RESEARCH ON THE INFLUENCE OF AQUEOUS EXTRACTS FROM THE GRAPE SEEDS ON THE DEVELOPMENT OF SOME SPECIE OF *RHODOTORULA* SPP.

CERCETĂRI PRIVIND INFLUENȚA EXTRACTELOR APOASE DIN SÂMBURI DE STRUGURI ASUPRA DEZVOLTĂRII UNEI SPECII DE *RHODOTORULA* SP.

HAINAL Anca Roxana, IGNAT Ioana, VOLF Irina, POPA V. I.
"Gheorghe Asachi" Technical University Iasi, Romania

Abstract. *This paper presents the results of research on the influence of aqueous extracts from the grape seeds on the development of strains of Rhodotorula spp. Extracts were obtained with different concentrations in dry matter and polyphenolic compounds, which were then characterized in terms of content of polyphenols and sugars by different analytical techniques. The cultures were monitored by determining every 24 hours the amount of wet biomass. After recovery of biomass, the culture broth was characterized during nine days of culture from the evolution of pH, and concentration of total polyphenols by Folin Ciocâlteu method every 24 hours. The results obtained reveal a differentiated consumption of polyphenolic compounds depending on their concentration in the culture medium, consumption which was reflected directly on the variation of the biomass amount compared to reference.*

Key words: grape seeds, *Rhodotorula* spp., wet biomass.

Rezumat: *În lucrare sunt prezentate rezultatele cercetărilor asupra influenței extractelor apoase din sâmburi de struguri asupra dezvoltării unei tulpini de Rhodotorula spp. S-au realizat extracte de diferite concentrații în substanță uscată şi compuşi polifenolici, care apoi au fost caracterizate din punct de vedere a conținutului de polifenoli şi a zaharurilor prin diferite tehnici analitice. Cultura a fost monitorizată prin determinarea la fiecare 24 de ore a cantității de biomasă umedă. După recuperarea biomasei, din mediul de cultură s-a determinat pH-ul pe parcursul celor nouă zile de cultură, şi concentrația de polifenoli totali prin metoda Folin-Ciocâlteu la fiecare 24 de ore. Rezultatele obținute evidențiază un consum diferențiat al compuşilor polifenolici funcție de concentrația acestora în mediul de cultură, consum care s-a reflectat direct asupra variației cantității de biomasă comparativ cu martorul.*

Cuvinte cheie: seminte de struguri, *Rhodotorula* sp., biomasa umeda

INTRODUCTION

The recovery of compounds with nutritive and antioxidant potential from plant biomass is an economic problem relevant to food and pharmaceutical industry. Currently there is little information regarding the use of polyphenolic compounds in fermentation yeasts. However, research

shows that yeasts have the potential to fragment the polyphenolic compounds and to use these as a carbon source (Dănăilă M. et al., 2007).

Polyphenols include several classes of compounds such as: phenols, phenolic acids, flavonoids, anthocyanins and more complex structures such as tannins and lignins. Polyphenols are secondary metabolites normally produced by plants or in response to stress conditions, e.g. due to infections, high doses of UV radiation action or other factors.

In other cases, oxidised polyphenols may have inhibitory effect on the growth and development of certain microbial strains. The mechanism for expression of polyphenol toxicity may be explained by inhibiting hydrolytic enzymes, or by other mechanisms such as blocking the protein transport, non-specific interactions with carbohydrates, etc.. By extraction with water the following chemical compounds can be extracted from plant material: carbohydrates, glycosides, tannins, proteins, alkaloids, salts (Chow P. Et al., 2008).

Dănăilă M. et al., 2007, tested for yeast *Rhodotorula glutinis 9.3* the influence of ethanol extract obtained from grape seeds. The authors introduced in the culture medium extract in different concentrations to study its influence on the development of biomass and carotenoids pigment biosynthesis. Research showed that the extract can be used as additional carbon source for the growth of these yeasts. Another property of *Rhodotorula* yeast species is to metabolize polysaccharides (Arroyo-Lopez F.N. et al., 2008).

The purpose of this study is to determine the influence of aqueous extracts from grape seeds on the growth and development of one strain of *Rhodotorula sp*., along with the consumption of polyphenols compounds of culture medium taking into account the existing literature data.

MATERIAL AND METHOD

We cultivated a yeast strain *Rhodotorula sp*., denoted by R2, which was selected by Biotechnology Applied in Food Industry – Integrated Center for Research and Education – Bioaliment, "Dunarea de Jos" University, Galati. The preculture was done in culture medium with the following composition: 10 g / L glucose, 5g / L peptone, 3 g / L malt extract, 3 g / L yeast extract. Fermentation was performed on a platform thermostatic mixers for 48h at 27 º C and 120 rpm. The cells were recovered by centrifugation at 5000 rpm, for 15 minutes, washed twice with distilled water and inoculated on culture medium with the following mineral and organic chemical composition: 15 g / L glucose, 2.5 g / L yeast extract, 3 g / L sodium acetate, 1 g / L $(NH_4)_2SO_4$, 1 g / L KH_2PO_4, 0.1 g / L $CaCl_2$, 0.25 g / L $MgSO_4 \cdot 7H_2O$, 0015 g / L $ZnSO_4$, 0015 g / L $CuSO_4 \cdot 5H_2O$.

In other variants, the culture medium was prepared in aqueous extract obtained from 0.5 and 5 g of seeds of red grape dry material (Merlot variety, harvest 2009), brought to 1L. The culture medium was distributed in 250 mL Erlenmayer falsks with a volume of 100mL, after which was inoculated. Determining the number of inoculated cells was performed by reading optical density at 620 nm. An absorbency of 0.5 is equivalent to 10^7 cells in 1mL inoculum (Buzzini P., 2001). Each flask was inoculated with 4 x 10^7 CFU (CFU = colony

forming unit), in part. The extract was obtained using 0.5 to 5 g air-dried plant material introduced in a 250 mL Erlenmeyer along with 125 mL of distilled water.

The Erlenmeyer is covered with a watch glass and heated on a water bath so that the temperature in the vessel is 85-90ºC. Allow at this temperature for 45 min shaking it from time to time. The material is decanted and the clear solution is filtered through a glass funnel fitted with filter paper. The operation is repeated 3-4 times until a colorless extract was obtained. All extracts were collected in a flask of 500mL and completed to the mark with distilled water.

Three cultures were performed in the following order: a control culture without polyphenol extract and two medium cultures containing polyphenolic extracts of 0.5 and 5 g of dry material. The cultures were noted as the following: R2- culture in which medium composition consists only of the components of the recipe, R2S0.5 - culture medium containing in addition to the basic recipe components and extracts of 0.5 g dry plant material, and R2S5 - culture performed in the presence of aqueous extract of 5 g of material with reference to the medium components.

Every 24 hours, cells were recovered by centrifugation at 4000 rpm for 15 min and washed twice with distilled water. Wet cell mass was determined by weighing, and expressed in g/L culture medium, the pH was not changed during cultivation of the yeast, being determined after recovery of cells through centrifugation. Also, in the culture medium the concentration of total polyphenols was determined by the Folin-Ciocalteu method (Liu X. et al., 2008).

RESULTS AND DISCUSSIONS

Aqueous extracts were characterized and the data obtained are presented in table 1. Naturally, solids and polyphenol concentrations are directly proportional to the amount of material subjected to extraction.

Table 1

Characteristics of extracts from grape seeds

Proba	Total polyphenol concentration, mg / L gallic acid	Content in dry substance, g / L extract	Content in organic matter, g / L extract	Ash content, g / L extract	Ash content on dry weight basis,%
VV 5	374.0	0.900	0.712	0.188	20.0
VV 0.5	19.2	0.087	0.0695	0.0179	1.9

VV 5 - aqueous extract frrom 5 g dry plant material brought to 1L;
VV 0.5 - aqueous extract frrom 0.5 g dry plant material brought to 1L;

The data presented in figure 1 show the effect of the two extracts of different concentrations on the biomass yield compared with the reference culture.

The extract obtained from 0.5 g material seems to have a stimulating effect on the growth and development of yeasts compared with the reference, for a period of up to 120 hours. After this period there is a slow decrease in biomass yield compared with the reference sample, which could be explained by the toxicity determined by metabolic products that may cause cell death and lysis. If we conducted the fermentation in extract from 5 g of grape seeds,

there was a sharp increase in the amount of biomass accumulated after 72 hours compared with the control, but it decreases after 168 hours.

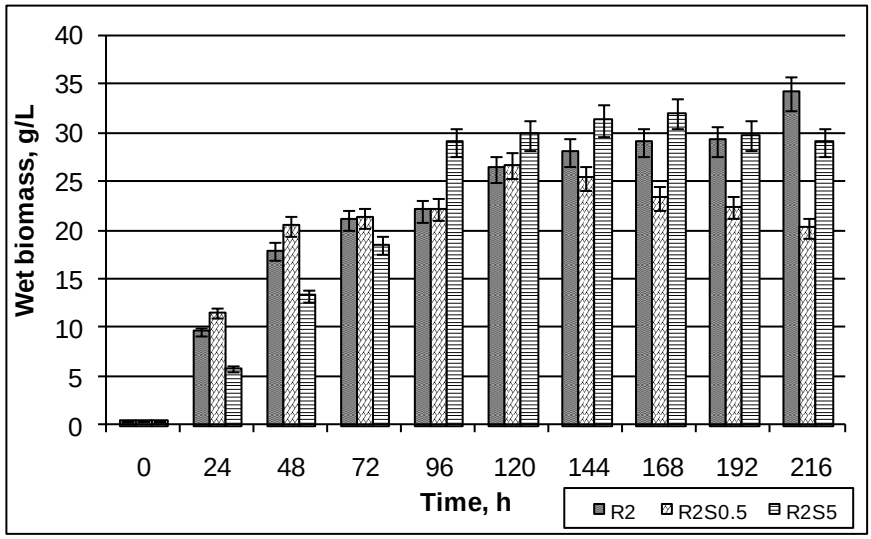

Fig. 1. Changes in the amount of wet biomass during the fermentation process

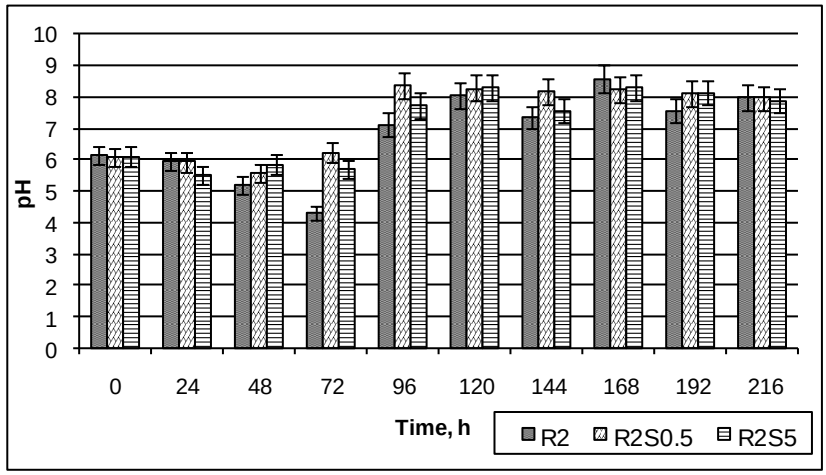

Fig. 2. The variation of pH during the fermentative process

In figure 2, we can follow the evolution of pH during fermentation. In this case the pH decrease is observed until 72 hours, reaching values up to 4. Subsequently, the pH jumps, remaining at values between 7 and 8 according to duration. The decreased of the pH could be explained by the

formation of acidic compounds in various stages of the glycolysis, and intermediate oxidation products for polyphenols, while increasing the duration to more than 96 hours may be due to the formation of phosphorylated compounds with a weak basic character.

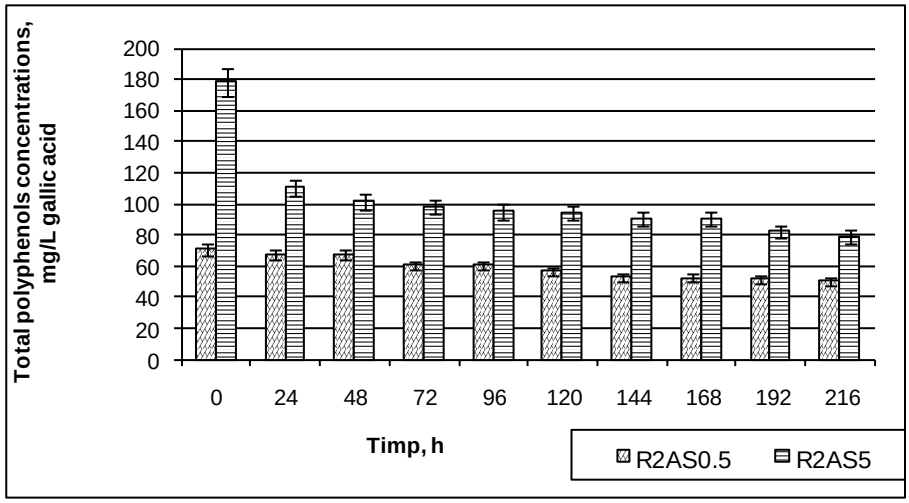

Fig. 3. Changes in total polyphenols concentration in culture medium during the experiment

In figure 3 one can observe a gradual decrease of the concentration of total polyphenols to the end of the process because of their possible metabolisation by yeasts. An interesting aspect is that, although the biomass yield started to decline, total polyphenol concentration continued to decrease and after 120 times respectively 168 hours for fermentation conducted in culture medium prepared in extract obtained from 5 g of material. This situation could be determined as following the action of oxidase enzymes released into the environment after cell lysis phenomena that may be involved in the degradation of polyphenols, aspect which will be further studied.

CONCLUSIONS

Using grape seed extracts containing polyphenols affect in a different way the developing *Rhodotorula* spp. from view point of yeld in biomass, which reached maximum values after 120 respectively 168 hours, depending on the concentration of polyphenols.

The data on changes in polyphenol content of the culture medium, highlights its low points during the process demonstrating that yeasts can consume additional aromatics compounds as carbon source.

REFERENCES

1. **Arroyo-Lopez F.N.,. Querol A, Bautista-Gallego J., Garrido-Fernandez A., 2008** - *Role of yeasts in table olive production – Review*, Int. J. Food Microbiol., Vol. 128, pg. 189–196;
2. **Buzzini P., 2001** - *Batch and fed-batch carotenoid production by Rhodotorula glutinis-Debaryomyces castellii co-cultures in corn syrup*. J. Appl. Microbiol., Vol. 90, pg. 843-847;
3. **Chow P., Nakayama F. S., Blahnik B., Youngquist J. A., Coffelt T. A., 2008** - *Chemical constituents and physical properties of guayule wood and bark*. Industrial crops and products 2 8, pg. 303–308;
4. **Dănăilă M., Popa V.I., Volf I., 2007** - *Influence of polyphenols on the biosynthesis of carotenoids by yeasts*. Proceedings of the 8 th ILI Forum, Rome, May, 10-12, p. 105-108;
5. **Liu X., Cui C., Zhao M., Wang J., Luo W., Yang B., Jiang Y., 2008** - *Identification of phenolics in the fruit of emblica (Phyllanthus emblica L.) and their antioxidant activities*. Food Chem., 109 (4), 909-915;