The importance of selenium from powder of skins and seeds of Italian grapes and wine, resulting from the vinification processes

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Abstract

Samples of grapes are originating from different Italian areas and purchased from local and Romanian markets. During the fermentation, yeasts convert most of the sugar in the grape juice into ethanol. Grapes may be potent against tumors because of their high concentration of tannin.

Selenium enriched yeasts may be a safe source as a diet supplement. *Saccharomyces cerevisiae* is normally present on grapes, often visible as a powdery appearance on the grapes’ surface. The fermentation can be accomplished with this natural yeasts complex.

Powder from grape skins and seeds is a processed by-product resulting from the wine-making process and could become a bio-product with antioxidant action as an important source of selenium and tannins.

The aim of this work was to analyze the synergism of the antioxidant action manifested by selenium enriched powder of skin and seeds of Italian grapes and tannins resulting from the wine-making process.

Keywords: antioxidant action, selenium, tannin

1. Introduction

The potential health benefits of red wine arose from the proposed “French paradox”[1]. Wines from vineyards located in an area with uniform soil composition were classified in 5 geochemical groups [2]. The soil parameters affecting solubility (pH, redox potential, complexing agents) were identified as dominant controls in element uptake. Wines from grapes of the same vineyard were most similar regardless of the vintage, grape variety or winery, suggesting that the wine element fingerprints are relict soil signatures that survive metabolic and winery process [3].

The analysis of the composition of wine demonstrated that it contains over 1000 beneficial substances for the organism.

*Wine*. Many studies have been published on how alcohol consumption may be associated with reduced mortality due to heart disease in some populations [4]. Winemaking or vinification is the production of wine, starting with the selection of grapes or other products ending with bottling the finished wine. Although most wine is made from grapes, it may also be made from other fruits or non-toxic plant materials.
The most common species of vine is *Vitis vinifera*, which includes nearly all the varieties of European origin [5]. One of the main problems encountered using the wild ferments is the non-completion of fermentation, meaning that some sugar remains unfermented. Wine yeast is efficiently assessed by converting sugar into ethanol [6].

Wine production in most countries is based on the use of commercial strains leading to the colonization of the wineries by these strains with the consequent reduction of autochthonous biodiversity [7]. A table grapes variety was created in Italy by professor A. Pirovano in 1991 from crossing Bican and Hamburg Muscat. It is one of the best varieties of table grapes in the world. The grape is big, of a conic-pyramidal shape, with one or two wings; average mass may be 10-12 g; the skin is rather thick and dense and has a beautiful golden-yellow color with a layer of pruin. The pulp is crunchy and succulent with a strong muscat flavor with 1-2 seeds.

**Yeast.** The yeast strain suitable for the wine production should have high fermentability, tolerance to ethanol, good sedimentation properties and no effect on titratable acidity.

Frequently, wild ferments lead to byproducts of unpleasant acetic (vinegar) flavor. Wines are nutritional drinks unlike distilled liquors. In recent studies, the specific health benefits to the consumption of wine, especially in the prevention of coronary hearth disease (CHD), were established.

**Saccharomyces cerevisiae** strains contribute to enological and sensorial characteristics of the wine; different yeast strains influence the physical-chemical characteristics of wines to a variable extent. Yeast cells need some nutrients for their development; many of these are trace elements that present a stimulating action for the yeasts growth. Some elements, at trace level, are essential for the growth of the grapes and the production of wine. The quality of the grapes determines the quality of the wine. Grapes’ quality is affected by the variety as well as the weather during the growth season, soil minerals and acidity [8].

**Polyphenols.** Red wines contain a range of water – soluble polyphenols that include phenolic acids, the resveratrol, the flavonols, procyanidins, and anthocyanins. Red wine has more antioxidant capacity than white wine due its phenolic content.

It is known that natural polyphenols posses such physical and chemical properties that contribute to a proper and efficient protection from oxidation of important biomolecules such as lipids, proteins, and nucleic acids.

Nutrition agents such as Vitamin E, selenium, vitamin D green tea and wine polyphenols have been found to inhibit proliferation or to induce apoptosis in some prostate cancer cell lines.

**Anthocyanins and tannins.** Many wines benefit from the addition of tannins, provided that the treatment is carried out at the most appropriate time. Since the different origins and proprieties of tannin can produce substantially different origins and proprieties of tannin can produced substantially different results, care must be taken to select the best tannin for each winemaking application.

Anthocyanins are guard systems of plants and protect them from UV damage. They form complex molecules with other phenolic molecules and strongly contribute to the colour and the aging of wine. Wines contain phenolic compounds that may be useful for preventing lipid oxidation as dietary antioxidants [9].

Anthocyanins contribute little to the taste of wine. Because anthocyanins readily polymerize with tannins, they play an important role in tannin retention and in aging.

It is known that the extraction of anthocyanins and tannins from red grapes takes place during fermentation on skins, and that the extent of extraction and the state of combination of these compounds determines the colour, structure and aging potential of red wines.

It was proposed that extraction of the anthocyanins, which are located exclusively in skin cell vacuoles, is due to degradation of the proteinaceous portions of cellular membranes caused by sulphur dioxide addition. Tannins found in vacuoles may be attached to the vacuolar membranes and to the polysaccharides of the cell wall.

It is possible that pectinolytic enzymes might favour the extractability of upper-middle molecular weight tannins, thus improving the structure of the wine.

**Selenium.** More than 30 years ago, Frost predicted that selenium will challenge the advances in biochemistry and nutrition [10]. Selenium, as a trace element, is known to be essential for life in nutrition but it is toxic at levels above certain limits.
The AFC recommends the use of L-selenomethionine up to 250 µg/day as a source for selenium in food supplements, and it would not be of safety concern in adults if used at levels up (supplying up to 100 µg selenium/day) [11].

Selenium supplementation will be beneficial for people with lower dietary selenium intake and for people with iodine deficiency. *Saccharomyces cerevisiae* enriched in selenium (selenium yeasts) accumulates from 1,200 to 1,400 mg/g DM in yeast material when the culture medium is supplemented with 30 mg/mL selenite added during growth [12]. Korhola et al. (1986), in their study on *S. cerevisiae*, proved that the yeast accumulates selenium at the rate of 500 mg Se/g DM [13].

*Saccharomyces cerevisiae* has a high protein convert, which is related to the ability of incorporating Se. SeY can be consumed as a nutritional supplement.

**Antioxidant action.** The polyphenolic compounds of wine are a source of dietary antioxidants and a possible link between a moderate intake of wine and the low incidence of arterosclerosis, cancer, neurodegenerative and heart disease.

The aromatic fraction of wines is composed by a wide variety of compounds with different aromatic properties and antioxidant power. Some of these compounds are already present in the musts, others are modified during the vinification process, and finally, others are produced during the fermentative process by yeast activity.

The study describes a natural fermentation for obtaining a bioactive compounds powder from grape skin and seeds. Powder from grape skin and seed enriched with selenium and with high content in polyphenols proved a high antioxidant action.

### 2. Materials and Method

**Obtainment of bioactive compounds based on powder of skin and seeds from Italian grapes.** Samples of grapes were harvested from the Italian region, from Romanian markets for the Hamburg wine brand production. During the fermentation, yeasts convert most of the sugar in the grape juice into ethanol.

**The bioprocess consisted in:**
- Winery process – with addition or non-addition of selenium salts;
- The pressed grapes were distributed with the juice in Erlenmeyer flasks (1000 ml must/juice in 1500 ml flasks) and were incubated at 18-24°C in Gerhardt incubator, static culture.
- At the end of the fermentation the juice was separated from the grapes’ skin by filtration.
- Processing the fermented medium
- Separation of the skin and seeds from wine and residual biomass,
- Obtaining 3 fractions (grapes skin and seeds, wine, residual biomass)
- Isolating of natural yeast of *Saccharomyces cerevisiae* who tolerated a high concentration of selenium
- For the preparation of culture media the grapes were crushed to a juicy form and allowed to stay in contact with the skins for 96-148 h in order to be used by the natural occurring yeasts for fermentation.

**The variants taken in study were:** Control culture – M – juice from wine and crushed grapes without selenium addition; P - juice and crushed grapes with 0,5 ml sodium selenite 10% addition at 48-72 hours of cultivation. Were study three variants for each of them

**Processing the winemaking bio products (figure 1).** Separation of the skin and seed of the grapes from the wine was done by filtration. There result three fractions.

1. Wine sample (F1) enriched with selenium and control in parallel;
2. Powder from grape skin and seeds enriched with selenium (F2) and control in parallel Residual biomasses enriched with selenium (F3) and control in parallel.

Our paper was focused on the determination of antioxidant action. At the end of the process, the obtained fractions were tested for their antioxidant activity.

The antioxidant activity was measured by Bradolini for wine from Basilicata region. The antioxidants was measured by photochemiluminescence [14].
Once the enriched residual biomass was separated by filtration, it was washed several times in order to remove the unprocessed extra cellular metallic residues [15]. After the residual biomass was purified, the selenium residual biomass cream was obtained. Yeast 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 selenium.

Antioxidant activity has been used in studies aiming to clarify the dominant factors controlling elements uptake by grapevine and the relationship between wine composition vineyard, and wine color.

Antioxidant action of wines is strictly related to the amount of phenolic compounds and selenium in wine and powder.

The antioxidant activity of methanol solutions of samples was calculated by using the relation [16]:

$$\% AA = \frac{I_0 - I_s}{I_0} \times 100$$

where: $I_0$ = the maximum CL for standard at $t = 5s$; $I_s$ = the maximum CL for sample at $t = 5s$. 

**Figure 1.** Biotechnological process for obtaining bioactive compounds with antioxidant action
3. Results and Discussion

After the fermentation of the grape juice we compared the samples which suffered the treatment with selenium with the control culture, regarding the wine and powder of grape skin and seeds by antioxidant action.

When we supplemented with 0,5 ml sodium selenite 10% during the exponential growth phase, the antioxidant action dried selenium powder of grape skin and seed.

Sodium selenite was added at different stages of yeast development such as during the growth phase influenced the color of the wine through the high content on tannins from wines.

The aim of this experiment was to analyze the antioxidant action in wine and powder grape skin and seeds, as an alternative to the known process for obtaining antioxidant bioproducts for producing at large scale of bioactive compounds based on by-products from wine making products enriched with selenium.

The effect of sodium selenite on natural yeast cells during cultivation was studied, in order to obtain a selenium enriched wine, powder of grape skin and seeds, residual biomass with a high antioxidant activity. We analyzed the antioxidant action from the supernatant (wine samples- 50μL) - Figure 2.

![Figure 2. a) Antioxidant action from wines sample (F1)](image)

![Figure 2. b) Chemoluminosecent time variation effect for wine sample and from powder of grape skin and seeds](image)

![Figure 3. a) Antioxidant action from powder of grapes skin and seed (F2)](image)

![Figure 3. b) Chemoluminosecent time variation effect for from powder of grapes skin and seed](image)
Antioxidant compounds in wine are mainly hydrophilic and their antioxidant activity could be well evaluated by the DMPD method [17]. Powder from grapes seed and skin has proved a certain antioxidant activity, higher than 90%.

The wine enriched with selenium presented a high antioxidant activity comparative with control sample, respectively 94.47%.

4. Conclusions
We suggest these laboratory trials be designed for scaling up to industry bioprocess in wine factories for the recovery of the grape skin and seeds and residual biomass for enriched yeast with minerals as a natural source in food supplements. This study can be an economic alternative to selenium enriched yeast produced usually in biotechnologies factories, with uptake of energy and raw materials.

Bio-products based on grapes and grapes were produced by the wine juice during fermentation. The process is efficient from the biotechnological point of view and economic at the same time because the powder of grape skin and seed is a byproduct and proved a high antioxidante action. The obtained results increase in the level of anthocyanins with increasing of intracellular selenium concentration. Rezultatele obtinate cresc nivelul de antociani odata cu cresterea concentratiei intracellularare de selenium. Selenised white wine can be considered an adequate source of SeMet, species of recognised healthy properties, in the diet [18].

Powder of grape skin and seed is a byproduct and could become a bio-product with potential use as an important source selenium and tannins for the people with these certain mineral deficiencies, with lower imunitar system, playing an important role human health safety. The bioactive compounds proved a antioxidant activity, for wine sample by 97% and for powder from grapes skin and seeds more than 91%.

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