IMPROVING WINE-MAKING PRODUCTS WITH SELENIUM AND TOTAL POLYPHENOLS

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IULIANA DIANA BARBULESCU1,2, MARIANA FERDES2, CARMEN BEJAN3, ANGHEL RODICA2, RAZVAN TEODORESCU4
1University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Manastur Street 3, Cluj-Napoca, Romania, barbulescudia@yahoo.com
2University Politehnica Bucharest, Splaiul Independentei 313, S6, 060042 Bucharest, Romania
3National Institute for Biotechnology Research and Development in Horticulture Stefanesti, Bucharest – Pitesti Avenue 37, 117715 - Arges, Romania
4University of Agronomical Sciences and Veterinary Medicine of Bucharest, 59, Marasti Ave., Bucharest, 010464 Romania

Corresponding author: Iuliana Diana Barbulescu*, Splaiul Independentei 313, S6, 060042 Bucharest, Romania, phone: + 40-0720198946, e-mail: barbulescudia@yahoo.com

Abstracts

In the final product of the wine-making process it has been observed a correlation between selenium and polyphenolic contents. The bioconversion of inorganic selenium during the wine making process brings a large amount of polyphenols in the final product.

Samples of grapes are originating from different Romanian areas, Hamburg brand and purchased from local and Romanian markets. All the grapes were crushed so that the yeast went into contact with the juice for the batch fermentation. Each obtained fraction was assessed for total polyphenols, flavonoids and anthocyanins content. Under natural fermentation, the yeast strains were able to improve the metabolic potential of the assimilation of polyphenolics compounds, as a consequence to the added selenium to grape wine during the wine making process. Sodium selenite, an inorganic form, was used for the addition of a 10 % solution during the exponential phase, in three portions, of the grape’s juice fermentation for improving the total phenolics for rising the added value of the winemaking bioproducts. It was observed that an addition of 600 ppm sodium selenite during fermentation, due to enhanced of the total phenolics in the final bioproducts of vinification process.

Keywords: total phenolics, selenium, total flavonoids, wine-making

Abbreviations: ROS reactive oxygen species; total phenolics (TP), total flavonoids (TF) anthocyanins (TA), A - total anthocyanin, Recommended Dietary Allowance (RDA), European Food Safety Authority (EFSA), white wine enriched with polyphenols (PEWW)

INTRODUCTION

Phenolics and selenium are new antioxidant compounds. Wines and grapes contain a number of polyphenolic constituents classified as flavonoids and non-flavonoids, which play a major role in enology.

Antioxidants including polyphenolics have been found to protect renal cells from the cellular injury induced by ischemia and reperfusion. Resveratrol, a stilbene polyphenol found in grapes and red wine, has recently been found to protect the isolated rat heart from ischemia reperfusion injury. [Giovannini L., 2001].

In vitro results suggest that resveratrol can protect LDL against oxidative damage resulting from the exposure to various environmental challenges, possibly by acting as a free radical scavenger.
The small dose of resveratrol (4.38 nM) used is attainable with a diet including red wine and vegetables confirming its protective role against some pathological processes such as inflammation, coronary heart disease, and cancer. Resveratrol, a naturally occurring hydroxystilbene identified in over 70 plant species including nuts, grapes, pine trees, certain vines and red wine, is thought to play a role in the prevention of heart disease. Attention was first drawn to resveratrol in 1992 when it was mentioned as a constituent of red wine. Humans have been consuming wine for approximately 7,000 years.

The effects of a white wine enriched with polyphenols (PEWW) from Chardonnay grapes and of a sparkling red wine (SRW) from Pinot Noir and Chardonnay grapes were studied for the first time on early atherosclerosis in hamsters.

Because wine phenolic compounds such as catechin, quercetin, and resveratrol given at nutritional doses mimicking a moderate consumption of two glasses of red wine per meal prevent the development of atherosclerosis through several indirect mechanisms independent of the inhibition of lipid peroxidation.

The use of selected *Saccharomyces* yeasts for wine-making has clear advantages over the traditional spontaneous fermentation. [Matilde Maqueda, 2011]. Yeast strains contribute to the oenological and sensorial characteristics of the wines they produce. Chemical and sensorial analysis were performed on the final wines, which differed depending on the yeast strain used.[ Mar Vilanova, 2005].

The biochemical transformation of flavour-inactive grape juice constituents into flavour-active components has emerged, in recent years, as an important, additional mechanism, whereby yeasts substantially impact on wine aroma and flavour and facilitate greater expression of grape varietal character.

Under appropriate conditions, yeast is capable of producing biomass with high protein content and meanwhile accumulating large amount of trace elements such as selenium, and transforming inorganic selenium (low bioavailability, potentially toxic) into organic form (safer and highly bioactive), mostly in the form of selenomethionine [G. N. Schrauzer, 2000]. Furthermore, selenium yeast has been recognized by the European Food Safety Authority (EFSA); its Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food has given a positive Scientific Opinion for the use of selenium yeast in food and food supplements and concluded that organically bound selenium yeast complying with
specific characteristics is safe to use in food, supplements and food for particular nutritional use (PARNUTS) [F. Aguilar, et al, 2008].

The Recommended Dietary Allowance (RDA) for an adult in USA is 55 μg per day. Selenomethionine (SeMet) is the major selenium-containing protein in cereal grains, corn, rice, and soybeans, which comprises between 45.5% and 86.5% of total selenium. According to the study of Rayman et al., [2000] the selenomethionine concentration ranged from 60% to 84% selenium in ten selenium-enriched yeast samples. Based on his results Yoshie Motomura, [2008], the sprouts of wheat grown with selenium under dark conditions and extracted with ethanol could be considered a potent and functional food ingredient or dietary food supplement for humans and animals because the selenium increased the total polyphenol content and antioxidative activity.

The work presents the accumulation of the phenolics compounds in the wine-making bioproducts as a result of the addition of inorganic selenium during fermentation.

The aim of this experiment was to analyze the PT, PF and PA in wine and powder grape skin and seeds, as an alternative to the known process for producing bioactive compounds as added value to winemaking products.

Material and methods

Process for obtaining added value to wine-making products

The study describes a natural fermentation for obtaining bioproducts based on the improvement of the bioproducts resulted from the winemaking process with phenolic and selenium organic compounds.

Wine-making process – with addition or non-addition of sodium selenite

Supplementation for using selenium-enriched microorganisms has received a lot of attention in the recent years.

1) Fermentation was performed in 2 l Erlenmeyer flasks containing 1 l must/juice of Hamburg grapes. Obtaining the grape juice, the best environment for the growth of wine yeast. The fermentation was effectuated in the presence of the skin and seed’s grape.

2) Fermentation was conducted at 20-22°C for 10-12 days, at static culture.

   All fermentations were performed in triplicate and the results concerning PT, PF and PA.

   As the other authors have reported, the characteristics of wine are influenced by the yeast strains used to ferment must [Mar Vilanova, 2005]

   For red wine production there are no skins removed and the pressing or setting operations aren’t done until after the beginning of the fermentation.

3) Addition of sodium selenite solution during fermentation

   The addition was effectuated in three portions of 200 ppm during the fermentation process. Selenomethionine was found to be the main Se-species in the selenised white wine. [M.T. Pérez-Corona, Y. Madrid, 2011]

   Selenium is an essential microelement in human and animal nutrition, whose intake can be in inorganic (e.g. selenite, selenate) or organic form (e.g. selenomethionine).
The prooxidant effect of inorganic selenium sources in the animal nutrition was found as a great disadvantage. [Ştefan Fujs, 2009]

Under appropriate conditions yeasts are capable of accumulating large amounts of trace elements, such as selenium, and incorporating them into organic compounds. It has been found that introducing water-soluble selenium salt as a component of the culture medium for yeasts produced by conventional batch processing results in a substantial amount of selenium being absorbed by the yeast. To discover the form of organically incorporated selenium in the yeast cells the amino acid content of the selenium-enriched bakers' yeast was determined and compared with normal bakers' yeast. It was expected that the amount of sulphur-containing amino acids (Met, Cys) would change because of the chemical similarity of sulphur and selenium. [A’ . Suhajda,2000]

4) Separation of the fermentated juice from the grapes’ skin and residual biomass by filtration.
5) Separation of the skin and seeds from wine and residual biomass.
6) Centrifugation of the residual biomass from separating the wine from biomass.
7) Obtaining 3 fractions (grape skins and seeds, wine, residual biomass).

The process for obtaining bioproducts enriched with phenolics compounds (figure 2)

Separation of the skin and seed of grapes from the wine was done by vacuum filtration. There were obtained three fractions enriched with phenolic compounds from Hamburg grapes.

- Wine sample enriched with phenolic compounds form (sample) and control
- Powder from grape skin and seeds enriched with phenolic compounds (sample) and control
- Residual biomasses enriched with phenolic compounds (sample) and control

Purification of residual biomass and of the powder from skin and seeds

1. Washing the residual biomass with buffer solution EDTA-Na 0,1 M
   Centrifugation: Effluent 1
   Residual biomass purified with EDTA- Na 0,1 M
2. Washing with buffer solution Na₂HPO₄ (0,01M)
   Centrifugation : Effluent 2
   Residual biomass purified with buffer solution Na₂HPO₄ (0,01M)
   SEVERAL WASHING WITH DISTILED WATER
3. Washing the residual biomasses with distilled water
   Centrifugation : Efuent 3
   Residual biomass purified with distilled water
4. Washing the residual biomasses with distilled water
   Centrifugation: Efluent 4
   Residual biomass purified with distilled water (Cream biomass and cream from skin and seeds).
After the residual biomass was purified, the selenium residual biomass cream was obtained.

Residual biomass (cream) was pasteurized at a temperature of 75-80°C for 45-50 minutes in order to inactivate the microorganisms, after that it was dried until 5% humidity, resulting in the residual biomass enriched in TP (total phenolics).

The study of T. Kaur and M. P. Bansal was designed with the aim to achieve a balance between selenium (Se) incorporation and optimal growth of yeast cells along with the effect of Se enrichment with antioxidant defense status of yeast cells.

Also, the balance between Se incorporation and optimum growth of yeast cells was achieved along with the yeast enhanced anti-oxidative defense status. [T. Kaur 2006].

The powder of skin and seeds were dried at 37-45 °C

**Analytical assay**

**Extraction of solid samples**

Samples were passed through fine mesh sieve (0.5 mm). At 0.100 g sample were added to more than one occasion (up to discoloration of the sample) 80% Et OH acidified with 1% HCl. It was centrifuged 20 min at 6000 rpm and the clear extract was collected in 100 ml flasks. Before carrying out analytical determinations, all samples were centrifuged 20 min at 6000 rpm.
Solid residues from winemaking processes have been subjected to extraction with superheated water–ethanol mixtures. Identification and characterization of the extracted compounds were achieved by spectrophotometry, gas chromatography with either flame-ionization or mass detectors, and high performance liquid chromatography with UV detection. [J. Gonzalez-Rodriguez, 2003].

Many authors have studied the phenolic compounds in grapes and wines using HPLC as the most suitable analytical technique. However, this technique is not available in wineries for routine analyses, whereas spectrophotometric methods, as more affordable techniques with lower expenses, lower reagent consumption and rapid measurements, can be used for wine and grape analyses to follow the changes in the polyphenols contents during grape ripening and their changes during the wine-making process. The most common used methods are methods for the determination of the total phenolic, anthocyanins, flavan-3-ols and flavonoids. Resveratrol (trans-3,4',5-trihydroxystibene) is a phytopolyphenol isolated from the seeds and skins of grapes.

Recent studies indicate that resveratrol can block the process of multistep carcinogenesis, namely, tumor initiation, promotion and progression. Resveratrol can also reduce the risk of cardiovascular disease in man. The molecular mechanisms of resveratrol in chemoprevention of cancer and cardiovascular disease are interesting and under intensive investigation. Reactive oxygen species (ROS) are regarded as having carcinogenic potential and have been associated with tumor promotion. Resveratrol may act as a reactive oxygen species scavenger to suppress tumor development. Meanwhile, efficient endogenous antioxidants, including superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase, are present in tissues. [Lin JK, Tsai SH., 1999].

**Instrumentation and reagents**

Analysis of the polyphenols was performed with a SPEKOL 11 spectrophotometer. The reagent tanic acid and (+) catechin were purchased and the Folin-Ciocâlteu reagent. All the other employed reagents were of analytical grade purity.

The common spectrophotometric method for the determination of the total phenolics content using the Folin-Ciocâlteu reagent has been widely used in the area of oenology and viticulture. This method is based on oxidation–reduction reactions in which phenolic are oxidised and show maximum absorbance in the wavelength region between 725 and 765 nm. In this study, a procedure based on the reported method 20 was used with some modifications based on testing the effects of temperature and time of the reaction between Folin-Ciocâlteu reagent and standard solutions of gallic acid. For the determination of the total flavonoide, a colorimetric method using AlCl₃ was applied for the analysis of the wines and grape extracts. This method is based on the formation of stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols, which exhibit maximum absorbance at 510 nm.

**Total phenolics assay**

The Folin-Ciocâlteu method was used for the determination of the total phenolic. In brief, an aliquot (1 mL) of the appropriate diluted extracts were added to a 10 mL volumetric flask, containing 5 mL of distilled water. Then, 0.5 mL of Folin-Ciocâlteu reagent was added and the contents mixed. After 3 min, 1.5 mL Na₂CO₃ solution of concentration 5 g/L was added and made up to a total volume of 10 mL distilled water. After keeping the samples at 50°C (water bath) for 16 min in sealed flasks and subsequent cooling, their absorbance was read at 765 nm against distilled water as the blank. A calibration curve was constructed using Tanic
acids standard solutions (0–100 mg/L). The concentration of total phenolic is expressed as the Tannic acid equivalent per 1 g of fresh sample. All samples were prepared in triplicate.

Violeta Ivanova, (2010) evaluates the polyphenolic contents of six commercial red and white Macedonian wines and four grape varieties. Spectrophotometric methods were applied for the determination of the total phenolics, the total flavonoids, the total anthocyanins and the total catechins.

The Folin–Ciocalteu method [K. Slinkard, 1977], was used for the determination of the total phenolics.

Resveratrol, and other polyphenols in wine, are thought to have been taken in part into account for the so-called French Paradox, the discovery that the rate of coronary heart disease mortality in France is lower than in other industrialized countries with a similar risk factor profile.

**Total flavonoids assay**

Total flavonoid content was evaluated according to a colorimetric assay with aluminium chloride.[ J. Zhishen, 1999]

A 1 mL aliquot of wine sample or grape extract (appropriately diluted) was added to a 10 mL volumetric flask containing 4 mL of distilled water, followed by the addition of 0.3 mL of solution of NaNO₂ (0.5 g/L). After 5 min, 0.3 mL of a 1 g/L solution of AlCl₃ was added and 6 min later, 2 mL of NaOH (1 mol/L) were added to the mixture. The total volume was made up to 10 mL with distilled water, the solution was mixed and the absorbance was measured at 510 nm against water blank. Catechin was used as the standard for the construction of a calibration curve and the concentrations are expressed as catechin equivalents (mg/g CE).

**Total anthocyanins assay**

The determination of the total anthocyanin was realised by the method proposed by Di Stefano et al. The samples were diluted with a solution consisting in 80/20/1 (v/v/v) ethanol/water/HCl concentrates) and the absorbance was measured at 520 nm.

\[
A = \frac{22.76 \times d \times V}{m} + 0.05
\]

where:

A - total anthocyanin content, mg × g⁻¹

**Results and discussions**

The polyphenolic compounds were mainly located in the grape seeds and skins, whereas the pulp contained a very low concentration of these components. It was observed that the seeds contained the highest contents of total phenolics (TP), total flavonoids (TF) anthocyanins (TA) were mainly located in the skins.

The concentrations of the phenolic families in wines depend not only on the grape variety, but also on additional factors, such as the edaphoclimatic conditions, the enological practices, the storage conditions, etc. During bottle aging of wine, modifications in the polyphenolic composition occurs as a result of different transformations, such as oxidation processes, condensation and polymerization reactions including direct reactions between
anthocyanins and flavanols or reactions between anthocyanins and flavanols through ethyl bridges, whereby stable pigments are formed which stabilizes the wine colour. All these reactions are related to changes in the colour and sensorial characteristics, such as the flavour, bitterness and astringency of the final wine.

**Figure no.3.** Influence of selenium for improving the total phenolics compounds from winemaking bioproducts

**Figure no.4.** Influence of selenium towards improving the flavonoids compounds from winemaking bioproducts
Red and white wines have a different phenolic composition, which is characteristically for each variety. The polyphenolic content of the final wine depends not only on the grape variety, but also on the different winemaking procedures applied for production. Red wine production includes the procedure of maceration, which is not applied for white wine production; white wines are produced without grape mash, having no contact with the grape skins. Therefore, white wines contain lower amounts of polyphenols. Polyphenols encompass several classes of weakly acidic chemicals related to, or built upon the phenyl ring. Polyphenols contain one or more phenolic hydroxyl groups directly attached to these carbon-based aromatic phenyl-ring compounds. These are easily oxidized to quinones by reactive oxygen species, a property that helps account for their free radical scavenging capacity. [Charles M. Benbrook, 2005]

Conclusions

1. The effect of sodium selenite on natural yeast cells during cultivation was studied, in order to improve the contents in phenolics, flavonoids and total anthocyanins as added value of the wine-making products (wine, powder of grape skin and seeds, residual biomass).
2. The addition of sodium selenite 10% solution in three parts by 200 ppm each of them were added during the exponential phase of fermentation.
3. The obtained results increase the level of anthocyanins by increasing the quantities of sodium selenite.

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References


12. KAUR AND M. P. BANSAL , Selenium enrichment and anti-oxidant status in baker’s yeast, Saccharomyces cerevisiae at different sodium selenite concentrations (Nutr Hosp. 21:704-708), 2006;


16. VIOLETA IVANOVA, MARINA STEFOVA AND FABIO CHINNICI, Determination of the polyphenol contents in Macedonian grapes and wines by standardized spectrophotometric methods, J. Serb. Chem. Soc. 75 (1) 45–59, 2010

17. J. ZHISHEN, T. MENGEHENG, W. JIANMING, Food Chem. 64 555, 1999

18. CHARLES M. BENBROOK, Elevating Antioxidant Levels in Food through Organic Farming and Food Processing, An Organic Center State of Science Review, 2005