ANNALS OF THE UNIVERSITY OF CRAIOVA

Series: ✓*Biology*
✓*Horticulture*
✓*Food products processing technology*
✓*Environmental engineering*

Vol. XXVII (LXIII) - 2022

HIGHLIGHTING EXTRACELLULAR ENZYMES FROM YEAST AND THEIR ROLE IN WINE MAKING

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Keywords*: enzymes; extracellular; yeasts; effect;*

ABSTRACT

The enzymes produced by yeasts in the extracellular environment are the main factors that influence the secondary aromas of the wines obtained according to the primary varietal character of the processed varieties. Pectolytic enzymes are essential in the depolymerization of pectins and the release of color compounds, especially anthocyanins from the epicarp of black grape berries. Proteolytic enzymes break down proteins into amino acid chains, which help the multiplication of yeasts and the formation of specific aromas.

INTRODUCTION

Beta-glucosidase is an enzyme that catalyzes the hydrolysis of terminal glycosidic bonds in beta-D-glucosides and oligosaccharides, releasing glucose. Beta-glucosidase is composed of two polypeptide chains. These two chains are chiral in nature, meaning the chains are asymmetric and cannot overlap. Each chain consists of 438 amino acids and constitutes a subunit of the enzyme. Each of these subunits contains an active center. The active center serves as the location where the enzyme binds to the substrate and an enzymatic reaction can occur. Culture medium with the following composition is used (Rosi et al., 1994; Rodriquez et al., 2004)

Pectinases (polygalacturonases) cleave pectin by random hydrolysis of (1-4)-□-D-galactosiduronic bonds in pectate or other galacturonans. The term pectin describes a broad family of soluble heterogeneous polysaccharides. These polysaccharides are an integral part of the primary cell wall structure. Pectins are located between the cells, in the middle lamella where they act as a cellular binder. Pectinases are used especially in red grapes and have the ability to break the cell wall in the skin of red grapes thus allowing the extraction of anthocyanins and tannins. These enzymes allow the improvement and stabilization of wine color by binding anthocyanins to tannins. Another benefit of pectinase treatment is that the particles settle much more quickly. Pectinase is highlighted on the culture medium developed by researchers in the field (Buzzini & Martini, 2002),

Protease whose activity is highlighted by using the YEPG culture medium (Strauss et al., 2001). Protease or proteinase) is an enzyme that catalyzes the process of proteolysis, the degradation of proteins into smaller chain polypeptides or even into free amino acids.

MATERIAL AND METHODS

Qualitative screening to highlight extracellular enzyme activity (Saccharomyces and non-Saccharomyces yeasts), a yeast suspension was used to be tested after 24 h of culture (A580 = 0.5 corresponding to a cell concentration of 106/ml). The yeast strain is inoculated on the surface of the medium (from place to place). Cultures are incubated at 25°C for 2-5 days. for ß-glucosidase. Components g/l of the culture medium: Yeast Nitrogen Base (YNB) 6.7; Arbutin 5; agar20. Autoclave at 120°C for 15 min. Immediately after autoclaving, add 2 ml of filtered 1% (W/V) ferric ammonium citrate solution to 100 ml of medium and distribute in sterile vessels.

The culture vessels are inoculated with the analyzed yeast strain in culture for 24 hours and incubated at 25°C. Observations are made daily for a week. The control is represented by an uninoculated culture dish. Yeasts with enzymatic activity hydrolyze the substrate and a dark brown halo is visible around the colonies.

Protease

YEPG culture medium is used and the following components are added: g/l; Casein 20; Agar 15; pH 6.5. The culture vessels are inoculated with the analyzed yeast strain in culture for 24 hours and incubated at 25°C. Observations are made daily for a week. The control is represented by an uninoculated culture dish. Yeasts with enzymatic activity hydrolyze the substrate and a clear zone is visible around the colonies.

Pectinase

Culture medium with the following composition is used (P. Buzzini and A. Martini, 2002), Components g/l: Yeast Nitrogen Base (YNB) 6.7; pectin 10; Agar 15; pH 7

The culture vessels are inoculated with the analyzed yeast strain in culture for 24 hours and incubated at 25°C.

After the growth of the cells, hexadecyltrimethylammonium is added to the surface of the medium

bromides (10 g/l) (Ankin and Anagnostakis 1975). The development of a clear halo around the colonies indicates pectinase activity of the strain.

RESULTS AND DISCUSSIONS

The extracellular enzymes of the yeasts are the main factors that intervene in the destructuring of the substrate, in our case the grape berries from which we extract aromas and aroma precursors as well as the coloring substances from the epicarp of the grape berries.

Table 1

Characterization sheet of Saccharomyces yeasts from the point of view of extracellular enzymatic activity

No.	(Code) Yest strain	Species	β Glucosidase	Protease	Pectinase
1	5	S.ellipsoideus	-	+	++
2	40	S.ellipsoideus	-	+	+
3	28	S.ellipsoideus	-	+	+
4	54	S.ellipsoideus	-	+	+
5	49	S.ellipsoideus	+	+	+
6	53	S.ellipsoideus	-	+	+

7	55	S.ellipsoideus	-	+	+
8	93	S.ellipsoideus	+	+	+
9	41	S.oviformis	-	+	+
10	71	S.oviformis	-	+	+
11	72	S.oviformis	-	+	+
12	39	S.oviformis	-	+	+
13	63	S.oviformis	-	+	+
14	61	S.oviformis	+	+	+
15	101	S.oviformis	+	+	+
16	60	S.oviformis	-	+	+
17	59	S.oviformis	-	+	+
18	57	S.oviformis	-	+	+
19	36	S.oviformis	-	+	+
20	58	S.oviformis	-	+	+
21	68	S.oviformis	-	+	+
22	66	S.oviformis	-	+	+
23	82	S.bayanus	-	+	-
24	83	S.bayanus	-	+	+
25	43	S. rosei	-	+	-
26	140	S. rosei	-	+	+
27	135	S. rosei	-	+	+
28	139	S. rosei	-	+	+
29	44	S. rosei	-	+	+
30	67	S. rosei	+	+	+
31	45	S. rosei	-	+	+
32	37	S. rouxi	+	+	+
33	143	S. rouxi	-	+	+

Legend: (-) without enzymatic activity;
(+) with enzymatic activity;
(++) with high enzymatic activity.

The extracellular enzymatic activity of the studied yeast strains (tables 1-4) reveals a weak ß-glucosidase enzyme presence, on the other hand, the protease and pectinase activity is well represented.

ß-glucosidase, forms a brown halo, proving to be present in Saccharomyces ellipsoideus, strains 49,93; in Saccharomyces oviformis in strains 61,101; in Saccharomyces rosei strain 67 and in Saccharomyces rouxi strain 37.

Protease, the enzyme that breaks down proteins into amino acids, is a growth enhancer through the released nitrogen and phosphorus, and some of the amino acids can generate specific odorous substances, it is present in all Saccharomyces and non-saccharomyces yeast strains.

Pectinase, the enzyme that breaks down the pectins in the epicarp of the grape berries, releases varietal aromas specific to each variety, but especially helps to release the coloring substances from the epicarp of the black grape berries by depolymerizing the pectins. It is present in a clear halo in most strains of grapes. both Saccharomyces and non-saccharomyces yeasts with the exception of S. Bayanus-82, S. rosei -43.

Table 2

Characterization sheet of non-saccharomyces yeasts from the point of view of extracellular enzymatic activity

Yeast Strain (Code)	Species	β Glucosidase	Protease	Pectinase
4	Candida utilis	-	+	+
5	Candida utilis	-	+	+
3	Candida utilis	-	+	+
39	Candida utilis	-	+	+
73	Candida utilis	-	+	+

Table 3

The intensity of extracellular enzyme activity recorded in Saccharomyces yeast strains

Enzyme	Positive strains		No activity		Very intense activity	
	No.	%	No.	%	No.	%
β Glucosidase	6	18,2	27	81,8	0	0
Protease	33	100	0	0	0	0
Pectinase	30	90,9	2	6,1	1	3

From table no.3 results that the activity intensity of the β Glucosidase enzyme is 18,2%, of all the analyzed yeasts, the activity intensity of the protease enzyme is 100%, and that of pectinase is 90.9%, the remaining 3% is with very intense activity and only 6 ,1% is without enzymatic activity.

Table 4

The intensity of extracellular enzyme activity recorded in non-saccharomyces yeast strains

Enzyme	Positive Strains	No activity		
	No.	%	No.	%
β Glucosidase	0	0	5	100
Protease	5	100	0	0
Pectinase	5	100	0	0

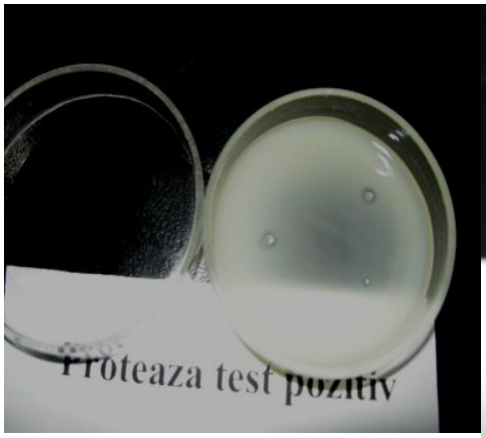
Table no. 4 shows that the intensity of β Glucosidase enzyme activity is zero, and the protease and pectinase activity is 100% in the case of the 5 non-saccharomyces strains of the Candida utilis species.

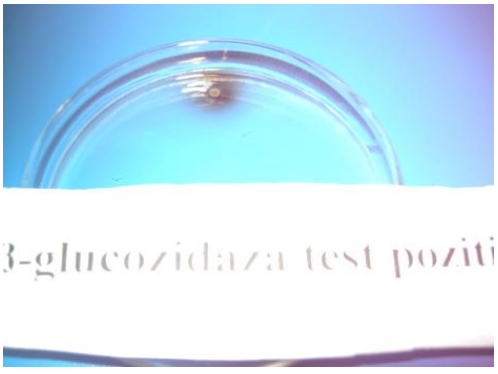
The role of extracellular enzymes in winemaking

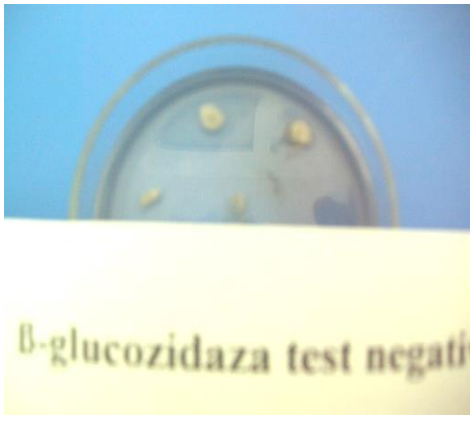
ß- Glucosidases, have a major role through their activity of forming aroma precursors in imprinting the varietal aromatic character of wines.

Proteases, enzymes that break down proteins to amino acids, including phenylalanine and histidine, which are floral aromatic precursors, give wines this character.

Pectinases are enzymes that break down pectins and contribute to the release of aromas from the epicarp of grape seeds, but also help to release coloring substances.







Pictures showing the enzyme activity in yeasts

CONCLUSIONS

The culture media used favored the development of cell cultures of both Saccharomyces and non-saccharomyces yeasts;

Extracellular enzymes, the most obvious in all yeast strains were proteases and pectinases, which through their activity can potentiate the varietal aromas in the wine, contribute to the formation of the aromatic precursors of some amino acids, making it more fruity, and by destructuring the pectins, the released substances dyes during the maceration-fermentation process of black grapes.

These enzymes give the secondary aromatic character of alcoholic fermentation to white wines as well as the colored appearance of red wines.

The intensity of β Glucosidase enzyme activity in the case of Saccharomyces and non-saccharomyces yeasts is quite low, while the protease and pectinase activity is very high

ACKNOWLEDGMENT

This scientific work constitutes one of the objectives from ADER 7.1.2./2019-2022 project.

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