PERIODICO di MINERALOGIA Volume 90, No. 3, 2021

The effect of adding natural polyphenolic compounds on yeast fermentation and wine quality

MIHAELA DĂNĂILĂ*, VALENTIN. I. POPA, IRINA VOLF
**"Technical University "Gh. Asachi", Jassy, Bd. Mangeron 71, 700050, Department of Natural and Synthetic Polymers,*

Abstract

The aim of this study was to evaluate the capacity of natural polyphenolic compounds to affect the yeast fermentative metabolism. Depending the used dosage, the polyphenolic compounds obtained from the grape seeds can influence the alcoholic fermentative process and therefore the quality of the obtained wines.

Keywords*:* yeast, polyphenols, fermentation, wine.

Introduction

In the complex process of winemaking, a major role is played by type and quantity of the polyphenolic compounds. The anthocyanins, the flavonoids, the cathechins as well as other polyphenolic substances contribute to the wine's sensorial characteristics, especially bitterness and color. In addition, the moderate wine consumption, a phenomenon that initially was known as the "French paradox", induces endothelial nitric-oxide-dependent vasorelaxation, inhibits oxidation of human low-density lipoproteins and platelet aggregation, effects that are associated with a lower incidence of cardiovascular diseases and the antioxidant and anti-inflammatory activities [2, 3, 4]. The quantity of polyphenolic compounds varies in large limits depending to source. For example, in the white wines polyphenol content varies in the range 200 to 300 mg/L, while in the red wines the quantity varies from 1000 to 4000 mg/L, due to the grape variety, maceration temperature, technological process, etc.

A series of vinification experiments were done in order to evaluate the addition of supplementary quantities from the polyphenolic compounds during the fermentation process. The goal was to determine some specific parameters from the alcoholic fermentative process [5] and to analyze the characteristics of the obtained wines.

Material and methods

Plant Extract: Grape seeds (Statiunea de Cercetare Dezvoltare pentru Viticultura si Vinificatie Iasi) from the *Chambourcin* type were separated from the marc, treated with $KMnO_4$, dried and sorted at dimension between 1-2 mm. The analysis of seeds was done by ISTA methods and according to this protocols the moisture content was establish to 8.51%, physical purity 99.5% and genetic purity 100% [6]. The seeds before extraction were ground resulting a brown-red powder. This powder was extracted in a device for continues extraction (Soxhlet) with ethyl- ether to degrease of vegetal material. The obtained residue treated with ethanol for the extraction of polyphenolic compounds, at the optimum ratio between the vegetal material and the solvent 1:3.5 and the extraction was conducted until the complete extraction of vegetal material.

Microorganisms: Two strains of *Saccharomyces sp.* from Statiunea de Cercetare Dezvoltare pentru Viticultura si Vinificatie Iasi collection were used. They belonging to the physiological strains *ellipsoideus* (A2B) and *oviformis* (7.2.), maintained on YPG medium and grape juice medium.

The yeast were cultivated on grape juice agar medium at 25°C for 72 hours, then on 100 mL grape juice medium for 48-72 hours at 25°C. At the end of this period the number of yeast cells/mL medium was determinate. The yeast inoculum density was established at $3{,}5x10^7$ cells/mL, and this was inoculated, in a ratio 1/100, in recipients with grape juice.

***Culture medium for fermentation trials*:** For the fermentative process the sample of juice from grapes utilized had the following characteristics: cultivar *Feteasca regala*, pH= 3.7, sugar content 192 (g/L), total acidity 3,0 (g/L tartric acid), total polyphenols 737.5 (mg/L eq. tannic acid), non- tannins polyphenols 525 (mg/L eq. tannic acid), total SO_2 98.0 (mg/L), free SO_2 6,0 (mg/L), citric acid 0.22 (mg/L), tartric acid 0.025 (mg/L), temperature (°C) 19.7.

Fermentation trials: Fermentation trials were carried out on 22 glass recipients (each with 3L capacity) that contained 2380 mL grape juice and 23.8 mL inoculum. The concentrations of polyphenols used in this study were 10; 20; 25; 30; 35; 70; 140; 740; 1240 and 4240 mg/L vegetal extract. As control, it was used a recipient with no extract added. The study was considered finished when the alcoholic process stopped.

Analysis and determinations: The total polyphenolic compounds were determined using the Folin-Ciocalteu method. Their quantitative expression was made using a standard curve with tannic acid (figure 1), where the polyphenols were quantified as mg. equivalent tannic acid/L.

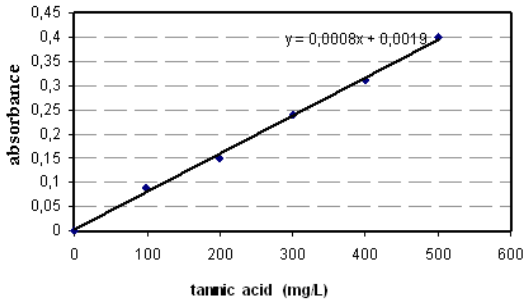

Figure 1. The standard curve with tannic acid

Concomitant to the determination of the total polyphenols, the non-tannin polyphenols were quantified, largely using the same procedure. The only difference was the use of a probe reaction using a solution of methyl – cellulose 0.4%, followed by the Folin-Ciocalteu reactive reaction. A Carl-Zeiss spectrophotometer at 420 nm wavelength was used in order to determine the color intensity from the wine probe. At the end of the alcoholic fermentation, the yeast biomass and dried substance were quantified. The yeast biomass was determined using a centrifuge at 3500 rpm, for 20 min. Later, the yeast biomass dried at a temperature of 105^0 C, until it reached constant mass and then used for dry substance determination.

Results and discussion

It's known that the yeasts are among the main reasons for the reduction of the polyphenolic compounds level in wines during the fermentative process. Among the possible mechanisms, there can be mentioned the physical ones (that involve the establishment of weak and reversible interactions between the polyphenolic compounds and the yeast wall), the chemical ones (by co-pigmentation and condensation reactions between the yeast metabolic products and different polyphenols classes) and/or enzymatic ones (action of antocyanin-β-D-glucoside type enzymes) [7].

During this study, if no extract added in the fermentation medium, the evolution of the polyphenolic compounds was not dependent on the yeast strain used, due to the quantity of the total polyphenolic compounds (737,5 mg eq. tannic acid/L) and non-tannins polyphenolic compounds (525 mg eq. tannic acid/L) in the grape juice. Because of the fermentative process, in both control samples, the quantity of polyphenolic compounds was identical, equal to 418 mg eq. tannic acid /L for the total polyphenolic compounds and 375 mg eq. tannic acid /L for the non-tannins polyphenolic compounds. In comparison to the control sample, the level for the total and non-tannins polyphenolic compounds is higher in all samples containing vegetal extract. Furthermore, the profile of polyphenols is uniform (figures 2 and 3).

In the case of adding in the fermentative media a variable quantity of vegetal extract, the behaviour of the two strains is different, the *Saccharomyces oviformis (7.2.)* strain being different by the (A_2B) strain by a lower level of the polyphenolic compounds. The highest concentration in polyphenolic compounds 1000 mg/L for the *Saccharomyces oviformis (7.2.)* (160% higher than the control sample) and 1025 mg/L for the *Saccharomyces ellipsoideus (A_2B)* strain (170% higher than the control sample) is obtained in both strains by adding 4240 mg/L vegetal extract.

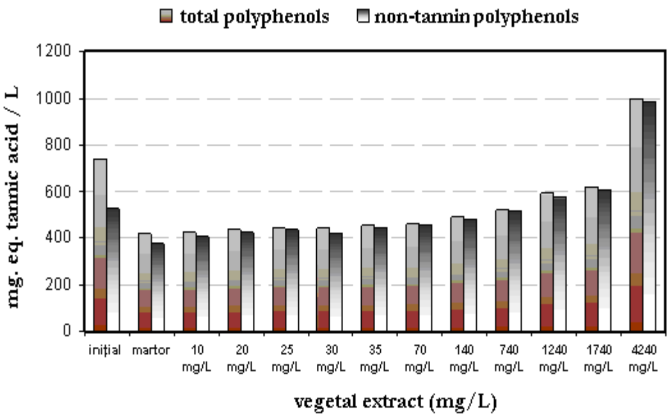

Figure 2. The polyphenolic profile of wines obtained with yeast Saccharomyces oviformis (7.2.)

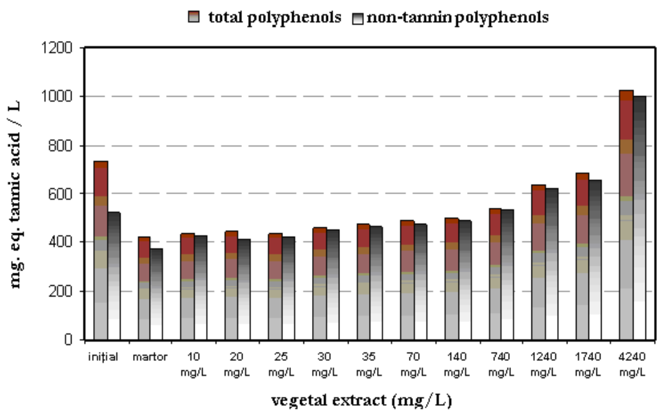

Figure 3. The polyphenolic profile of wines obtained with yeast Saccharomyces ellipsoideus (A_2B)

As for the level of the polyphenolic compounds, it is possible to use them partially as a carbon energy source by the yeasts, as shown by the evolution of both mass quantity and yeast dry substance.

The *Saccharomyces oviformis (7.2.)* strain is different from *Saccharomyces ellipsoideus* (A_2B) by the higher quantity of biomass and the obtained dry substance, which depends on the extract quantity added. The biomass quantity grows proportionally with the added extract quantity (10-35 mg/L extract). Beyond a 70 mg/L extract addition, the biomass quantity shows a descendant evolution).

The behaviour of the *Saccharomyces ellipsoideus* (A_2B) strain is similar ascendant at 10-35 mg /L and descendant over 70 mg/L (figure 4 and 5).

The difference is that for this strain the obtained values are not significantly different as a response to the quantity of extract used for adding in the media (figure 6 and 7).

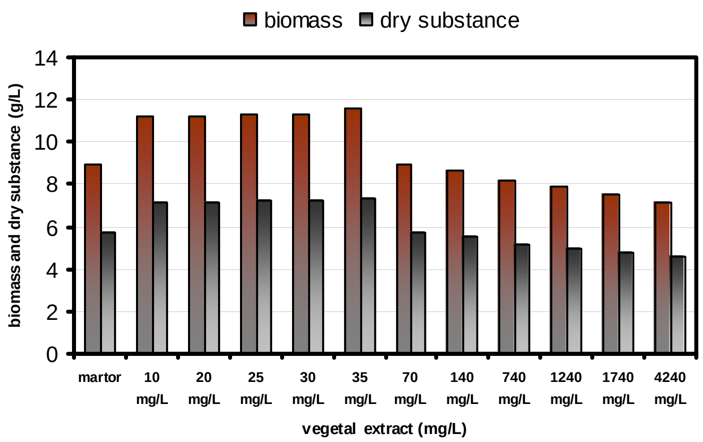

Figure 4. Saccharomyces oviformis (7.2.). The biomass and dry substance accumulation under the influence of natural polyphenolic compounds

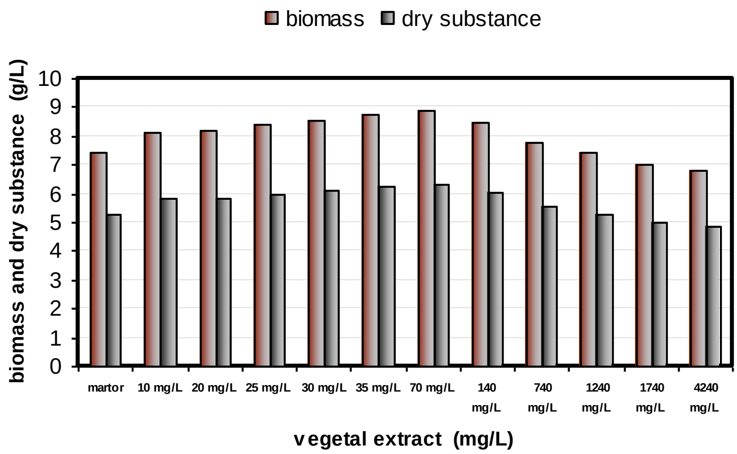

Figure 5. Saccharomyces ellipsoideus (A_2B). The biomass and dry substance accumulation under the influence of natural polyphenolic compounds

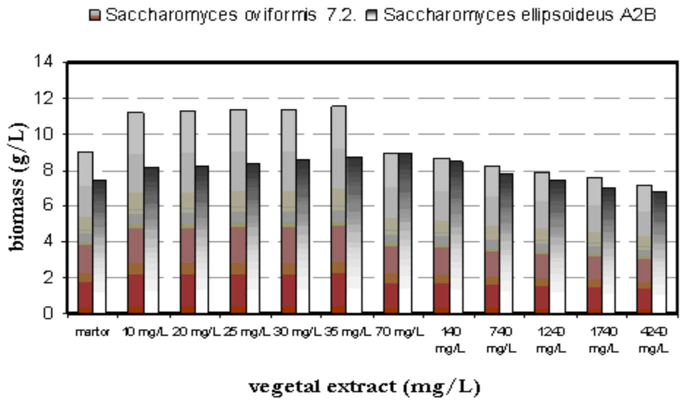

Figure 6. Comparative analyses of yeast biomass

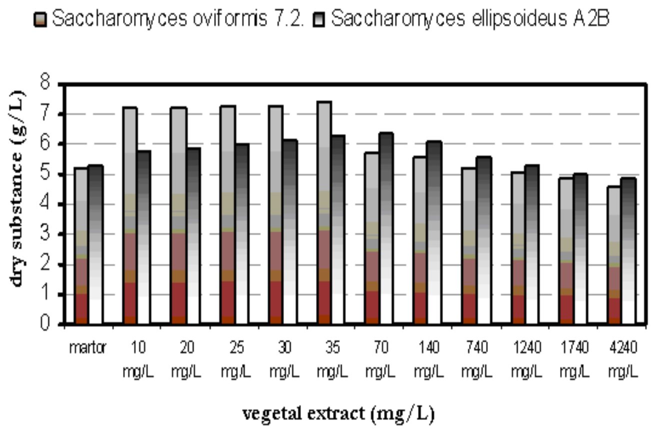

Figure 7. Comparative analyses of yeast dry substance

The pH and temperature in dynamic study, which can provide data regarding the nutritive substrate metabolism, have pointed different values, depending on the yeast strain and extract quantity. The experimental data shows an intense metabolic activity for the *Saccharomyces oviformis (7.2.)* strain, while the *Saccharomyces ellipsoideus* (A_2B) strain proves a lower adaptive capacity in a media that was added polyphenolic extract. The reduction of pH and the temperature level for quantities from 10 to 70 mg/L, depending on the extract quantity used, sustain the existing data on the polyphenolic compounds and the accumulation of higher quantities of biomass. The inhibitor effect grape seed extract on yeasts showed through slight increase of the pH and temperature decrease, for the samples containing from 140 to 4240 mg/L vegetal extract.

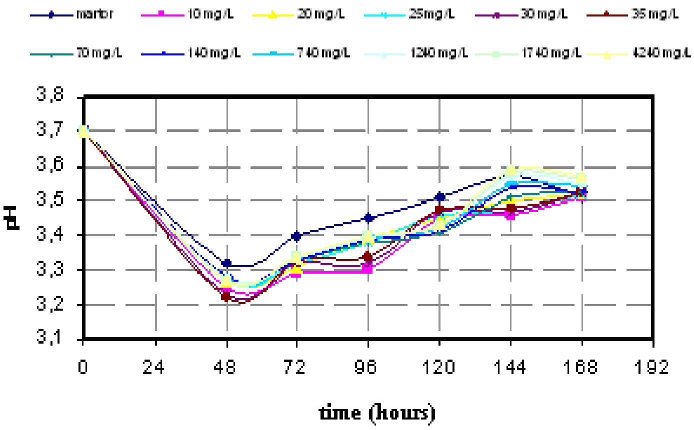

Figure 8. pH evolution in grape juice inoculated with *S. oviformis (7.2.)*

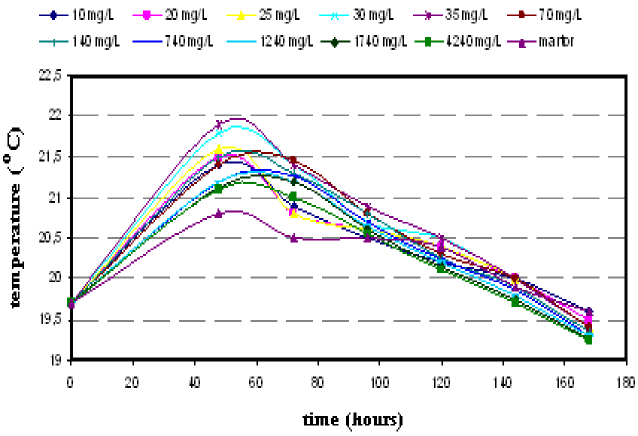

Figure 9. Temperature evolution in grape juice inoculated with *S. oviformis (7.2.)*

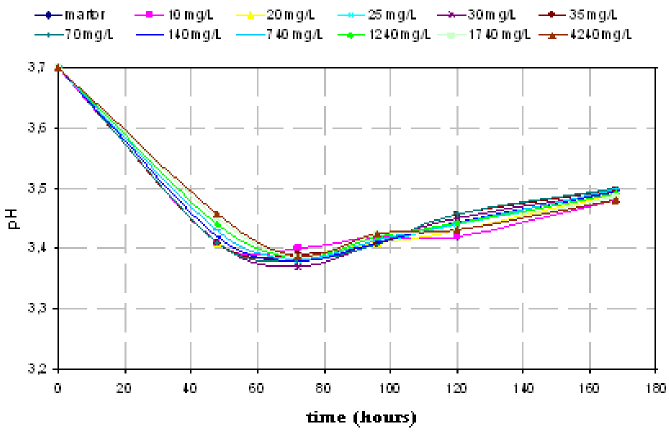

Figure 10. pH evolution in grape juice inoculated with *S. ellipsoideus (A_2B)*

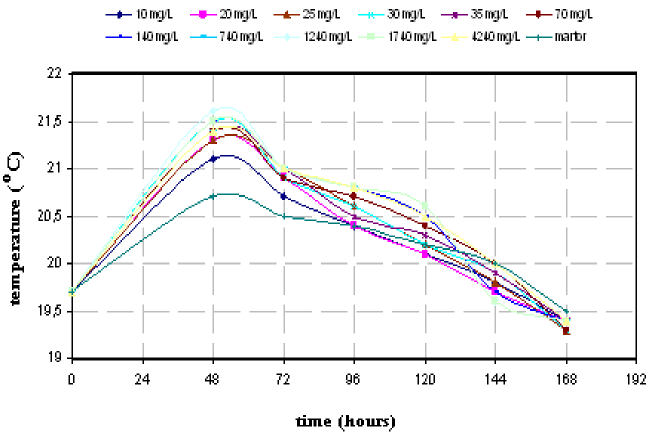

Figure 11. Temperature evolution in grape juice inoculated with *S. ellipsoideus*(A_2B)

Because the polyphenolic compounds plays a major role in wine color formation and stability, the colorant intensity is strictly dependent on the polyphenolic compound's level that settled at the end of the fermentative process. For the wines obtained with the use of both strains, in case of adding 10 to 30 mg/L vegetal extract in the grape juice, the color intensity was close to the control sample, at a difference of only 10%. For quantities, exceeding 30 mg/L the color intensity was superior to control sample. Also, the color intensity was different in the case of the two yeast strains, *Saccharomyces ellipsoideus* (A_2B) strain showing an increase up to 10% in case of the maximum extract dose, in comparison to the same dose used in the *Saccharomyces oviformis (7.2.)* strain (figure 12 and 13).

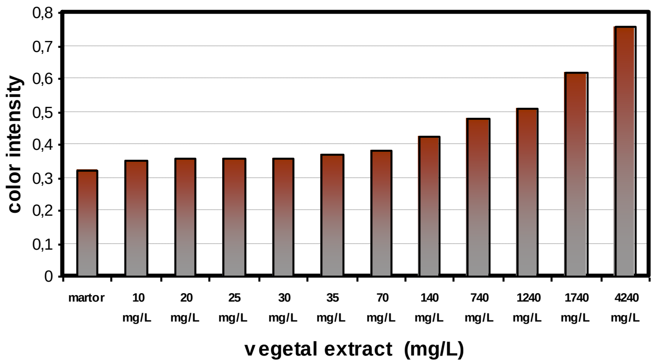

Figure 12. Color intensity for wines obtained with yeast *S. oviformis (7.2)*

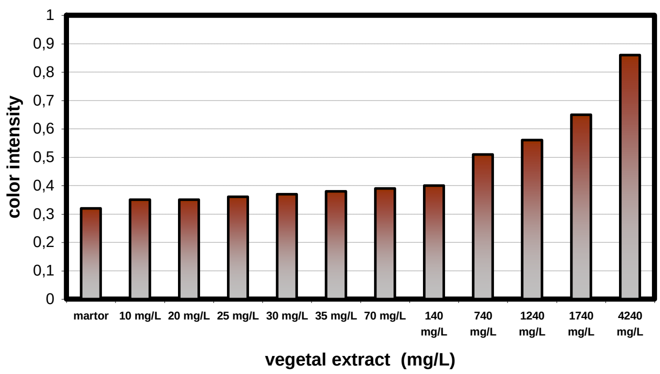

Figure 13. Color intensity for wines obtained with yeast *S. ellipsoideus* (A_2B)

Conclusions

Depending on the used dosage, the polyphenolic compounds obtained from the grape seeds can influence the alcoholic fermentative process and therefore the quality of the obtained wines, by promoting the accumulation of higher quantities of total and non-tannins polyphenolic compounds and modifying their sensorial and organoleptic properties.

In addition, a high quantity of biomass formed by the use of the vegetal extract as a carbon and energy source by the yeasts is a fact that shown also by both pH and temperature in dynamic evolution.

References

1. J.E. KINSELLA, E. FRANKEL, B. GERMAN, J. KANNER, Possible mechanisms for the protective role of antioxidants in wine and plant food - *Food Technology*, 85-89 (1993).
2. E.M. CONNER, M.B. GRISHA., Inflammation, free radicals, and antioxidants - *Nutrition*, **12**, 274-277 (1996).
3. C.A. RICE-EVANS, N.J. MILLER, G. PAGANGA, Antioxidant properties of phenolic compounds, *Trends in plan science,* **2,** 152-159 (1997).
4. E.N. FRANKEL, AL.WATERHOUSE, PL. TEISSEDRE, Principal phenolic phytochemicals in selected Californian wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins, *J Agric Food Chem*, **43**, 890-894 (1997).
5. V.I. POPA, MIHAELA DANAILA, IRINA VOLF, Natural polyphenolic compounds – modulators of yeast fermentative activities, *Buletinul Institutului Politehnic din Iasi*, In press.
6. ISTA ORG., http://www.seedtest.org..
7. M. CARIDI, Influence of Yeast on Polyphenols in Wine, *Food Technol. Biotechnol.***, 42** (1), 37–40 (2004).