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# Influence of enzymes action on chromatic characteristics and aromatic profile of pre fermented grape juice

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***Abstract***

Was tested the limit of perception, the amplitude of the aromatic profile on nine flavor descriptors and the chromatic characteristics for the liquid obtained from white and black grapes, table varieties, which were processed by the action of the enzymes which acted as pectin lyase, pectin esterase, polygalacturonase, hemicellulase, in the form of enzymatic preparations of known action. The chromatic features were highlighted by means of the spectrophotometric technique with optical density measurements in the UV/Vis range and calculating color intensity and hue. The odorants were identified by sensory analysis with the development of the aromatic profile. Although only the Lallzyme EX-V enzymatic preparation has been recommended for the improvement of the chromatic properties, others such as Lallzyme HC (for clarification) and Rohavin Clear (for clarification) have contributed to changing the color perception of pre-fermented juices. They were highlighted the following flavor descriptors: a (fruit, apple, wax), b (citrus, floral, lemon, wax, magnolia), e (lilac, citrus, floral, woody); f (mint, cold, woody), h (sweetish, fruit tutti frutti). The highest values in the intensity of the perception of the flavor components were obtained in the Rohavin Clear variant and ranged between 2.4 and 2.5 out of maximum 3 points, thus proving a synergistic action of the pectinase and the polygalacturonase.

***Keywords***: grapes, pectinolytic enzymes, UV/Vis spectrophotometer, pre-fermented juices, ODE (odor description**)**

## 1. Introduction

Since ancient times, natural enzymes produced by microorganisms have been used to process grapes. Nowadays, enzymes from exogenous sources are widely used in different industrial processes. Pectinases are used in making fruit or vegetable juice, in the wine industry, for treating wastewater, or in extracting vegetable oil [1-3].

The enzymatic hydrolysis is the most appropriate technique to generate color and flavor precursors in grape juice and then wine [4].

The color and the flavor components of the grape juice are given by the chemical substances that form the precursors for the same characteristics in wine as well [5].

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An important role in the extraction of flavor and color components is played by the use of exogenous enzymes in the form of enzymatic preparations. Their action is to release flavonoids, tannin, anthocyanins from the cells of the tissues from the skin of the grain and its vicinity. The preparations with similar activity in winemaking [7] are also used for juice production [8].

There are two categories of commercial preparations that contain clarification enzymes and color extraction enzymes.

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Therefore, there are lyases that catalyze the depolymerization, pentin esterases which deesterify the methyl groups of the pectin producing methyl alcohol and pectinic acids and the polygalacturonases (endo and exopolygalacturonases) which hydrolyze the bonds between the galacturonic acid residues. Thus, these enzymes are: endopolygalacturonases, pectin-methyl esterases, arabinases [3, 9-11].

These preparations are a mixture of several enzymes from these groups that act on sedimentation, clarifying, pressing, color extraction, flavor release and filtration. Thus the color increases in intensity by augmenting the content of anthocyanins which also leads to a flavor improvement [12]. The preparations for color extraction, during maceration, are a cocktail of enzymes such as pectin lyases, pectin methyl esterases, polygalacturonases, hemicellulases, cellulases and proteases, which also have glucosidase activity [13-16].

Flavor is made of volatile components, chemical stimuli detected by the receptors placed in the olfactory epithelium in the nasal cavity. They are transmitted directly to the receptors on the retronasal olfaction in the mouth when swallowing. In order to consider the flavor components, the sensory analysis is used with differentiation methods for different characteristics.

For the quantitative determination of chemicals which producesensations odoorante using flame ionization detector (CG-FID) and gas chromatography-mass spectrometry (CG-MS) [17].

The present study followed the action of three enzyme preparations used for the processing of white and black grapes in four samples and the chromatic characteristics and the aromatic profile of the pre-fermented juices have been obtained. A comparative analysis was made with the values obtained from the quantitative study of the flavor components from the literature.

## 2. Materials and Methods

### *2.1 Materials*

Two grape varieties were used for this study: white (Italy) and red (Republic of Moldova) which have been procured from commerce.

#### *2.1.1 Enzyme action*

Pectolytic enzyme preparations and their operational parameters

Rohavin Clear - clearing white and rose juice. It contains pectinase, polygalacturonase and it does not have secondary enzymatic activities. It is fungal in nature and has an enzyme activity of 1440 PE/g (Georgia), 8 h at 20°C.

Lallzyme HC- clarifying the juice by pectin hydrolysis, pectinylase (PL) (100 PL u / g), pectin esterase (PE) (800 PE ug/g) and polygalacturonase (PG) (3500 PG ug / g) and very low cinnamyl esterase activity. It is fungal (Southern Australia), 12 h at 20 °C.

LELYMEME EX-V-release of intracellular content by the action of: pectin-lysa (PL) (100 PL u/g), pectin esterase (PE) (800 PE ug / g) and polygalacturonase (PG) a very low secondary activity of cellulase and hemicellulase (southern AUSTRALIA), 8 days at 28°C.

In order to analyze the consequences of the enzymatic activity of the three preparations were used samples coded as in Table 1.

#### *2.1.2 Sample preparation*

Juice making and samples was carried out according to the following scheme: destemming→ crushing→enzymatic treatment (specific as duration and temperature)→separation of juice by pressing→centrifugal clarification (2930 rpm, 10 minutes, 20°C)→grape juice preferment. Four fractions of juice preferment of by 200 ml were analyzed.

### *2.2.Methods of analysis*

#### *2.2.1 Chromatic Characteristics*

Used to determine, the following methods of analysis: chromatic characteristics [18] ((18OIV-MA-AS2-07B: 2009) using the T80 UV/VIS spectrophotometer (United Kingdom). Measurement was done in cuvettes with optical path of 1 mm.

#### *2.2.2 Sensory analysis*

The aromatic profile of the analyzed liquids was perceived by analyzing the flavor descriptors such as: a-fruits, apples, wax; b-fruit with whipped cream; c-tea, mint, fruit, berries; d-citrus, floral, lemon, wax, magnolia; e-lilac, citrus, floral, floral woody, Lily of the valley; f-cold mint, woody; g-fresh, citrus, lemon peel; h-sweetish, fruit, tutti frutti; i-fusel, cognac, fruit, banana oils [19, 20]. The aromatic profile method was used to determine. There were questionnaires that were completed by a number of 15 trained panellists, boys and girls.

Their training was long, for 4 weeks, and consisted in analyzing some grape samples and identifying standard (references) flavor components. These references have been described using terms of comparison with the flavors perceived by panellists throughout their lives. Specific components were studied on table grapes, white, and tomato varieties used for determinations. The results of 4 determinations were used.

The expression of the flavor was perceived by odor and taste (by the retronasal perception of the flavor components sought) [21].

*2.2.3 Statistical analysis*

The data that were obtained by analysis were collected and the variance was calculated for each determined parameter and the probable differences ($p \leq 0.05$) were determined using the t-test sample. Work with 4 replies for each work variant.

## 3.Results and discussions

The analyzed samples are pre-fermented grape juices. Their chemical composition is somewhere between grape juice and young wine.

*3.1 Determination of chromatic properties*

According to the method used chromatic characteristics of liquids to be analyzed are given by the brightness dependent on transmittance. This is inversely proportional to the intensity of color and chromaticity which depends on the wavelength where reading is done and purity.

Selected wavelengths were those specific to the wine industry [21]

Absorption values for enzyme-free samples are found in the literature and range from 0.428-0.894 at $\lambda$ = 420 nm and 0.456-0.969 at $\lambda$ = 520 nm in red wines [22].

It can be seen from Figure 1 that at the studied samples the absorbance is different for the enzyme-free variants (abs = 0.327-$S_{ref}$ LHC and abs = 0.66 $S_{ref}$RC) although the same variety of white grapes was used. The duration and temperature of the particular treatment have led to a different behavior of extraction of chemical compounds and the development of fermentation metabolites.

If a higher dose of enzyme is used, the absorbance value increases relative to the recommended dose (abs = 0.301 - $S_2$LEXV +; abs = 0.297 - $S_2$LHC+ at preparation). The same trend is not valid after 3 days of liquid storage. On samples with fermentation maceration (LEXV) the absorbance value decreases to $\lambda$=520 nm, at the recommended dose of 0.976 to 0.959.

The behavior of pre-fermented liquid is different. These different optical densities are influenced by the chemical composition. For LEXV we have obtained a more intense color samples at $\lambda$ = 420 nm $S_2$LEXV- preparation (abs = 0.301); $S_1$LEXV, after 3 days (abs = 0.633); $S_1$LEXV at $\lambda$ = 520 nm (abs = 0.976); $S_1$LEXV, 3 days after preparation (abs = 0.959). So if the time is longer then the efficiency of the enzyme is higher even if the dose does not increase.

For liquids from white grapes the best results were obtained at the recommended dose and over time. Thus at $S_{ref}$ LHC at $\lambda$ = 420 mn (abs = 0.327) and $S_1$LHC at $\lambda$ = 420 nm after 3 days (abs = 0.482).

For RC time is depletion of enzyme action values obtained after 3 days being smaller by about 49%. So the RC enzyme's efficiency was greater, its rinsing power also manifests over time even if operating parameters change. By reducing the molecular mass of the macromolecular components, it reduces turbidity, increases clarity and reduces components which are able to absorb the light intensity of the beam and therefore the absorption values are smaller.

The intensity of the coloring that was studied for red grapes only is a sum of the absorbance values (DO-optical density) [5] which was read at $\lambda$ = 420 nm (for the complementary yellow green color and purple absorbed ) and $\lambda$ = 520 nm (for purple complementary color and green absorbed) [23]. At $\lambda$ = 620 nm (for blue green complementary and red absorbed) absorbance was not read.

**Color intensity(I)=$A_{420}$+$A_{520}$** **(1)**

The wine industry is used the value of the intensity of the coloration calculated using formula 1. If < 0,4 then the liquid is rosy, if >1 the liquid is intense red, and if < 0,5 and >1 then the liquid is red [24].

Hee is the conventional ratio between absorbance readings at $\lambda$ = 420 nm and $\lambda$ = 520 nm [11].

**Hue=A420/A520** **(2)**

According to the literature, and using formula 2, if H < 1 the liquid to be analyzed or pre-fermented juice from red grapes has violet shades. If H >1 then the hue is ruby with slight brick inflections [24].

***Table 1.*** Abbreviations used to study the chromatic characteristics and the aromatic profile

| Abbreviating | code | Explaining the code and the abbreviation |
|---|---|---|
| $S_{ref}$LEXV | 127 | Reference without enzyme action using red grapes |
| $S_1$LEXV | 271 | The Lallzyme EX-V sample recommended by the manufacturer-2g (g/100 g) |
| $S_2$LEXV+ | 721 | The Lallzyme EX-V sample higher than the recommended dose 4g (g/100 g) |
| $S_3$LEXV- | 217 | The Lallzyme EX-V sample less than the recommended dose 0,5g (g/100 g) |
| $S_{ref}$LHC | 283 | Reference without enzyme action using white grapes |
| $S_1$LHC | 382 | The Lallzyme HC sample recommended by the manufacturer - 1g (g/100 g) |
| $S_2$LHC+ | 823 | The Lallzyme HC sample higher than the recommended dose 2g (g/100 g) |
| $S_3$LHC- | 832 | The Lallzyme HC sample less than the recommended dose 0,1g (g/100 g) |
| $S_{ref}$RC | 473 | Reference without enzyme action using white grapes |
| $S_1$RC | 437 | The Rohavin Clear sample recommended by the manufacturer - 1g (g/100 g) |
| $S_2$RC+ | 347 | The Rohavin Clear sample higher than the recommended dose 2g (g/100 g) |
| $S_3$RC- | 743 | The Rohavin Clear sample less than the recommended dose 0,1g (g/100 g) |

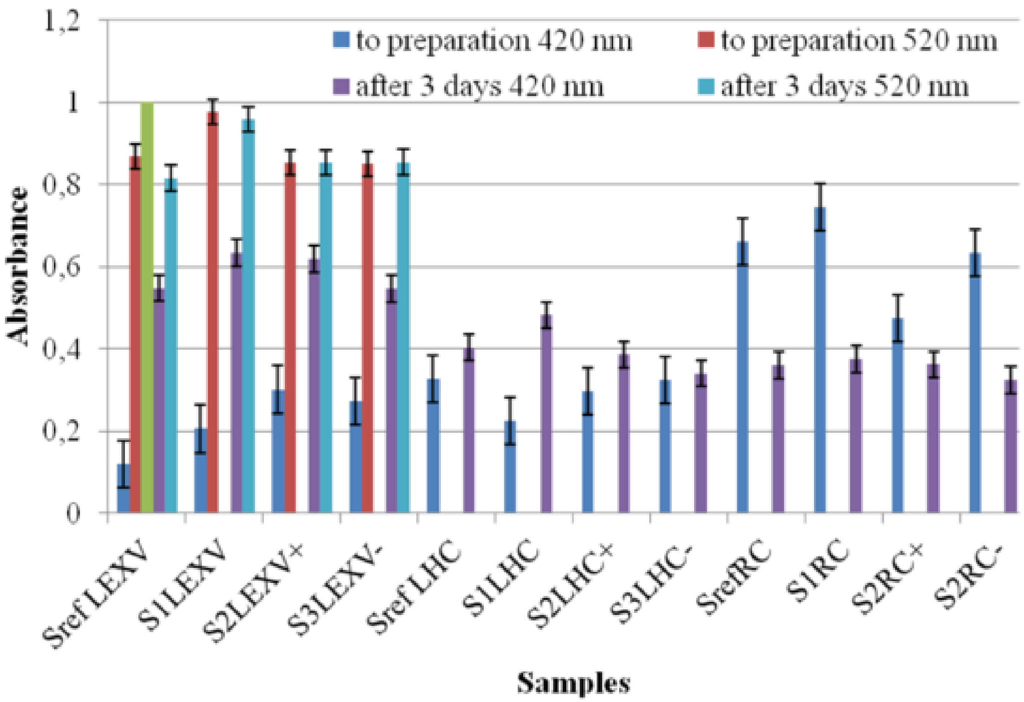



***Figure 1***. Evolution of the optical density of white and red grape juice studied according to the enzyme dose (recommended $S_1$, higher $S_2$, lower $S_3$ compared to the $S_{ref}$) at specific wavelengths (420 nm and 520 nm)(significance level $p < 0.05$, all value have standard deviation, n = 4)

***Table 2.*** The chromatic characteristics evaluated mathematically based on the absorbance measured for prefermented red grape juices treated with Lallzyme EX-V

| Samples | To preparation | | After 3 days | |
|---|---|---|---|---|
| | Color Intensity | Color hue | Color Intensity | Color hue |
| [a]$S_{ref}$ LEXV | 0,988 ± 0,011 | 0,13 ± 0,0042 | 1,36 ± 0,0058 | 0,66 ± 0,0064 |
| [b]$S_1$LEXV | 1,18 ± 0,0051 | 0,200 ± 0,0051 | 1,59 ± 0,0054 | 0,660 ± 0,0063 |
| [c]$S_2$LEXV+ | 1,15 ± 0,0026 | 0,35 ± 0,0055 | 1,47 ± 0,00126 | 0,72 ± 0,013 |
| [d]$S_3$LEXV- | 1,12 ± 0,0022 | 0,32 ± 0,005 | 1,42 ± 0,028 | 0,64 ± 0,0048 |

[a]Reference without enzyme action using red grapes; [b] The Lallzyme EX-V sample recommended by the manufacturer, [c]The Lallzyme EX-V sample higher than the recommended dose, [d]The Lallzyme EX-V sample less than the recommended dose (significance level $p < 0.05$, n = 4, mean, ± standard deviation)

For the studied samples the values are shown in Table 2. This shows that the intensity value is greater than 1, so the coloration is intense red. It is noted that the highest intensity value of 1.18 (at the preparation) and 1.59 (after 3 days) is obtained for the working variant with the recommended dose of the manufacturer.

With increasing storage life and the modification of the chemical composition of the medium the intensity of coloration increases by approximately 35% for $S_1$LEXV. From this point of view, the same growth trend is observed for the $S_2$LEXV+ and $S_3$LEXV- variants. In literature specialized it is noted that the quantitative variation of pigments (the anthocyanin monoglucoside fraction) responsible for color is perceived differently.

Quantitative variation is perceived by varying the luminous and qualitative intensity by hue [25].

From the studied samples, it is observed that the values obtained for hue are subunit. The highest value was obtained for the $S_2$LEXV sample (0.35 to the preparation) and the same sample and after a retention period of 3 days (0.72). Over time the value of this parameter tends to reach 1 and exceed this value which means that fluid will get shades red-orange of maturing.

So a greater amount of enzyme, leads to the release of a larger amount of pigments. Although only the LEXV enzyme preparation has been recommended to improve chromatic properties, others as LHC (for clarifying) and RC (for clarification) have helped to modify the color perception of the liquids to be analyzed.

### *3.2 Sensory analysis*

In sensory analysis, to highlight the aromatic profile a questionnaire was written which was completed by each panelist trained in part. The method used is of analytical description of quality with the recording of a score. A single sensory feature, namely flavor, has been analyzed in this case. The Structured Scale Method, with simple scoring, with very few points completed the questionnaire and contributed expressing the limit of perception and the amplitude of the aromatic profile on descriptors of flavour [26-29].

The samples were coded and the meaning of the codes was shown in table 1. These determinations were made four times, against the results presented are an average.It can be seen from Figure 2, that in samples with LEXV added, for flavor, significant results were obtained for sample 271 ($S_1$LEXV) for flavor descriptor h (sweet, fruit, tutti frutti) with a value of the highest score 5 of 5.

With the increase in the amount of enzyme (LEXV) is profiled to the advantage flavor descriptors: b (whipped cream), e (lilac, citrus, floral woody, Lily of the valley), g (fresh, citrus, lemon peel) and disappear (fusel, cognac, fruit, banana). If the dose decreases, the intensity of the perception of the aromatic descriptors is higher than the control sample and it is remarkable: a (fruit, apple, wax), e (lilac, citrus, floral, woody, Lily of the valley) and i (fusel oil, cognac, fruit, banana).

So the addition of LEXV enzyme to red grapes at the recommended dose, highlight the sweetish flavor of tutti frutti. This according to the literature is given by ethyl butyrate [20]. The surplus of enzyme highlights the lilac, citrus, floral, woody aroma. It has been shown to be given by α-terpineol [20] and the lower dose of enzyme preparation of fruit, apple, wax. This latter is given by ethyl caprate [20].

When macerating fermentation the flavor components is due to volatile grape components and pre-fermented juice. The scientific literature highlights the presence of α-terpineol in grape varieties in the amount of 3.26-30.77% [20] of ethyl caprate in the amount of 1.5-2.91%, specific to white varieties of grapes. Percent expression is the result of GC/MS chromatographic analysis and represents the percentage of the area represented [20].

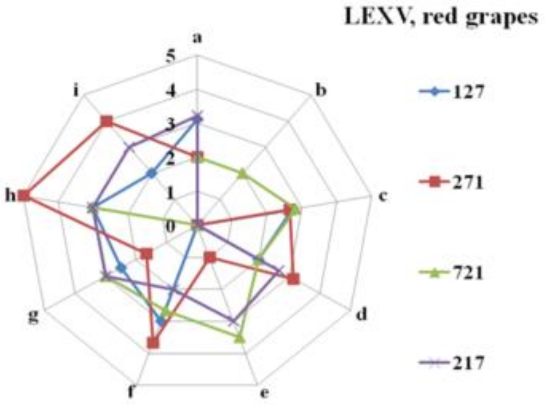
Only these substances have been imposed, because in the case studied, they were olfactory and it was wanted a quantitative positioning knowing that the threshold of perception for α-Terpineol is 250 μg/l [30] and the retention time is 13.163 minutes [20]. For fermentation maceration to give α-terpineol in a higher amount it is recommended to work with a larger dose of LEXV enzyme preparation namely of 2,5 g/100 kg of crushed grapes instead of 2g/100 kg (recommended dose). This is, because the value of the general impression of pre-fermented juice had the highest value for the $S_2$LEXV variant (2.4 out of 3 points) (Figure 3) with the highest amount of α-terpineol.

It can be seen from Figure 2 that in LHC-added samples significant results, from the aromatic point of view were obtained for sample 283 ($S_{ref}$LHC) for flavor descriptor f (mint, cold, woody) with a value of intensity of 5 out of 5, distinct from the RC variant (3 to f, 5 to c (tea, mint, fruit, berries). This is due to a different working period although it is the same variety of white grapes.

The highest intensity was obtained for descriptors: f (mint, cold, woody) at work variant 382 ($S_1$LHC) (4 of 5)and g (fresh, citrus, lemon peel), h (sweetish, fruit, tutti frutti) at 832 ($S_3$LHC) (3.3 of 5). The highest intensity value, maximum 5 was obtained at the $S_2$LHC sample for the descriptor e (lemon, citrus, floral, woody) (5 of 5), with increasing the amount of enzyme.

If the dose is lower than recommended, was noted the flavor descriptor g (fresh, citrus, lemon peel) with an intensity value of 3.3 out of 5). So the addition of LHC enzyme in the recommended dose highlight the mint, cold, woody aroma, which is given by DL-Menthol. The surplus of enzyme highlights the aroma of lemon, citrus, floral, wood which is given by α-terpineol and the lower dose of enzyme preparation of the fresh citrus flavor, the lemon peel which is given terpinolen.

When processing grapes using clarifying enzyme preparations (RC and LEXV), juice flavor components are due to volatile components and flavor descriptors of grapes. The scientific literature highlights the presence of DL menthol in white grape varieties in the amount of 16.63-24.14% **[20]** of α-terpineol which was presented above and terpinolen in the amount of 5.98% [20], specific to the white grape varieties. Only these substances have been imposed, because in the studied case they have been noted olfactory these and a positioning was desired from the quantitative point of view knowing that the retention time is 12,809 minutes [20].



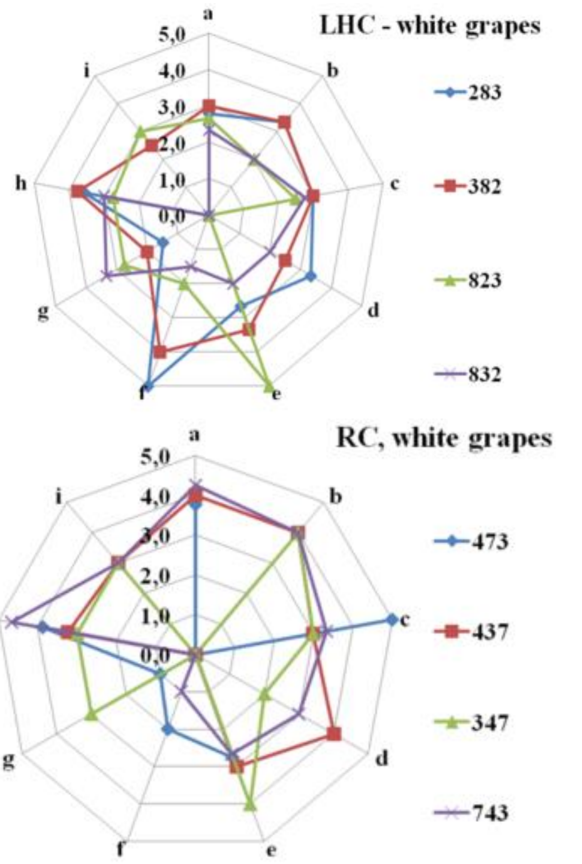




***Figure 2***. Aromatic profile on ODE (odor description) using the QDA method (qualitative descriptive analysis) of the panel action score enzyme preparations on white and red grapes (codes presented in table 1) a-fruits, apples, wax; b-fruit with whipped cream; c-tea, mint, fruit, berries; d-citrus, floral, lemon, wax, magnolia; e-lilac, citrus, floral, floral woody, Lily of the valley; f-cold mint, woody; g-fresh, citrus, lemon peel; h-sweet fruit, tutti frutti; i-fusel, cognac, fruit, banana oils.

So the addition of LHC enzyme in the recommended dose highlights the mint, cold. When processing white grapes with the LHC enzyme preparation to obtain DL-menthol in a higher amount it is recommended to work without the addition of LHC enzyme preparation. This one, because the value of the overall impression of the analyzed wort was the highest for the $S_{ref}$LHC work variant (with a value of 2 of 3) (Figure 3), so with the highest amount of DL - menthol.

In samples with RC added (Figure 2) recommended for clarification are obtained significant results for sample 437 ($S_1$RC).They were remarked the following flavor descriptors: a (fruit, apples, wax) (with a value of 4 of 5), b (fruit with cream) (4 of

5), d (citrus, floral, lemon, wax, magnolia) (4 of 5), h (sweetish, fruit, tutti fruit) (3.2 of 5). At sample 473 ($S_{ref}$RC) we noted: a (with a value of the intensity of 3.8 of 5), c (tea, mint, fruit) (5 of 5), h (with an intensity value of 3.9 of 5). With the increase in the amount of enzyme is profitable to the advantage aroma descriptors b, e (lemon, citrus, floral, woody) and disappears f (mint, cold, woody). If the enzyme preparation ($S_3$RC) decreases, then the intensity of the aromatic descriptors that are of higher perceptual value compared to the control sample are: a (with a value of the intensity of 4.3 of 5), b (4 of 5) and h (4.7 of 5).

So from this study it is concluded that the addition of enzyme, RC, in the recommended dose, reveals the flavor of: a (fruit, apple, wax), b (fruit with whipped cream), d (citrus, floral, lemon, wax, magnolia). In literature, it is said that are given by ethyl caprate, ethyl decanoate and nerol. The surplus of enzyme highlights the mint flavor, cold, woody that is given by DL menthol, and the lower dose of enzyme preparation of sweet flavor, fruits tutti frutti which It is given by ethyl butyrate.

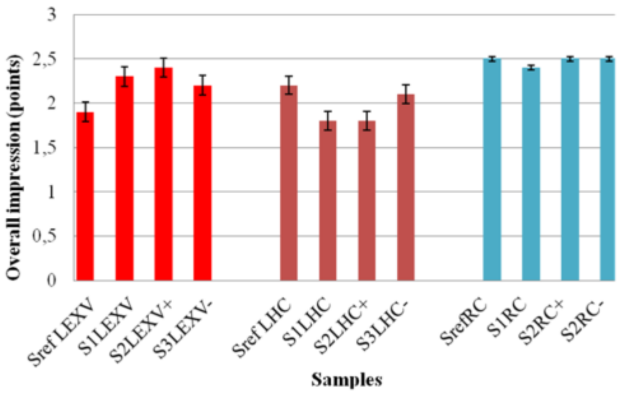



***Figure 3***. The graphical representation of the general aromatic sensory profile (general impression) of grape juices treated with pectinolytic enzyme preparations (general impression - scale with 3 points: 1 - weak, 2 - medium, 3 - high) (significance level $p < 0.05$ , $n = 4$)

In the scientific literature is highlighted the presence of ethyl caprate, above mentioned in grape varieties, of ethyl 9-decenoate in an amount of 0.32-1.07% with a retention time of 16.515 minutes [20] and nerol in an amount of 1.14-3.09% with a retention time of 14.261 minutes [20]. When processing the white grapes with the enzymatic preparation RC to obtain flavor components according to the aroma descriptors studied it is recommended to use the enzyme preparation at the doses studied because the aromatic profile is balanced. In support of this statement stay and the results of the general impression of the fermented pre-fermented juice analyzed which were ranging from 2.4 to 2.5 from 3 points (Figure 3).

Grapes contain terpineols linked to diglycosides. An enzyme complex is involved in the release of terpineol. The grape flavor characterizes the grape variety and is therefore a key element of the aromatic profile, typically, and hence of the quality of the wines. Certain aromatic compounds are found both free and as carbohydrates in the case of residues. Free aromas can be detected by odor, while the bound form is odorless. In the grape berries, the combined fraction is predominant compared to the free form. Thus, by releasing the bound form it is possible to increase the aromatic potential of the wines [7].

## 4. Conclusion

Therefore an overdose of the enzyme in the enzyme preparation leads to the release of a larger amount of pigments. Although only the LXV enzyme preparation was recommended for the improvement of the chromatic properties, the others such as LHC (for clarification) and RC (for clarification) contributed to the change in the color perception of the analyzed liquids. Their presence is influenced by the use of the following enzyme preparations: LEXV, LHC, RC, by their specific action on the white and black grapes, which resulted in a pre-fermented liquid.

**Compliance with Ethics Requirements.** Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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