DEVELOPMENT OF *RHODOTORULA* YEAST STRAIN UNDER THE INFLUENCE OF POLYPHENOLIC COMPOUNDS IN THE PRESENCE OF COPPER IONS

DEZVOLTAREA TULPINEI DE DROJDIE *RHODOTORULA* SUB INFLUENŢA COMPUŞILOR POLIFENOLICI ŞI A IONILOR DE CUPRU

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Abstract. *The paper presents the results of a study on the influence of polyphenolic compounds in combination with free metal ions on the growth, development and biosynthesis of specific pigments by Rhodotorula spp. yeast strain. For this purpose, the aqueous extract obtained from Vitis vinifera (Merlot) was characterized from the point of view of the total and individual phenolic content. We added in the aqueous extract different concentrations of copper ions, the solution obtained was used for preparation of culture medium for Rhodotorula yeast in a fermentative process. Under these circumstances, we monitored the effect of polyphenolic aqueous extracts on the amount of wet biomass and the biosynthesis of carotenoid pigments. Thus, it was noticed that the same metal concentration in the extract might have a stimulating effect on the amount of wet biomass while an inhibitory effect was registered on the carotenoid pigments biosynthesis.*

Key words: *Vitis vinifera* seeds, *Rhodotorula* spp., polyphenols, carotenoid pigments, copper ions

Rezumat. *În lucrare sunt prezentate rezultatele unui studiu privind influenţa compuşilor polifenolici în asociere cu ioni metalici liberi, asupra creşterii, dezvoltării şi biosintezei de pigmenţi specifici a unor tulpini de drojdie Rhodotorula sp. În acest scop s-au preparat extracte apoase din seminţe de Vitis vinifera, (soiul Merlot), care au fost caracterizate din punct de vedere al conţinutului total şi individual de polifenoli. Aceste soluţii apoase în care s-au introdus diferite concentraţii de ioni de cupru, au fost folosite pentru prepararea mediului de cultură necesar cultivarii tulpinii de drojdie Rhodotorula sp. În aceste condiţii s-a urmărit efectul extractelor apoase polifenolice si a ionilor de cupru asupra cantităţii de biomasă umedă şi asupra biosintezei de β-caroten. Astfel, s-a observat că aceeaşi concentraţie a metalului în extract poate avea un efect de stimulare asupra randamentului în biomasa umedă şi un efect de inhibare asupra biosintezei pigmenţiloi carotenoizi*

Cuvinte cheie: seminţe de *Vitis vinifera*, *Rodotorula* sp., polifenoli, pigmenţi carotenoizi, ioni de cupru.

INTRODUCTION

Polyphenolic compounds are some of the most common classes of secondary metabolites present in plants, generally known and appreciated because of

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strong antioxidant properties that prevent free radical formation (Kong J. et al., 2003). In recent years, natural antioxidants have aroused particular interest because of potential applications in industry as food and pharmaceuticals additives and because of multiple beneficial effects in treating and preventing various diseases (Bleve M. et al., 2008; Caridi D. et al., 2007).

Due to the character of their biological activity, natural polyphenols are compounds essential to stimulating plant growth and development as well as of some species of microorganisms. The ability to stimulate or inhibit the development of plants and microorganisms is closely correlated with the concentrations of polyphenolic compounds applied. Thus, in some cases, the presence of these compounds in low concentrations may have beneficial effects on the development of microorganisms; in cases when concentrations are higher, there is a phenomenon of inhibition (Popa V.I. et al., 2007).

Another important property of these compounds is the ability to complex with heavy metals. On the other hand, free metal ions are the most important chemicals used in the metabolism of microorganisms like microelements (Bartacek J. et al., 2008).

In addition, yeasts are well known for their potential to remove heavy metal cations from aqueous solutions. Recently, it was reported that yeasts of the genus *Rhodotorula* spp. are resistant to heavy metals and can play an important role in their mineral cycle (Li Z. et al., 2006). Their cell wall is composed of mannan, chitin, traces of glucans and glucoproteins. The destruction of this wall by alkali treatment leads to a considerable decrease in the adsorption of heavy metals, suggesting that the yeast cell wall is responsible for biosorption of metal ions.

By using hot water extraction of plant materials the inorganic salts, oligosaccharides, sugars and polyphenols are removed (P. Chow et al., 2008), and extracts can be successfully used as a carbon source in fermentation processes. To this end, we tested aqueous extracts of *Vitis vinifera* seed in combination with Cu^{2+} ions in different concentrations in the cultivation of a strain of yeast *Rhodotorula* sp. The effect of these extracts and ions copper on wet biomass yield, the consumption of polyphenolic compounds in the extract and retaining existing copper ions in the culture medium were followed.

MATERIAL AND METHOD

The extraction of polyphenolic compounds was performed using 20 g of dry material and 125 mL distilled water. Successive extractions were performed in the same conditions (70°C, 45min) until a colorless extract was obtained. The resulting solution was filtered and brought to 500 mL in a calibrated flask. In order to identify the polyphenolic compounds we used as high performance liquid chromatography analytical method. Before to the HPLC analysis the extract obtained was concentrated and subjected to purification steps carried out by liquid-liquid extraction with ethyl acetate. Chromatographic analysis was performed on a HPLC Dionex UltiMate 3000 system equipped with a UV-VIS PDA detector. Separation was achieved on a Dionex Acclaim 120, C18 RP (4.6x150 mm, particle size 5 µm) column and the temperature was maintained at 30 °C±1. The flow rate was 0.5 mL/min. The mobile phase used was 1% acetic acid in water (A) versus 1% acetic acid in methanol (B) for a total running time

of 30 min, and the gradient changed as follows: solvent B started at 10% and increased immediately to 40% in 30 min. The determination of total polyphenols, tannins, flavonoids, flavonols and anthocyanins was also achieved in the concentrated extract (Hainal A.R. et al., 2010a).

For cultivation of yeast strain and carotenoid pigment extraction and analysis were used procedures described in a previous paper (Hainal A.R. et al., 2010b).

RESULTS AND DISCUSSIONS

In table 1 the total concentrations of polyphenolic compounds, tannins, flavonols, flavonoids and anthocyans expressed in mg/100 g dry plant material are shown.

Table 1

Total amount of phenols, tannins, flavonoids, flavonols and antocyanins for concentrated extracts

Aqueous extracts:	Total phenols mg /100g GAE	Taninns mg /100g GAE	Flavonoids mg /100g RE	Flavonols mg /100g RE	Antocyanins (mg/100g RE)
Vitis vinifera seeds	506.25	198.38	27.73	7.11	18.52

The identification and quantification of polyphenols existing in *Vitis vinifera* seed extract was done according to the calibration straight lines obtained for a series of standard polyphenols. Chromatographic profile of standards used is that of fig.1 and the chromatogram recorded for the plant extract is represented in fig.2.

The main compounds identified in the extract obtained from *Vitis vinifera* seeds were gallic acid (6.12 mg/100g plant material) and catechin (44.36 mg/100g plant material). We can also see the presence of another major element to Tr = 9.8 which according to literature (Maier T.et al., 2009, Montealegre et al., 2009) can be attributed to epicatechine.

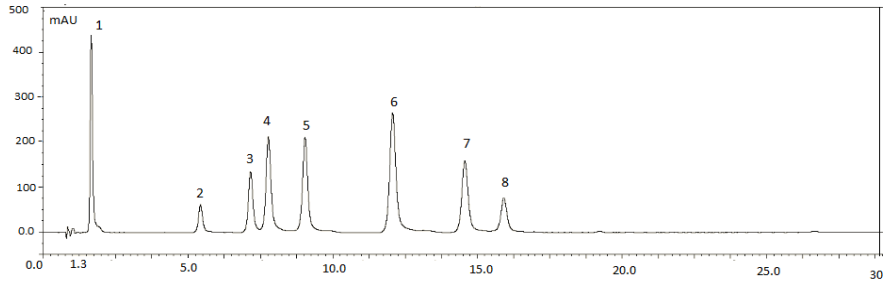

Fig. 1 - Typical chromatogram at 280 nm obtained for polyphenol standards. Identified compounds of peaks 1–8 are gallic acid, catechin, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid and sinapic acid respectively

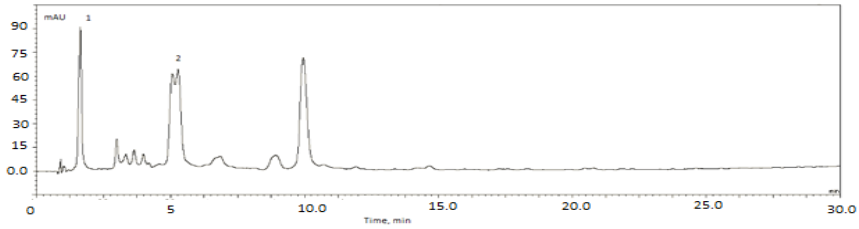

Fig. 2 - HPLC profile of *Vitis vinifera* seeds aqueous extract; Identified compounds 1-gallic acid; 2-catechine

In fig. 3 we can see changes in the amount of wet biomass for fermentation performed in an aqueous extract of *Vitis vinifera* seed. These extracts have a total of 259.14 mg /100g GAE polyphenols. In these solutions, we added a solution of copper ions in concentration of 10, 25, 50 and 100 mg/L. It appears that the best yield in the wet biomass is recorded when we add in the medium 10 mg/L copper ions, which is the optimum condition for cultivation of yeast *Rhodotorula spp*.

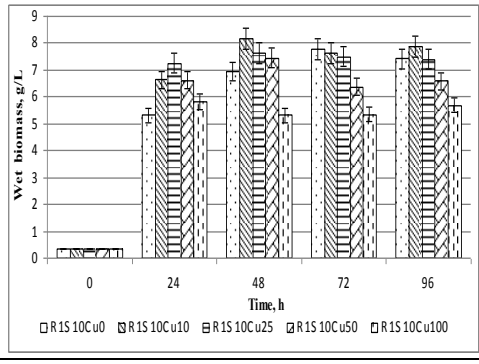

Fig. 3 - Variation in the amount of wet biomass during fermentation

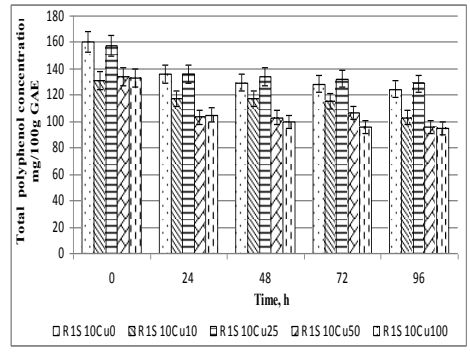

Fig.4 - Variation of total polyphenol concentration determined from the residual culture medium

Fig. 4 shows that the concentration in total polyphenols is lower than that measured before the addition of copper ions. It also shows that with increasing concentration of copper ions added in the extract, the concentration of polyphenolic compounds decreases; this confirms literature data that say that the polyphenols complex with copper ions (Hainal A.R., 2009).

However, the lowest concentrations in total polyphenols during fermentation processes are identified for the experiment in which we obtained the maximum amount of wet biomass. This leads to the conclusion that the yeast degrades polyphenols and uses them as carbon source.

At the same time, fig. 5 shows that, at baseline there was a low concentration of copper ions in comparison to the one introduced in the environment. Their

concentration decreased until the end of the process, which proves that biomass can retain free copper ions in the environment.

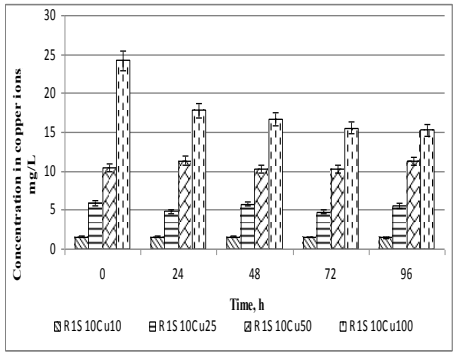

Fig. 5 - Variation of concentration in copper ion determined from the residual culture medium

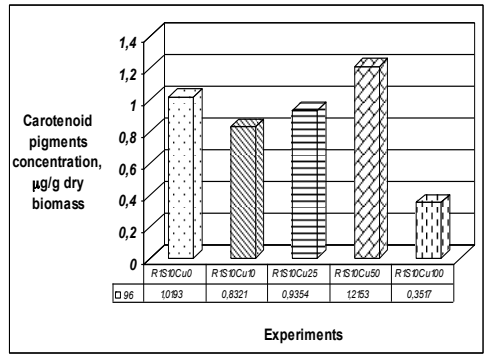

Fig. 6 - Variation of carotenoid pigments concentration extracted from the biomass resulting at the end of the fermentation processes

In fig. 6 we can observe the variation of carotenoid pigments concentration biosynthesized by yeast strain *Rhodotorula spp*. Thus, it appears that a maximum of pigments extracted at the end of fermentation is carried out in the presence of 50 mg/L copper ions added, an experiment in which we obtained a small quantity of wet biomass. This can be taken into account when intend to obtain a maximum of carotenoid pigments.

CONCLUSIONS

1. Aqueous extract of *Vitis vinifera* seeds contain high concentrations of catechins and gallic acid along with other unidentified polyphenolic compounds.

2. The best conditions for obtaining a maximum yield in wet biomass are those when a total polyphenol concentration of 258.95 mg GAE/100g has been used with an addition of 10 mg/L copper ions.

3. To assure conditions for biosynthesis of carotenoid pigments, it is recommended a culture medium containing 258.95 mg GAE/100g total polyphenols to which was added 50 mg / L copper ions

4. Polyphenolic compounds existing in the culture medium were used as carbon source by yeasts.

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