and left in the vials to rest for 6 minutes; the absorbance is measured at a wavelength of 734 nm. The spectrophotometer is zeroed with methanol.

From the two absorbances, the % difference in absorbance is calculated according to the formula:

$$\%\Delta A\ (734nm) = [A(\delta) - A(6min)/\ A(\delta)] \times 100 \qquad (2)$$

The Trolox reference curve expresses the antioxidant capacity in Trolox equivalents.

Trolox solution: 0.0250g of Trolox is dissolved in 90 ml of methanol and 10 ml of deionized water.

A Trolox solution with a concentration of 2 mM (solution A) is prepared for the standard reference curve.

In 10 ml volumetric flasks, the corresponding amounts of 8, 6, 4, and 2 ml of solution A are added, and the volume is made up of pure methanol.

2.2.3. Measurement of phenolic compounds by the Folin-Ciocalteau method.

2.2.3.1. Anhydrous sodium carbonate solution.

The scale is set to zero with the beaker to be used, and with the help of a special spatula, 50.00 g of anhydrous sodium carbonate is added to it, which is dissolved in deionized water using very mild heating and simultaneous stirring. Once dissolved, it is placed in a 250 ml volumetric bottle, and the bottle is filled with deionized water up to the mark. It is then left at room temperature and without light for 24 hours. Then, using filter paper, the content is filtered and not diluted. The solution can be used immediately after its preparation. It keeps for many days [34,35].

2.2.3.2. Folin-Ciocalteu solution.

Folin-Ciocalteu solution with normality 1N is commercially available, but we have not prepared it (8).

2.2.3.3. Gallic acid solution.

The balance is set to zero with the beaker to be used, and 0.50 g of gallic acid is added to it with the help of a special spatula. These are dissolved in 90 ml of deionized water, and the

solution is transferred to a 100 ml volumetric flask and made up to the mark with ethanol (10 ml). The solution stays for two weeks.

2.2.3.4. Dilution of samples.

The initial concentration of the substances is 300 mg in 1000 ml of alcoholic beverage. Several samples were taken, diluted, and measured at a specified time. Red wine was diluted with 100 µl of red wine in 99.9 ml of deionized water, and rosé wine was diluted with 100 µl of rosé wine in 49.9 ml of deionized water. White wine was diluted with 100 µl of white wine in 49.9 ml of deionized water, and tsipouro was diluted with 100 µl of tsipouro in 24 ml of deionized water.

2.2.3.5. Experiment procedure.

20 µl of the sample (standard or sample) and 1450 µl of deionized water are added to an Eppendorf and 100 µl with the help of pipettes. We vigorously stir for 30 seconds in the vortex and let it rest for 8 minutes, add 300 µl of anhydrous sodium carbonate, and again vigorously stir for 30 sec in the vortex.

We place the samples in the wells, cover them with parafilm, and place them for 30 minutes in the water bath at 40°C. As soon as they acquire a blue color and have returned to room temperature, the spectrophotometer is at 750 nm. The spectrophotometer is zeroed with deionized water.

Phenolics are expressed through the reference curve in gallic acid.

Gallic Acid Solution: 0.50 g gallic acid is dissolved in 90 ml deionized water and 10 ml ethanol.

A gallic acid solution (solution A) is prepared for the standard reference curve.

In 10 ml volumetric flasks, add the proportional amounts of 2.5/2.0/1.5/1.0/0.5 ml of solution A and make up the volume with deionized water.

3. Results and Discussion

The same treatment and the Measurement of their antioxidant activity were applied to all samples. However, each method applied (DPPH, ABTS, Folin-Ciocalteu) has a different photometric response from the samples, while the dilutions made were found experimentally and applied for each sample separately.

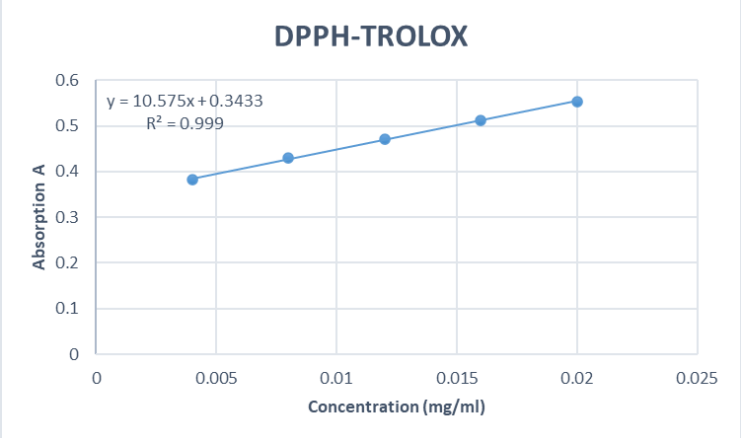

Figure 2. Standard reference curve with Trolox for the DPPH method.

3.1. Evaluation of DPPH radical scavenging capacity of wine and tsipouro samples.

Assessment of the DPPH radical scavenging capacity of samples of vitamins A, E, C, and resveratrol is widely used to assess the antioxidant capacity of samples of plant origin (Figure 3,4,5,6). To estimate the antiradical capacity of the samples, mg Trolox equivalents per 100 ml of solution were calculated based on a standard Trolox curve (Figure 2).

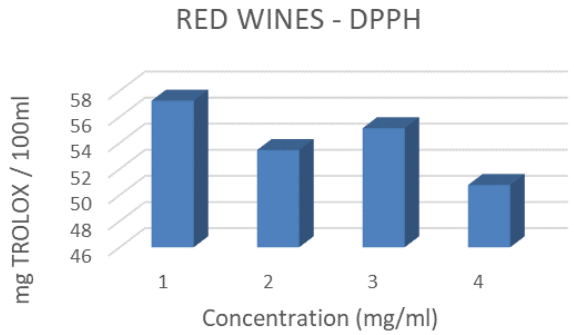

Figure 3. Final results (after 1500 hours) of red wine with **(1)** resveratrol; **(2)** vitamin C; **(3)** vitamin E; **(4)** vitamin A for the DPPH method.

Based on the diagram above, we notice that all four substances we added to the wine had positive results. More specifically, resveratrol, as expected, gives the best rates of antioxidant capacity based on the reference curve and troloχ equivalents, followed by the sample with vitamin E, which gives excellent results, the following vitamin is C with satisfactory results, and finally, it is vitamin A whose results are also satisfactory.

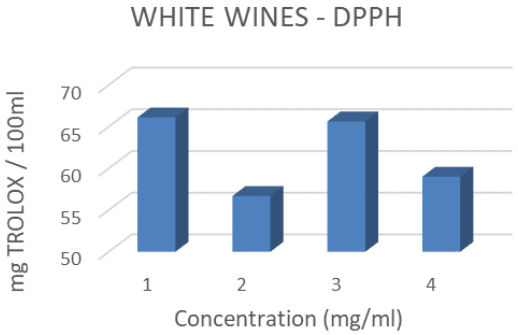

Figure 4. Final results (after 1500 hours) of white wine with **(1)** resveratrol; **(2)** vitamin C; **(3)** vitamin E; **(4)** vitamin A for the DPPH method.

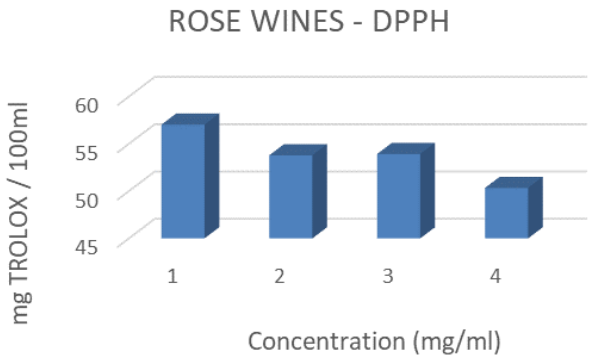

Figure 5. Final results (after 1500 hours) of rose wine with **(1)** resveratrol; **(2)** vitamin C; **(3)** vitamin E; **(4)** vitamin A for the DPPH method.

Based on the diagram above, we notice that all four substances we added to the wine had positive results. More specifically, resveratrol, as expected, gives the best rates of antioxidant capacity based on the reference curve and trolox equivalents, followed by the sample with vitamin E, which gives perfect results, the following vitamin is C with satisfactory results, and finally, it is vitamin A whose results are also satisfactory.

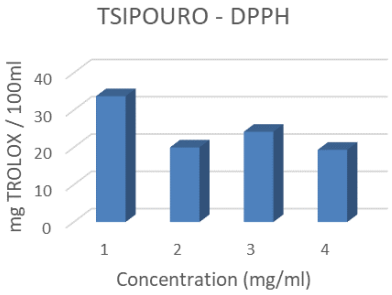

Figure 6. Final results (after 1500 hours) of tsipouro with **(1)** resveratrol; **(2)** vitamin C; **(3)** vitamin E; **(4)** vitamin A for the DPPH method.

Based on the diagram above, we notice that all four substances we added to the tsipouro had positive results. More specifically, resveratrol, as expected, gives the best rates of antioxidant capacity based on the reference curve and trolox equivalents, followed by the sample with vitamin E, which gives excellent results, the following vitamin is C with satisfactory results, and finally, it is vitamin A whose results are also satisfactory.

3.2. Estimation of ABTS free radical scavenging of wine and tsipouro samples.

The ABTS+ radical inhibition test gives an estimate of the studied samples' antioxidant capacity (Figure 8,9,10,11). The results are expressed as mg trolox equivalents per 100 ml of solution and with the percentages of root inhibition from each sample. The concentration of the samples is calculated using a Trolox standard curve (Figure 7).

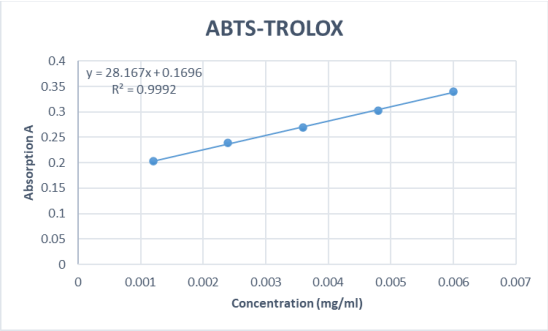

Figure 7. Standard reference curve with trolox for the ABTS method.

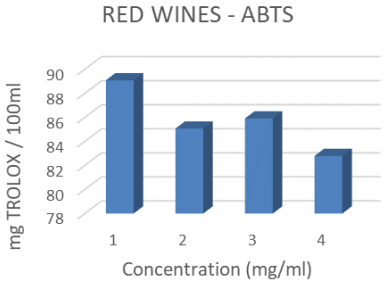

Figure 8. Final results (after 1500 hours) of red wine with (1) resveratrol; (2) vitamin C; (3) vitamin E; (4) vitamin A for the ABTS method.

Based on the diagram above, we notice that all four substances we added to the wine had positive results. More specifically, resveratrol, as expected, gives the best rates of antioxidant capacity based on the reference curve and trolox equivalents, followed by the sample with vitamin E, which gives very good results, the following vitamin is C with satisfactory results, and finally, it is vitamin A whose results are also satisfactory.

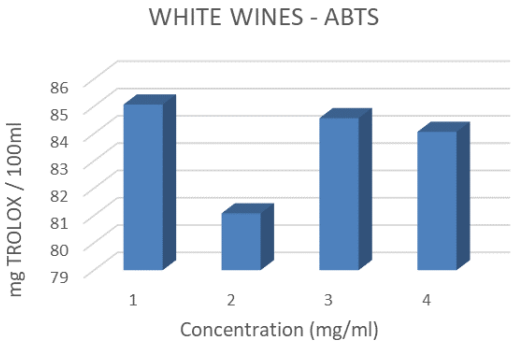

Figure 9. Final results (after 1500 hours) of white wine with **(1)** resveratrol; **(2)** vitamin C; **(3)** vitamin E; **(4)** vitamin A for the ABTS method.

Based on the diagram above, we notice that all four substances we added to the wine had positive results. More specifically, resveratrol, as expected, gives the best rates of antioxidant capacity based on the reference curve and trolox equivalents, followed by the sample with vitamin E, which gives very good results, the next vitamin is A with satisfactory results, and finally, it is vitamin C whose results are also satisfactory.

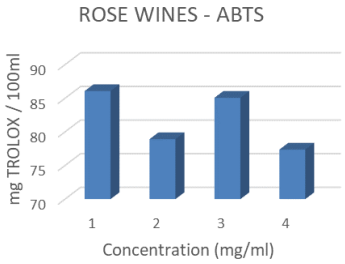

Figure 10. Final results (after 1500 hours) of rose wine with **(1)** resveratrol; **(2)** vitamin C; **(3)** vitamin E; **(4)** vitamin A for the ABTS method.

Based on the diagram above, we notice that all four substances we added to the wine had positive results. More specifically, resveratrol, as expected, gives the best rates of antioxidant capacity based on the reference curve and trolox equivalents, followed by the sample with vitamin E, which gives very good results, the next vitamin is C with satisfactory results, and finally, it is vitamin A whose results are also satisfactory.

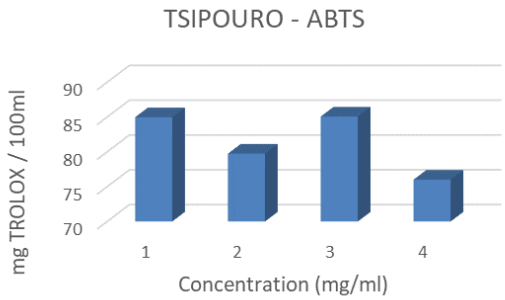

Figure 11. Final results (after 1500 hours) of tsipouro with **(1)** resveratrol; **(2)** vitamin C; **(3)** vitamin E; **(4)** vitamin A for the ABTS method.

Based on the diagram above, we notice that all four substances we added to the tsipouro had positive results. More specifically, resveratrol, as expected, gives the best rates of antioxidant capacity based on the reference curve and trolox equivalents, followed by the sample with vitamin E, which gives very good results, the next vitamin is C with satisfactory results, and finally, it is vitamin A whose results are also satisfactory.

3.3. Calculation of the total phenolic components (Total Phenolic Content, TPC) determined by the FOLIN-CIOCALTEU method.

The estimation of the total phenolic content was done using the Folin-Ciocalteu method (Figures 13,14,15,16). To extract the results, the standard curve is constructed graphically, through which the concentration of the phenolic components of the samples is calculated and expressed in gallic acid equivalents (Gallic Acid Equivalents, GAE) (Figure 12).

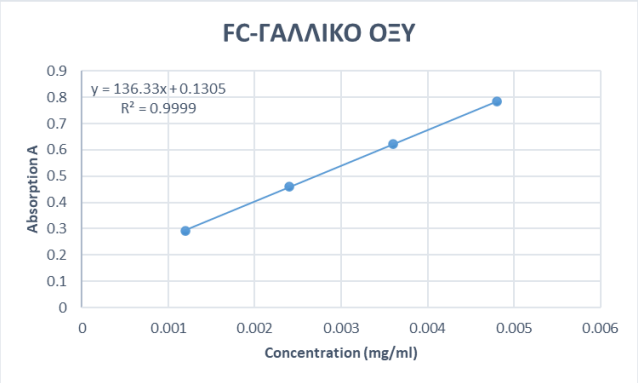

Figure 12. Standard reference curve with gallic acid for the FC method.

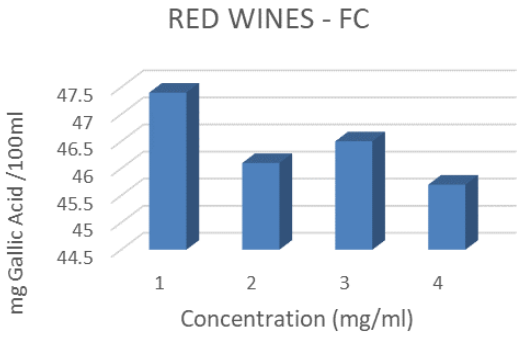

Figure 13. Final results (after 1500 hours) of red wine with **(1)** resveratrol; **(2)** vitamin C; **(3)** vitamin E; **(4)** vitamin A for the FC method.

Based on the diagram above, we notice that all four substances we added to the wine had positive results. More specifically, resveratrol, as expected, gives the best percentages of phenolics based on the reference curve and gallic acid equivalents, followed by the sample with vitamin E, which gives very good results, the next vitamin is C with satisfactory results, and finally, the vitamin A whose results are also satisfactory.

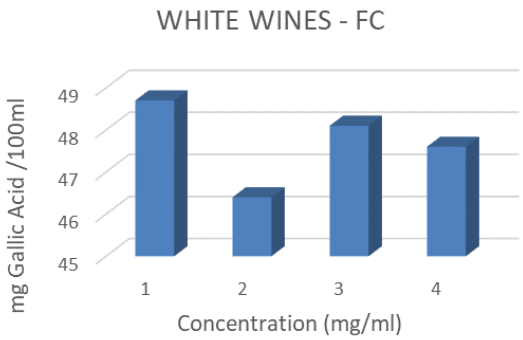

Figure 14. Final results (after 1500 hours) of white wine with **(1)** resveratrol; **(2)** vitamin C; **(3)** vitamin E; **(4)** vitamin A for the FC method.

Based on the diagram above, we notice that all four substances we added to the wine had positive results. More specifically, resveratrol, as expected, gives the best percentages of phenolics based on the reference curve and gallic acid equivalents, followed by the sample with vitamin E, which gives very good results, the next vitamin is A with satisfactory results, and finally, the vitamin C whose results are also satisfactory.

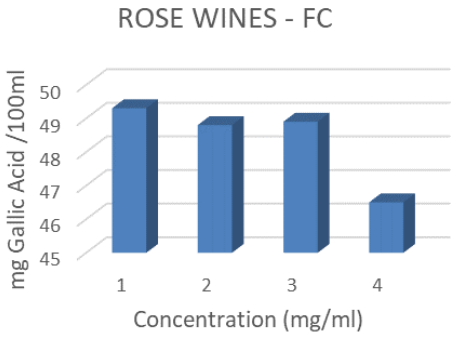

Figure 15. Final results (after 1500 hours) of rose wine with **(1)** resveratrol; **(2)** vitamin C; **(3)** vitamin E; **(4)** vitamin A for the FC method.

Based on the diagram above, we notice that all four substances we added to the wine had positive results. More specifically, resveratrol, as expected, gives the best percentages of phenolics based on the reference curve and gallic acid equivalents, followed by the sample with vitamin E, which gives very good results, the next vitamin is C with satisfactory results, and finally, the vitamin A whose results are also satisfactory.

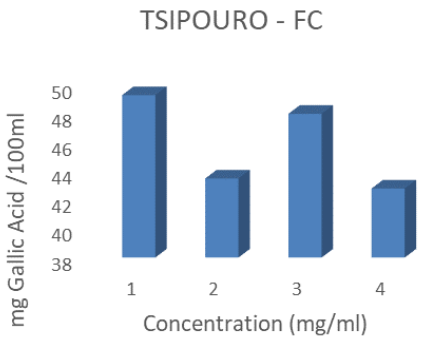

Figure 16. Final results (after 1500 hours) of tsipouro with **(1)** resveratrol; **(2)** vitamin C; **(3)** vitamin E; **(4)** vitamin A for the FC method.

Based on the diagram above, we notice that all four substances we added to the tsipouro had positive results. More specifically, resveratrol, as expected, gives the best percentages of phenolics based on the reference curve and gallic acid equivalents, followed by the sample with vitamin E, which gives very good results, the next vitamin is C with satisfactory results, and finally, the vitamin A whose results are also satisfactory.

3.4. Organoleptic test.

Finally, an organoleptic test was carried out, and it was found that the organoleptic characteristics of the wines and the tsipouro had not suffered any unpleasant effects. The taste, the aroma, and the color have no change.

3.5. Discussion of results(10).

It is observed that resveratrol and vitamin E in all samples of alcoholic beverages and in the two methods of antioxidant capacity DDPI and ABTS, consistently give the highest percentages; this is due to the fact that they are solid antioxidant substances and perhaps stronger than vitamins A and And. Finally, the same is observed for the method of phenolic substances in alcoholic samples. Resveratrol is, again, the substance with the largest amounts, which was expected since it is probably the most important substance in the category of non-flavonoid phenols.

4. Conclusions

The recorded results are the final ones after 1500 hours of experiments. We observe that with both DPPH and ABTS methods, red, white, and rosé wine, as well as tsipouro with the addition of the four substances that are resveratrol, vitamin C, vitamin E, and vitamin A, show us that the increase of their antioxidant capacity has been achieved and with the best percentages as expected in the red wine samples.

In more detail, we see that in relation to the prices of base wine, there are increases in white wine, rosé, and red wine, as well as the four substances that were incorporated. We have more significant increases in darker wines (rosé, red) compared to white wine and tsipouro. You demonstrate the positive effect of the antioxidant components of the four substances included in them.

Total phenolics were measured using the Folin Ciocalteu method. We also observe the increase in phenolics, and we see that the results are the same as those of antioxidants; the highest value is observed in red wines, followed by rosés and then whites and tsipouras. Darker wines also show a more significant increase than white wine and tsipouro.

The correlation of the results for the total phenolics of the wine and its antioxidant capacity is high.

Red wine has the largest percentage increase in all three methods, followed by rosé, white wine, and tsipouro. The best results are shown by the samples with resveratrol, followed by the samples with vitamin C, and finally, we have the samples of vitamin E and vitamin A, which are similar. The results of the DPPH and ABTS methods are identical, which means that the results are correct; the same applies to the results of the Folin Ciocalteu method.

Finally, we should mention that the wines and the tsipouro have not undergone oxidation during the experiment or any other alteration because the experiment was completed in a relatively short time after the integration of the substances and with great care so that they

do not come into contact with the air, protected from light and at a suitable temperature (ambient temperatures).

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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