Antifungal activity of natural phenolic compounds on *Saccharomyces Cerevisiae*

MIHAELA DĂNĂILĂ[1], VALENTIN. I. POPA[1]
[1] "Technical University "Gheorghe Asachi", Department of Natural and Synthetic Polymers Iassy, Bd. Mangeron 71, 70005, Romania , e-mail: dnl2mihaela@yahoo.com

Abstract

This study evaluated the behavior of Saccharomyces cerevisiae yeast in the presence of different selected natural polyphenols, in order to provide an indication of the suitability of such compounds as potential antimicrobials, or influencing agents in the fermentation. Thus the obtained results of the study could sustain other directions for the natural polyphenolic compounds in which they can be used, like in biotechnological processes as fermentation or in order to yield biomass economically from different raw materials.

Keywords*: Saccharomyces cerevisiae,* natural polyphenols, minimum microbiocidal concentration.

Introduction

In last years, a special concern is manifested toward the environment protection and the use of new waste sources to obtain biological active compounds which can be used in different fields (medicine, agriculture, food industry). The agricultural wastes, resulted from the primary processing of different kind of resources represent an important source of natural compounds (especially polyphenolics) with valuable biological properties. The term "phenolic" or "polyphenolic" describes the compounds that possess a benzene ring substituted by one or several hydroxyl groups (-OH). Phenolics are secondary metabolites synthesized by plants, both during normal development and as response to stress conditions such as infection, wounding and UV radiation [1]. The accumulation of compounds derived from the phenylpropanoid biosynthetic pathway is controlled in a way sensitive to the plant environment involving controls at the genomic level and by regulation of a highly specific set of enzymes [2].

In plants, phenolics may act as phytoalexins, antifeedants, and attractants for pollinators, contributors to plant pigmentation, antioxidants and protective agents against UV light [3, 4].

Plant polyphenol composition is highly variable both qualitatively and quantitatively; some of these compounds are ubiquitous, whereas others are restricted to specific families or species. In the case of a single species, large variations may also occur, particularly because of genetic factors, environmental conditions, and growth or maturation stages [5, 6]. From the agroindustrial wastes category, grape seeds as well as compounds derived from spruce bark are important by-products after winemaking and wood processing and are considered as accessible industrial wastes, from the economic point of view and biological implications.

Taking into account the practical implication derived from biological properties of polyphenolic compounds, and in order to provide an indication of the suitability of such compounds as potential antimicrobials, or influencing agents in the aerobic and anaerobic yeast processes, this study evaluated the behavior of *Saccharomyces cerevisiae* in the presence of different selected natural polyphenols, from the minimum microbiocidal concentration point of view. Thus, the obtained results of the study could sustain other directions for the natural polyphenolic compounds in which they can be used, like in biotechnological processes as fermentation or in order to yield biomass economically from different raw materials [7, 8, 9].

In order to reveal the relationship between the structure and antifungal activity of natural polyphenols we have used crude extracts obtained from grape seeds and spruce bark, anthocyanins extracted from blueberry and grape seeds, KPN - Normal Chestnut Extract and pure compound like gallic, vanillic and tannic acids. The antifungal activity was assessed in mineral medium, on a rotary shaker, by using minimum microbiocidal concentration (MMC) as a criterion.

Material and Methods

Microorganism and test conditions: Minimum microbiocidal concentration (MMC) values were evaluated by the dilution susceptibility test. Test strain *Saccharomyces cerevisiae* was cultivated on a synthetic medium containing progressively lower concentrations of the test compounds, and incubated at 25 °C. Last two tubes were free of test polyphenols and they have been used as a growth control in broth and 70% ethanol. The prepared fungal cultures (1 mL) after 72 h were poured into Petri dishes (9 cm diameter), and then the YPG agar was allowed to solidify. After another 72 h the tests plates were analyzed and the lowest concentration of the test extract (m/V) preventing appearance of growth was considered to be MMC.

Pure polyphenolic compounds: Gallic, tannic and vanillic acids were purchased from Sigma-Aldrich. Tannins were obtained by kindliness of Tannin Svenica-Slovenia.

Crude extracts from vegetal materials: Grape seeds *Vitis vinifera – Chambourcin* variety and spruce bark (*Picea abies)* were selected, dried and screened in a dimensions range of 1-2 mm. Vegetal materials were ground into powder by a blender, and stored at 0-5ºC for later use. ***Solvent extraction. Step I.*** The red grape seeds and spruce bark powder was extracted in a device for continues extraction (Soxhlet) with ethyl-ether to degrease of vegetal material. The extraction conditions were solid to liquid ratio (1:3.5 g/mL), temperature 95°C. ***Solvent extraction. Step II.*** The extraction procedures consist in a optimum solid to liquid ratio (1:3.5 g/mL), number of extractions (complete extraction of vegetal material), temperature 95°C and ethanol (1:1, v:v) as organic solvent were used.

Anthocyanins extracts: The anthocyanins were obtained by maceration of 2.5 g of milled grape seeds and blueberry fruits in 50 mL of methanol/formic acid (volume ratio of 95:5) for 12 h. The colored liquid was separated from the solid residue, which was further twice treated with fresh solvent. The three combined solutions were concentrated in a rotary evaporator up to 10 mL, avoiding temperatures higher than 35 °C. The extracts were dried and kept at a temperature of 4 °C.

Analysis and determinations: Total phenolics determination. Total polyphenols in the extracts were determined by Folin-Ciocalteu method, expressed as mg gallic acid equivalent (GAE/mL) [10]. ***Total Anthocyanins:*** a classic spectrophotometric method was used, based on the pH- differential structure [11]. **Total flavonoids:** was measured by aluminum chloride colorimetric assay [12]. ***The HPLC analyses of the grape seed extract*** were performed at room temperature using a 4.6 -250 mm Beckmann column (Ultrasphere) C18. The mobile phase was a mixture of two solvents A (water:formic acid 2.5: 97.5 ratio) and B (A : acetonitrile 20:80 ratio). The eluent flow rate was 0.7 mL/min and the duration of analysis was 80 min. The UV detector was tuned to 280 nm.

Results and Discussion

The physical and chemical characterization of the grape seeds, spruce bark and blueberry extracts (table 1) shows an extremely rich phenolic composition, which promotes the used raw material on a higher rank in the hierarchy of the available vegetable resources that contain polyphenolic compounds. From the vegetal material composition, the spruce bark had the higher content in total polyphenols and flavonoids substance, not a bit surprising if we take into account the natural protective role played in nature by this structure, and blueberry fruits are superior to grape seed from the anthocyanins and total polyphenols content point of view.

Table 1. Extraction of natural materials and analysis of polyphenolic profile

Analysis	Blueberry anthocyanins	Grape seed anthocyanins	Grape seed extract	Spruce bark extract
Flavonids (mg echiv. catechin / L)	-	-	44.67	56.23
Anthocyanins (mg/L)	46.52	25.34	89.77	36.65
Total polyphenols (g echiv. ac. galic/L)	0.94	0.67	2.14	3.09

The results of HPLC analyses are shown as a typical chromatogram in figure 1. Based on these experimental data, it is possible to draw the conclusion that the phenolic compounds in grape seed extract are represented primarily by gallic acid.

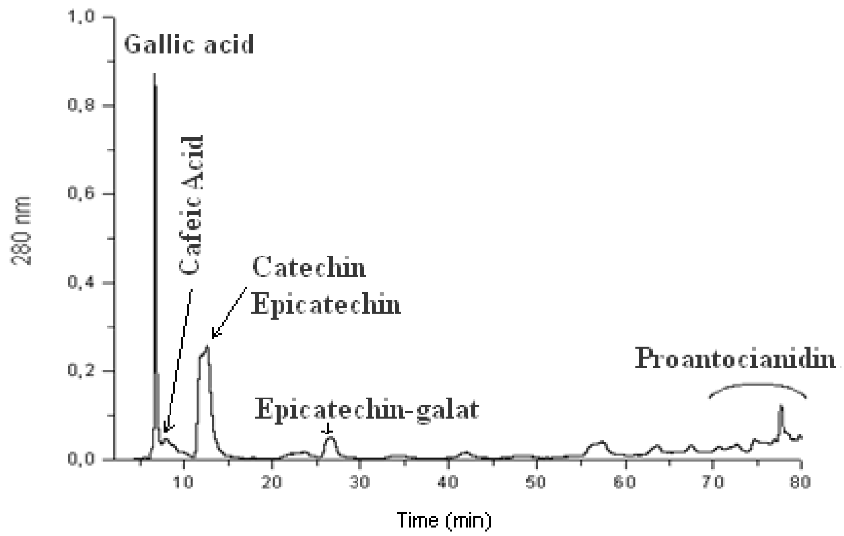

Figure 1. The HPLC analysis of grape seed crude extract

The antifungal activity of tannic acid, present in many foods including tea, cocoa beans, grapes, strawberry and persimmon and categorized as a 'generally recognized as safe' (GRAS) food additive., is increased compared with KPN tannins. Tannic acid, but not gallic acid, may action also like a siderophore to chelate iron from the medium and make iron unavailable to microorganisms [14]. Our results strongly suggested that phenolic acids with more hydroxyl groups show higher antifungal activity (between phenolic acids the tannic acid had the most pronounced activity). As expecting the MMC values for gallic acid were lower comparing with vanillic acid, probably because of hydroxyl group presence (figure 2).

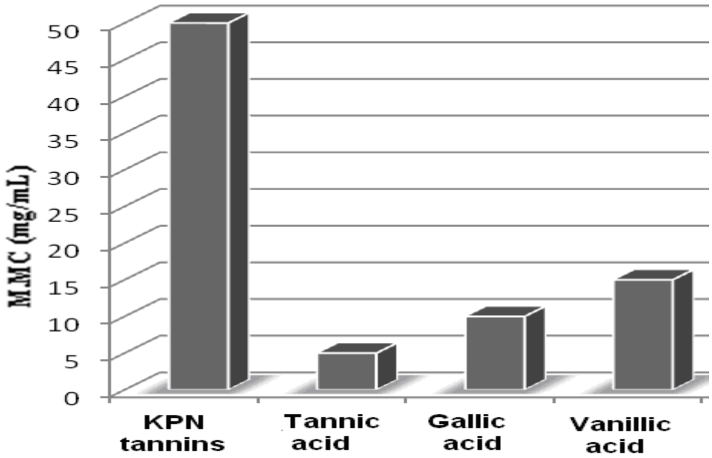

Figure 2. MMC of pure polyphenolic compounds on *Saccharomyces cerevisiae* (mg/mL)

However, there was no clear relationship between the total polyphenolic content and the antifungal activity of the polyphenols, in the case of Normal Chestnut Extract, which was the less efficient antimicrobial in this study.

When the antifungal activity of compounds or plant extracts cannot simply be estimated by the number of hydroxyl groups, it is necessary to take into account other factors, such as extraction source and polyphenolic compositions. Thus, in case of global extracts, spruce bark extract has proved a better antimicrobial as compared with the grape seed extract.

These aspects are not visible in the MMC values for anthocyanins, knowing that the differences between individual anthocyanins found in plants are related to the number of hydroxyl groups, the nature and number of sugars attached to the molecule, the position of this attachment, and the nature and number of aliphatic or aromatic acids attached to sugars in the molecule (figure 3).

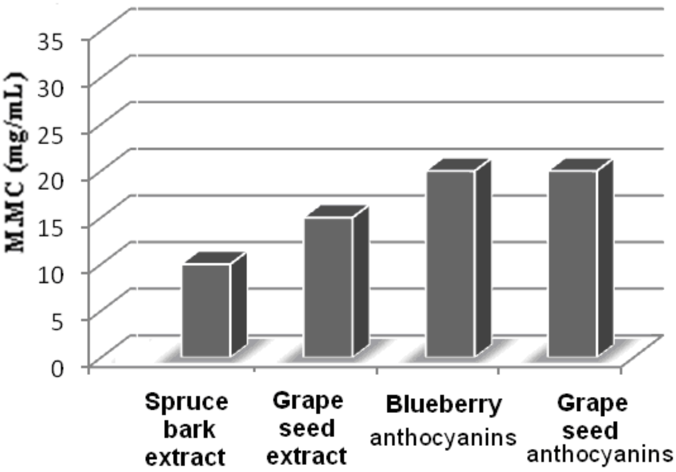

Figure 3. MMC of polyphenolic extracts on *Saccharomyces cerevisiae* (mg/mL)

Polyphenols could chelates metal ions and they are therefore different from simple phenols and the antimicrobial mechanisms can be summarized as follows. (i) The astringent property of the polyphenols may induce complex-forming with enzymes or substrates. Many microbial enzymes in raw culture filtrates or in purified forms are inhibited when are mixed with phenols. (ii) Toxicity of a polyphenol may be related to its action on the membranes of the microorganisms. (iii) Complex-forming with metal ions by polyphenols may account for tannin toxicity [13].

Conclusions

The study of the influence of polyphenols on *Sacchaoromyces cerevisae* development could have practical implication in the field of alcoholic fermentation or in biomass obtaining when acid hydrolysis products are used as substrates. At the same time, this strain represents a model recommended to be used with the aim to obtain information about role played by the polyphenols in its lifespan when the microorganism is cultivated in the presence of different stress factors.

References

1. SHI, J., YU, J., POHORLY, J.E., KAKUDA, Y., Polyphenolics in Grape Seeds—Biochemistry and Functionality, *J Med Food* **6 (4)**, 291–299, 2003.
2. KEFELI1, V.I., KALEVITCH, M.V., BORSARI, B., Phenolic cycle in plants and environment, *Journal of Cell and Molecular Biology*, **2,** 13-18, 2003.
3. POPA, V.I., ANGHEL, N., DANAILA, M., VOLF, I, Natural polyphenolic compounds. Structure, bioavailability and microbial interactions, International Lignin Institute, *ILI 8th Forum*, Rome, 2007.
4. DANAILA, M., VOLF, I., POPA V.I., POPA, M.I., Natural antioxidants from agroindustrial wastes. Sources, separations and practical implications, International Lignin Institute, *ILI 8th Forum*, Rome 2007.
5. MANACH, C., SCALBERT, A., MORAND, C., RÉMÉSY, C., JIMÉNEZ, L., Polyphenols: food sources and bioavailability, *American Journal of Clinical Nutrition*, **79 (5),** 727-747, 2004.
6. CHEYNIER, V., Polyphenols in foods are more complex than often thought, *American Journal of Clinical Nutrition*, **81(1),** 223S-229S, 2005.
7. POPA, V.I., DANAILA, M., VOLF, I., Natural polyphenolic compounds – modulators of yeast fermentative activities, *Buletinul Institutului Politehnic din Iasi*, **3 (2)**, 2006.
8. DANAILA, M., POPA, V.I., VOLF, I., The effect of adding natural polyphenolic compounds on yeast fermentation and wine quality, *Roumanian Biotechnological Letters,* **11** (4), 2833-2840, 2006.
9. DANAILA, M., POPA, V.I., VOLF, I., Influence of polyphenols on the biosynthesis of carotenoids by yeasts, International Lignin Institute, *ILI 8th Forum*, Rome 2007.
10. RECUEIL INTERNATIONAL DES METHODES D'ANALYSES – OIV, MA-F-AS2-07-CARCHR, **1**, 2006.
11. RIBÉ-GAYON, P., STONESTREET, E., Le dosage des anthocyanes dans les vins rouges, *Bulletin de la Societé Chimique de France*, **9**, 2649-2652, 1975.
12. SATU VUORELA, Analysis, isolation, and bioactivities of rapeseed phenolics, University of Helsinki Department of Applied Chemistry and Microbiology Food Chemistry Helsinki, PhD thesis, (2005).
13. JACOB, E.H., PIGNAL, M.C., Interactions levure–tanins. Croissance et survie de diverses levures dans des solutions tannantes, *Mycopathol. Mycol. Appl.* **48:** 121–142, 1972.
14. WAUTERS, T., ISERENTANT, D., VERACHTERT, H., Sensitivity of *Saccharomyces cerevisiae* to tannic acid is due to iron deprivation, *Can. J. Microbiol.* **47:** 290–293, 2001.