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LACCASE ENZYME IN DISEASED GRAPES AND ITS IMPACT ON WINE QUALITY

Tamazi Bagatrishvili[1], Olani Gotsiridze[1], Mariam Khositashvili[1], Marika Mikiashvili[1], Medea Ormotsadze [2], Lia Kotorashvili[2]

[1]Caucasus International University, 73, Chargali street, Tbilisi, Georgia

[2]Georgian Research Institute of Food Industry, 17, Davit Guramishvili street, Tbilisi, Georgia

E-mail: marina_khositashvili@yahoo.com, marika.mikiashvili@ciu.edu.ge, lia.kotorashvili@gmail.com

Abstract

In this study, there are given biochemical characteristics and activity of the enzyme laccase in grape juice and wine obtained from healthy and diseased grapes, which is caused by Botrytis cinerea. The study provides chemical indicators of healthy and diseased grapes and wine and the obtained data are compared with each other. The enzyme laccase belongs to the oxidase type of enzyme, that can chemically activate phenolic compounds and promote their oxidation. As a result, this process could affect on the taste, aroma, and color of wine. The main goal of this work was to determine the level of laccase activity in diseased grapes and its impact on the main characteristics of wine: chemical composition, color, aroma, taste, structure, body and others. In the study a spectroscopic method was used to determine laccase activity. The same method was used to determine the color intensity of wines obtained from healthy and diseased grapes. It was determined, that the wine was oxidized and brownish pigments predominated. In addition, glucose and fructose were determined in the samples by HPLC. It was determined, that the juice of grapes infected with Botrytis cinerea contained 1.2% more sugar, than the juice of healthy grapes.

Keywords: Laccase, Botrytis cinerea, phenolic compounds, wine taste and color, sensory characteristics

1. INTRODUCTION

The condition of grapes and their damage, which can be caused by biotic or abiotic factors, often leads to the activation of enzymatic processes, in which one of the main enzymes is laccase.

Enzyme laccase belongs to the class of polyphenol oxidase that are produced by Botrytis cinerea and cause “gray rot”. Laccase oxidizes phenolic compounds, as a result in wine decrease aroma, color and significantly disrupting the structure of the wine.

This process is especially in white wines received from diseased grape.

Nowadays, modern winemaking demands high competition, and high competition requires quality-oriented approaches.

Therefore, grapes especially diseased grapes, require a complete analysis of microbiological and enzymatic profiles. Determination of the enzyme laccase in decreased grapes is one of the effective mechanisms to assess the initial suitability of grapes and correctly plan future technological processes.

The main goal of our research was to determine the activity of the laccase enzyme in diseased grapes using enzymatic and spectroscopic methods, to study the effect of the enzyme on the sensory and physicochemical properties of wine, which will allow us to maintain and improve the quality of wine.

2. EXPERIMENTAL PART

The research object were Georgian grape varieties such as: Rkatsiteli and Budeshuri Saperavi grapes and the wine made from these grapes. Enzyme laccase on local Georgian varieties, such as Saperavi Budeshuri and Rkatsitli, has not been studied yet, especially in the case of diseased grapes.

In Georgian-language sources, we rarely come across information on laccase activity in general and its impact on wine quality. In addition, in this research modern methods were used, which allowed us to determine the activity of the enzyme laccase.

Diseased grapes can radically deteriorate the quality of wine, they can contribute to the spread of microorganisms undesirable for wine, which will produce negative changes in chemical terms. Its impact can be detected by both sensory and chemical technology characteristics.

Nowadays, winemaking is a complex biochemical process in which many factors can determine the chemical and organoleptic properties of wine. Therefore, in this process, great attention is necessarily paid to the enzyme systems, that are naturally found in grapes.

One of the most important enzymes is laccase, which has a significant impact on polyphenolic compounds, in particular on changes in the stability, color and antioxidant properties of wine. Laccase acts as a catalyst, that participates in the oxidation of phenolic compounds.

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When grapes are damaged, this enzyme becomes particularly active. Damage can occur to grapes mechanically.

This can occur during the grape harvest, during processing or transportation. It is during this damage that enzyme actively increases in the grapes, which leads to further undesirable reactions, which are: excessive oxidation, unpleasant taste properties, color instability and accelerated spoilage of the product.

It is also worth noting, that the action of the enzyme laccase is not always harmful and moreover, its controlled use may even give positive results both for color stabilization and for improving the color and structure of wine during the aging process. However, if control is violated and excessive enzyme activity is observed, it will inevitably cause excessive bitterness and darkening of the color of the wine.

The chemical composition of wine is quite complex, but phenolic compounds are one of the most important components. These compounds are responsible for the color, structure and aging ability of wine. The enzyme laccase oxidizes phenolic compounds such as catechin, caffeic acid and Tyrosol. In this process, the enzyme laccase oxidizes these phenolic molecules and converts them into quinones. Quinones react with amino acids — the nitrogen balance and aromatic profile of the wine change, and quinones also bind to thiols — aromatic components lose their intensity (especially tropical fruit and floral notes), as a result, the color, taste, aroma, and structure of the wine change — which can have both beneficial and destructive effects.

3. DISCUSSIONS

The following parameters were determined in fresh and diseased grape juice (Table 1).

Table 1. Chemical composition of the grape samples

Name	Acidity g/l	Sugars %	Phenolic compunds mg/l
Rkatsiteli	6.3	22.2	320
Rkatsiteli diseased	5.0	23.5	280
Budeshuri saperavi	6.1	23.1	920
Budeshuri saperavi diseased	4.3	24.3	760

As can be seen from the Table 1, the amount of sugar in the diseased grape samples increased to 22.2-23.5% in Rkatsiteli, and to 23.1-24.3% in Saperavi Budeshuri.

It was found, that the juice of grapes infected with Botrytis cinerea contained 1.2% more sugar, than the juice of healthy grapes. In our opinion, this is due to the fact, that the skin of the diseased grape berries cracks and the evaporation of juice from the berries occurs and the concentration of sugar in the juice increases.

This moment was reflected in the alcohol content in the wines made from these grapes juice. The alcohol content of the diseased wine exceeded the alcohol content of the wine obtained from healthy grapes by 0.7%. As for the content of total phenols, in the white wine obtained from healthy grapes it was 320 mg/l, and in the diseased one it was 280 mg/l.

This data coincides with the literature data that the enzyme laccase acts on phenolic compounds, oxidizing them to quinones. A similar relationship is observed in red wine.

The activity of the enzyme laccase was also determined in wines from both healthy and diseased grapes of Rkatsiteli and Saperavi Budeshuri (Table 2).

Table 2. Laccase activity in wines received from healthy and diseased grapes

Wine types	Grape condition	Laccase activity, OD 420nm
Rkatsiteli	healty	18.1
Rkatsiteli	diseased	63.3
Saperavi Budeshuri	healty	19.4
Saperavi Budeshuri	diseased	74.2

Wines obtained from Saperavi Budeshuri grapes differed in color intensity. Therefore, we determined the chromatic indices (color intensity) of wines obtained from healthy and diseased Saperavi Budeshuri grapes according to the OIV method. The color intensity was determined at 420 nm, 520 nm and 620 nm. The optical density at the wavelengths indicated at these nanometers represents the intensity of brownish, yellow and red hues, the sum of which determines the overall color intensity of the wine (Table 3).

Table 3. Color intensity of healthy and diseased grapes

Grapes type	OD 420 nm (yellow)	OD 520 nm (red)	OD 620 nm (blue/brownish)	Total colour intensity (Σ OD)
Healthy	0.15	0.75	0.20	1.10
Diseased	0.28	0.55	0.35	1.18

4. CONCLUSIONS

In this study were analyzed the impact of the fungal disease Botrytis cinerea on the physicochemical, enzymatic and sensory characteristics of wines made from Saperavi Budeshuri and Rkatsiteli grape varieties. For each variety, wines obtained from both healthy and diseased samples were compared. Based on the analysis conducted, significant differences were identified in various parameters, which indicates that Botrytis cinerea infection directly and in many ways affects the quality of the final product. The results of the study revealed that the physicochemical parameters of wines made from deseased grapes change significantly.

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In particular:

- Sugar content is significantly high in diseased samples, which is likely caused by damage to the skin of the grapes by Botrytis.
- Acidity increases in diseased samples of both varieties, indicating enzymatic destruction and microbial metabolism processes.
- Changes in pH are observed at a relatively small level, although an imbalance is still observed compared to healthy samples.

These changes are technologically important factors, as they directly affect the stability, fermentation processes, and storage capabilities of wine.

The study showed that laccase activity in both healthy and diseased samples is variable and depends on the condition of the grapes:

- Healthy samples: Laccase activity is relatively low, which is logical since microbial contamination is minimal.
- Diseased samples: Enzyme activity is sharply increased, which is associated with the microbiota and enzymatic load produced by the fungal infection.

It is noteworthy, that laccase is functionally stable over a wide pH range and its maximum activity is observed in wines damaged by botrytis. This suggests, that laccase may play a role not only in the oxidation of polyphenols, but also in the transformation of structural components.

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