SPRUCE BARK POLYPHENOLS AS METABOLIC BOOSTERS FOR YEAST DEVELOPMENT

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Dedicated to the 70th anniversary of the Department of Pulp and Paper, "Cristofor Simionescu" Faculty of Chemical Engineering and Environmental Protection, "Gheorghe Asachi" Technical University of Iasi

The present study has aimed to investigate the influence of anthocyanidins extracted from spruce bark (*Picea abies*) on the development of *Saccharomyces cerevisiae* yeast. The biostimulatory effect follows the so-called dose-effect relationship. At a concentration of 400 mg/L bioactive product, the yield in the substrate increases by 16% with an increase in protein biomass of approximately 80%. These results are encouraging, if the possibilities of developing larger scale applications in terms of protein production are considered.

Keywords: anthocyanidins, polyphenols, spruce bark, yeasts

INTRODUCTION

Modern methods used in the field of agricultural science and technology grant an important place to the processes of temporary acceleration or inhibition of plant growth, development and metabolism by means of different physical and chemical factors. Among these factors, the substances that act as biostimulators have recently gained wide applicability in plant culture.

Due to the remarkable biological properties, as well as the many possibilities of use in practice, in the last two decades, natural compounds with aromatic structure have been the subject of numerous studies undertaken worldwide.

Polyphenols are one of the main classes of secondary metabolites with aromatic structure in plants. These substances are widespread in the plant kingdom and perhaps only carbohydrate substances outnumber them. Of great importance for the life of plants, polyphenols appear in their metabolism, from the simplest phenols to macromolecules as lignin.[1-6]

Research carried out in recent years, aiming at discovering new biostimulatory products compatible with the environment, has drawn attention to the possibility of intervention of natural polyphenolic products, separated from phytomass with different chemical agents, in the process of plant growth.[7]

Recent studies have focused, on the one hand, on the separation and characterization of these constituents, and on the other, on the establishment of their role in the development of plants. It is known that the substances that stimulate the growth of plants change the redox character of the environment.[8-11] Also, the activity of phytohormones, especially their role in cell division, is well known. In the case of polyphenolic products extracted from plants, establishing their mechanism of action is much more difficult because of their complex structure, as well as the difficulties regarding the separation and purification of the components.[12-13]

If polyphenolic substances are essential for the growth and development of higher plants, the idea arose that these natural products could somehow influence the development of microorganisms.[14-16]

Microorganisms commonly used as a source of protein in human and animal nutrition are yeasts.[17-18] More and more experimental data

show that yeast protein can replace traditional plant and animal proteins. Yeasts have been found to be able to synthesize water-soluble B vitamins, but store them in the cell in amounts as large as, or even larger than those in animal tissues recognized as important sources of vitamins.

Considering the deepening food crisis, the manufacture of biosynthetic proteins is one of the prospects for ensuring the protein needs.

For the reasons stated above, it is appropriate to use yeast growth bioregulators that lead to an improvement in the efficiency of use (metabolization) of the substrate and, implicitly, to an increase in productivity.

The aim of this experiment was to study the influence of some natural polyphenolic products (anthocyanidins) on the development of *Saccharomyces cerevisiae* yeast. As a source of polyphenols, spruce bark (*Picea abies*) was chosen, a waste resulting from the pulp and paper industry.

EXPERIMENTAL

Materials

Spruce bark was received from SC SOMEŞ SA, Romania, and *Saccharomyces cerevisiae* yeast – from a domestic commercial source (Rompak Ltd. Pakmaya, Romania).

Anthocyanidins

The spruce bark was ground and subjected to extraction with a hydro-alcoholic acidified solution of 75% ethanol (0.1% HCl) at room temperature and in the dark. The extract obtained by filtration was concentrated *in vacuo* at a temperature of 40 °C.

The acidified hydro-alcoholic extract was subjected to acid hydrolysis, by heating to 90 degrees Celsius for one hour to remove the sugar moyeties present in the anthocyanin structure.

The anthocyanidins (aglycons) thus obtained were purified by selective adsorption on polystyrene-coated silica gel.

The extract was dried by evaporation under vacuum at 40 °C.

Preparation of polystyrene coated silica gel particles

10 g of silica gel was added to a solution of 50 g of polystyrene dissolved in 100 mL of dichloromethane. The solvent was evaporated *in vacuo* and the solid was dried in a free atmosphere.

Purification of anthocyanidins

4 g of powdered alcoholic extract from the bark of *Picea abies* was dissolved in 20 mL of 0.1% ethanolic HCl solution. To the solution thus prepared, 5 g of polystyrene coated silica gel and 10 mL of distilled water were added and stirred for 30 minutes. The solid was filtered, washed with water (to remove sugars and other ballast substances) on the filter, dried in vacuum, and then resuspended in absolute ethanol. The mixture was stirred for approx. 30 min and filtered under vacuum. The filtrate was evaporated to dryness under vacuum at a temperature not exceeding 40 °C. An intensely colored red powder was obtained, which represented anthocyanidins.

Methods

Culture medium: carbon source – glucose (5 g/L), ammonium source: nitrogen-sulfate (2.5 g/L), growth factor – vitamin H (100 μg/L), anthocyanidins concentration ranging from 200 to 400 mg/L.

Cultivation method: the yeast was grown in liquid medium (200 mL) under aerobic conditions (air flow rate of 10 L/h) in an oven at 30 °C for 72 h. The initial concentration of the yeast in the culture medium was 0.5 g/L.

Glucose dosing in the culture medium was made photocolorimetrically (the ortho-toluidine method) by reading the absorbance at 630 nm and determining the concentration according to a standard curve.[20]

The yeast concentration in the culture medium was determined based on a standard curve by reading the optical density at 600 nm.[21]

The quantitative analysis of anthocyanidins was performed spectrophotometrically by reading the absorbance at 520 nm and determining the concentration according to a standard curve using cyanidin as reference.[22]

RESULTS AND DISCUSSION

As mentioned above, the present study investigated the possible influence of a certain category of polyphenols, namely anthocyanidins, on the metabolic processes of yeasts. This was not exactly the case, because, in a preliminary study, polyphenols have been shown to act in a dose-dependent manner on enzymatic systems involved in the carbohydrate metabolism and cellular respiration.[23] At a certain concentration in the culture medium, these substances can influence the entire biochemical machinery of plants, with quite interesting results on the process of protein biosynthesis.

As a parenthesis, the echoes in the scientific market regarding the action of anthocyanins on yeasts refer only to the winemaking process.[24] It seems that not much is known about the action of anthocyanidins (aglycons of anthocyanins) on the development of yeasts under aerobic conditions, which means almost nothing. The interesting thing is that, the yeast cultivation experiments in

the presence of anthocyanidins performed in different two laboratories, at a time lapse of a few years, had almost identical results.

As stated by Griesbach and Santamour,[25,26] by studying the anthocyanin composition of 27 species from the category *Abies*, *Picea*, *Pinus*, *Pseudotsuga* and *Tsuga* (*Pinaceae*), only four anthocyanins were found: cyanidin 3-glucoside, delphinidin 3-glucoside, peonidin 3-glucoside and petunidin 3-glucoside.

The reason that the separated anthocyanins were hydrolyzed at the level of anthocyanidins was to study the influence of aglycon, suspected of biological activity, on the development of the yeast.

The experimental data presented in Figures 1 and 2 show that although the consumption of glucose in the presence of anthocyanidins is high, compared to the control, the amount of biomass obtained is almost double. The consumption rate of anthocyanidins is proportional to their concentration in the culture medium (Fig. 3). It becomes obvious that anthocyanins are actively involved in yeast biochemistry by intensifying the anabolic processes.

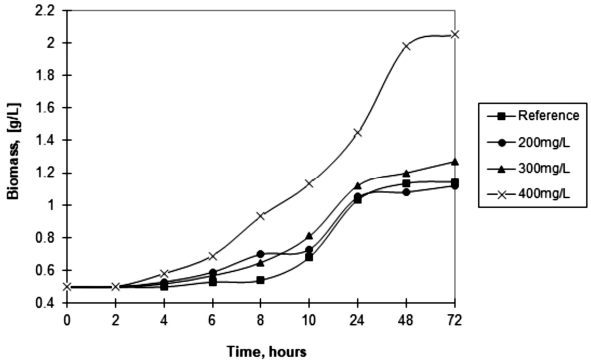

Figure 1: Evolution of yeast culture in the presence of anthocyanidins

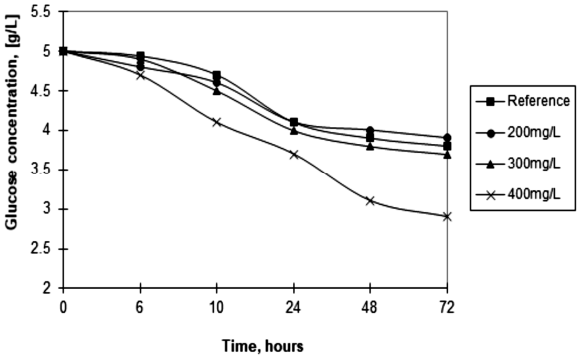

Figure 2: Influence of anthocyanidin addition on glucose consumption

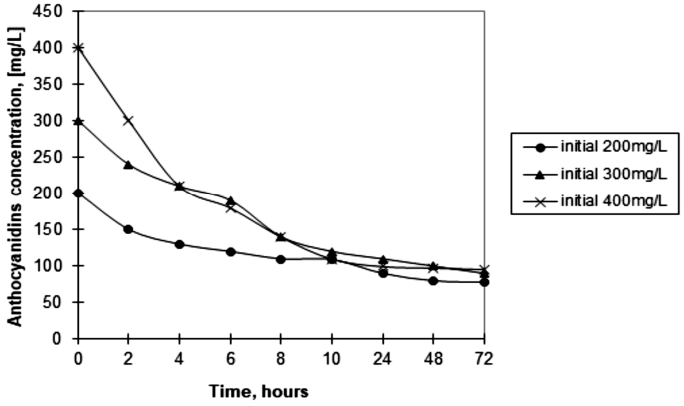

Figure 3: Variation in time of the concentration of anthocyanidins in the culture environment

Table 1
Substrate yields for control sample and polyphenolic products

	Substrate yield, %		
	Anthocyanidins		
Reference	200 mg/L	300 mg/L	400 mg/L
58	59	63	74

The presence of anthocyanidins in the yeast culture medium at a concentration of 400 mg/L causes an increase of biomass of 80%, compared to the control sample, as well as an improvement of the substrate yield of about 16% (Table 1).

These results are encouraging, if the possibilities of developing larger scale applications, in terms of protein production, are considered.

However, the mechanism by which these polyphenolic products act on the biochemistry of brewer's yeast remains an unresolved issue. However, if it works, the goal is achieved. Further research is necessary to obtain an insight into the mechanism underlying this phenomenon.

CONCLUSION

The present study has demonstrated that anthocyanidins extracted from spruce bark can act as metabolic boosters for yeast development. The biostimulatory effect follows the so-called dose-effect relationship. At a concentration of 400 mg/L bioactive product, the yield in the substrate increases by 16%, with an increase of protein biomass of approximately 80%. However, to be honest, the extraction, purification and optimal dosage lead us to the idea of looking for analogues of these anthocyanidins, preferably, easier to synthesize and mass-produce.

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