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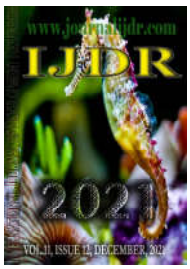

RESEARCH ARTICLE OPEN ACCESS

CHARACTERIZATION OF VOLATILE AND SENSORY PROFILE DESCRIPTION OF DRY RED TABLE WINE (*CV. ISABEL*) PRODUCED IN ARTISANAL AND INDUSTRIAL SCALE WITH ADDITION OF PECTINASE

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ABSTRACT

Due to the importance of the wine industry in Santa Teresa - ES and the limited availability of information on the composition of volatile and sensory profile of these wine, we performed the present study aiming to characterize the composition of volatile and sensory profile of dry red table wines of two cellars of Santa Teresa - ES, produced in artisanal and industrial scale without addition of pectinolytic enzyme, and microvinification with and without addition of pectinolytic enzyme. Wines produced in the cellars constituted 6 treatments with 3 replications, totaling 18 experimental units, with treatments: AE1 (artisanal without enzyme), AE2 (artisanal microvinificated without enzyme), AE3 (artisanal microvinificated with enzyme), BE1 (industrial without enzyme), BE2 (microvinificated industrial without enzyme), and BE3 (microvinificated industrial with enzyme). In AE1 and BE1, tanks of varying volumes in microvinification (treatments: AE2, AE3, BE2 and BE3) were used with polypropylene tanks of 50L. The soaking period was 5 days (cellar A) and 7 days (cellar B), with 3 pumpings per day. Twenty (20) g of potassium metabisulfite in hL^{-1} were applied, where active dry yeast Maurivin™ - UCD 522/AB *Mauri* (*Saccharomyces cerevisiae*) was inoculated, and the pectinolytic enzyme Coavin MX®/*AB* was added to treatments E3 and BE3. After racking and pressing, the wines were chaptalized with crystal sugar and transferred to 6 polypropylene tanks of varying volumes in artisanal (AE1) and industrial (BE1) winemaking. In microvinification (treatments: AE2, AE3, BE2 and BE3), they were transferred to 12 polypropylene tanks of 30 L, with 3 rackings being performed. Bottling was conducted in manual bottler and packaged in new and dark bottles of 750 ml, sealed with a cork stopper and identified according their treatments. The bottles were stored in horizontal position, remaining wrapped in a dry, ventilated area, protected from light and at room temperature. Gas chromatography volatiles analysis (GC-FID), descriptive analysis and principal component analysis were performed. The cellar factor (A) showed higher concentration of 2-phenylethanol, Monosuccinate acetate, Linalool, and lower concentration of Trans-3-hexen-1-ol. The enzyme factor (E3) had the highest concentration of ethyl lactate, and lower concentration of phenylethyl acetate. The results show that the concentrations of volatiles in E3 and BE3 treatments were lower than in the other treatments, with the exception of the 2-Methyl-1-butanol volatile. The Principal Component Analysis (PCA) applied to the volatiles of alcohol, ether, and aldehyde + acetate + monoterpenic classes discriminated treatment BE3, and when applied to the class C_6, it discriminated treatment AE3. The cellar factor did not influence the aroma (foxy, fruity and floral) and flavor descriptors (sweetness); and the enzyme factor did not influence the aroma (foxy, fruity and floral) and flavor was descriptors (sweet, bitter, persistence, and astringency). The PCA applied to the intensity of sensory taxes managed to discriminate the wines produced in cellars A and B after the introduction of pectinolytic enzyme. The wine of treatment AE3 showed higher intensity of Odor (undesirable), Flavor (acidity, bitterness, and astringency) and poor appearance (clarity) attributes. The wine of treatment BE3 was characterized by lower intensities in Appearance (clarity), Aroma (fruity and floral), and Flavor (sweetness and Isabel typicality).

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INTRODUCTION

The quality and ranking of wine can also be provided by the volatile compounds that distinguish its aroma. This, in turn, is given by the presence of volatile substances with odoriferous activity that reach the olfactory receptors, which can discriminate thousands of compounds. The flavor of the wine is due to many volatile and nonvolatile organic compounds, which may be divided into several groups, according to their chemical nature. Higher alcohols, fatty acids and esters form, quantitatively and qualitatively, the largest group in the fraction of volatile aroma of alcohol drinks, with higher alcohols being the most abundant (BERRY, 1995). Among the various sensory attributes of wine, aroma is one of the most important, and it can also induce flavor sensations. Falcão *et al*. (2008) mention that compounds of aromatic impacts can assist and guide the development of higher-quality wines. Over 700 compounds have been isolated and identified in the volatile fraction of the various wines (JACKSON, 2008) at concentrations ranging from hundreds of $mg.L^{-1}$ to the level of $ng.L^{-1}$. Sensory analysis is used to evoke, measure, analyze and interpret reactions of the characteristics of food or other materials the way they are perceived by the senses of sight, smell, flavor, touch and hearing (JESUS, 2010). Human perception of the attributes of wine can be measured using traditional practices of sensory evaluation in order to detect differences among product samples, quantifying the sensory attributes by the application of descriptive evaluation and testing the refusal or acceptance of wines by consumers (STONE and SIDEL, 2004). Pectinolytic enzymes may be used in the vinification process, since they have the advantages of facilitating the extraction of polyphenols (especially anthocyanins), enhancing color, favoring pressing, increasing yield in must and promoting the clarification/filtration of wine (AMORIM *et al.*, 2006). According to Ducret and Glories (2002), the use of pectinase enzyme provides greater extraction of coloring matter and chemical compounds in general. Due to the importance of the wine industry in Santa Teresa – ES and the limited availability of information on the composition of volatile and sensory profiles of this wine, we carried out the present study aiming to characterize the volatiles composition and the sensory profile of dry red table wines from two cellars of Santa Teresa - ES, produced in artisanal and industrial scale without adding pectinolytic enzyme and microvinification with and without addition of pectinolytic enzyme.

MATERIALS AND METHODS

Choice of cellars: Initial contacts made with the Association of Producers of Grape and Wine of Santa Teresa (APRUVIT) led to the selection of two designated cellars: A (artisanal) – winemaking in adapted premise and in polyethylene/polypropylene tanks; and B (industrial) – winemaking in appropriate facility and in polypropylene tanks.

Experimental planning: Wines produced in two cellars, the first being artisanal (A) and the other one industrial (B), consisted in six treatments with three replicates each, totaling 18 experimental units, with the following treatments (Figure 1):

Figure 1. Treatments

AE1: artisanal cellar/without enzyme AE2: artisanal cellar/microvinificated without enzyme AE3: artisanal cellar/microvinificated with enzyme
BE1: industrial cellar/without enzyme BE2: industrial cellar/microvinificated without enzyme BE3: industrial cellar/microvinificated with enzyme

Experimental design and statistical analysis: In the analysis of volatile compounds, the experimental delineation was completely randomized (DIC) in a 2x3 factorial design (cellar factor x enzyme factor) with three replications.

Data were subjected to analysis of variance (ANOVA), Tukey test (enzyme) at 5% probability. Besides these, a Principal Component Analysis (PCA) was performed. In sensory analysis, the experimental delineation was the randomized block (DBC) and having the judges as a block in a 2x3 factorial design (cellar factor x enzyme factor) with three replications. Data were subjected to analysis of variance (ANOVA) (F.V.: sample, judge and sample*judge), mean comparison test (*Tukey*) at 5% probability and Principal Component Analysis (PCA). Procedures of the SAS program (Statistical Analysis System - SAS Institute Inc., North Carolina, USA 1992), version 9.2, licensed to the Federal University of Viçosa/UFV – MG, were used for the statistical analysis.

JUDGE:
DATE:
RATE (note 1-9) THE PERCEIVED INTENSITY

1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9
Not Intense								Very Intense								

Evaluation	
Appearance (Visual Aspect)	
Clarity	
Full intensity	
Violet hue	
Aroma (Olfactory Appearance)	
Full intensity	
Foxy (labrusca)	
Fruity (strawberry / raspberry)	
Floral	
Undesirable odor	
Flavor (Palate Appearance)	
Full intensity	
Sweetness	
Acidity	
Adstringency	
Bitterness	
Persistence	
Isabel typicality (labrusca)	
Texture (Oral Sensations)	
Body / Structure	

Figure 2. Descriptive sheet used for analysis of dry red table wines

Production of wines: The dry red table wines produced in artisanal (A) and industrial (B) cellar are from commercial fields (crop: summer 2012) of grapes from *cv. Isabel* (*Vitis labrusca* L.) in the municipality of Santa Teresa – ES. The classic method of vinification in red wine, modified from references by Rosier (1995), Rizzon, Meneguzzo and Manfroi (2003) was used. The grape was transported to cellars A and B in polyethylene boxes with capacity for 20 kg of grapes, weighed in platform scales of the Cauduro brand, model 118PL. The berries were separated from the rachis and crushed in an inox stemmer-crusher of the brand Japa, model DZ-35 (3000 $kg.h^{-1}$) with attached pump. A sample of must was transferred to a 500 ml graduated cylinder, where the amount of sugar in gram, contained in 100 g of must, was determined with Babo winemeter. In artisanal (E1: no enzyme) and industrial (BE1: no enzyme) scale winemaking, fermentation tanks of polyethylene / polypropylene and polypropylene of varying volumes were used, respectively. In microvinification (treatments: AE2, AE3, BE2 and BE3), 50L polypropylene tanks were used. The period of maceration (fermentation in tumultuous phase) was between 5 days (industrial cellar) and 7 days (artisanal cellar) with three daily pumping over it. During this stage, 20 g of potassium metabisulphite per hL^{-1} of must was applied. This was inoculated with active dry yeast (*Saccharomyces cerevisiae*) Maurivin™ - UCD 522 produced by *AB Mauri* in the amount of 20 $g.hL^{-1}$ of must, with Coavin MX® pectinolytic enzyme, produced by *AB Enzymes* in the recommended dosage of 3-1 $mL.hL^{-1}$ of must, being added to treatments E3 and BE3. After separating the liquid from the solid and pressing, the musts were chaptalized with crystal sugar (5.4 kg sugar per hL of must). The fermented musts were recollected and transferred to six fermentation tanks of polyethylene/polypropylene in various amounts of winemaking in artisan scale (AE1) and industrial scale (BE1), and

in twelve polypropylene tanks of 30 L in microvinification (AE2, AE3, BE2 and BE3), all fitted with hydraulic bung. Fermentation in its slow phase lasted 20 days, with the first racking performed 15 days after the end of the slow phase of fermentation. The second racking was performed 30 days after the first racking, adding 8 g of potassium metabisulfite per hL of must. The third racking was performed 30 days after the second racking. Topping was performed after each racking. Bottling in cellars was held in the JAPA semiautomatic filler brand. Wine was put into new and dark 750 mL bottles, sealed with cork stopper and identified according to their respective treatments. The bottles were stored in horizontal position, remaining wrapped in a dry, ventilated place, protected from light and at temperature of 25±1°C.

Determination of volatile compounds: The determination of volatile compounds was performed at the Laboratory of Physiology and Genetics of Microorganisms in the Department of Biology at the Federal University of Lavras (UFLA), in Lavras – MG. Bottles with 750 mL of dry red table wine from the cellars (A and B), properly identified from each treatment (AE1, AE2, AE3, BE1, BE2 and BE3) with three replications, totaling 18 bottles, were randomly collected. The bottles were stored in horizontal position and transported to the laboratory in cardboard box, remaining wrapped at temperature of 25±1°C until the analyses began. The micro extraction (HS-SPME) was performed using 5 mL sample added with 1 g of NaCl packaged in amber vials. The sealed vials were stored at 60°C with exposure of fiber for 15 minutes. The analyses were performed using Shimadzu gas chromatograph (GC), model 17^A, equipped with flame ionization detector (FID) and DB Wax silica capillary column (30 m x 0.25 mm i.d. x 0.25 μm) (J&W Scientific, Folsom, Calif., USA). The operating conditions were as follows: oven temperature maintained at 50°C.5 min-1, increased to 190°C in increments of 3°C min^{-1} and then held at 190°C.10 min^{-1}. The temperatures of the injector and detector were maintained at 240°C; for desorption of the compounds, the fiber was maintained for 5 minutes in the gun; the carrier gas (N_2) was kept in a flow of 1.2 $mL.min^{-1}$; and injections were made in split mode (1:10). The identification of volatiles was performed by comparison of retention times of the compounds of the samples with those of the same compounds injected under the same conditions. The internal calibration method was used for quantification (semi-quantitative analysis) of the identified volatile compounds, and the concentrations were expressed as equivalents of 4-nonanol (internal standard) in a final concentration of 249.50 □$g.L^{-1}$ (Santos *et al.*, 2013). The 1-Hexanol, Ethyl lactate, Ethyl octanoate, Diethyl succinate, 4-Nonanol, 2-Phenylethanol, Geraniol, Menthol, α-Terpeniol b-Citronellol and Trans-3-hexen-1-ol compounds were purchased from Aldrich Chemical (Munich, Germany). The Acetaldehyde, 1-Butanol, 1-Propanol, 2-Methyl-1-propanol, 2-Methyl-1-butanol, Phenylethyl acetate, Isoamyl acetate, Butyric acid, Isobutyric acid, Hexanoic acid, Octanoic acid, Decanoic acid compounds were purchased from Fluka Analyticals (Seelze, Germany). The 3-Methyl-pentanol, Ethyl Monosuccinate and Benzoic acid compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA). The Linalool acetate and butyrate compounds were purchased from Acros Organics (Geel, Belgium).

Sensory Analysis: Sensory analysis was performed at the Laboratory for Sensory Analysis of the Brazilian Agricultural Research Corporation (EMBRAPA) / National Research Center of Grape and Wine (CNPUV), in Bento Gonçalves – RS. Bottles with 750 mL of dry red table wine from the cellars (A and B), properly identified from each treatment (AE1, AE2, AE3, BE1, BE2 and BE3) were randomly collected with three replications, totaling 36 bottles. The bottles were shipped in cardboard box into the room for sample preparation of sensory analysis laboratory. The wines were stored in the freezer in horizontal position and at temperature of 18 ±1°C until the analyses began. The wines were analyzed in October 2012, through the tasting panel of EMBRAPA/CNPUV, composed of eleven trained judges (nine men and two women aged between 25 and 58 years) with extensive experience in sensory description of wine.

The sensory evaluation was based on the modified Quantitative Descriptive Analysis (QDA) used by EMBRAPA/CNPUV in other analyzes of fine wine and table. With the objective of exercising the sensory memory, the judges were provided two samples of dry red wine table, with one of a commercial brand and another of the wine to be analyzed, randomly chosen among treatments. This exercise allowed the use and familiarity of the evaluation form, allowing judges to practice and identify the descriptive terminology developed (16 descriptors). After this exercise, a meeting was held to discuss the results, to clarify the terminology and use of the scale and to discuss any questions. The samples (treatments: AE1, AE2, AE3, BE1, BE2, and BE3) with three replications, were coded with random three-digit numbers on the bottles. Twenty (20) ml of wine at a temperature of 18±1°C in monadic and balanced way were served to judges, in crystal glasses (model ISO 3591: 1977), in individual booths under white light. Between one sample and another, the judges drank mineral water at 22±1°C, and wrote down the answers in a description form (Figure 2). The intensity of the attributes of the samples was evaluated in a structured nine-point scale, with terms of intensity anchored at their ends.

RESULTS AND DISCUSSION

Characteristics of volatile compounds in red wine dry table: Among the compounds separated and detected by GC-FID, 27 were identified forming 7 classes (alcohols, esters, volatile fatty acids, monoterpene, C6 compounds, aldehyde and acetate). The internal calibration method was used for quantification, and the concentrations were expressed as equivalents of 4-nonanol (internal standard). Of the volatile compounds identified, 6 belong to the alcohol class (1-Propanol, 2-Methyl-1-propanol, 1-Butanol, 2-Methyl-1-butanol, 3-Methyl-1-propanol, and 2-Phenylethanol); 6 to esters (Isoamyl acetate, Ethyl butyrate, Diethyl succinate, Ethyl lactate, Mono ethyl succinate, and Ethyl octanoate); 6 to volatile fatty acids (Benzoic acid, Butyric acid, Isobutyric acid, Hexanoic acid, Octanoic acid and Decanoic acid); 5 to monoterpenIcs (Geraniol, Linalool, Menthol, α-Terpeniol, and b-Citronellol), 2 to compounds of C_6 (1-Hexanol and Trans-3-hexen-1-ol); 1 to Aldehyde (Acetaldehyde); and 1 to Acetate (Isoamyl acetate).

The most abundant compounds in wines produced in cellars A (artisanal) and B (industrial) in Santa Teresa/ES were 2-methyl-1-butanol (amyl alcohol) and 2-methyl-1-propanol (isobutyl alcohol), followed by 2-Phenyl-ethanol, Ethyl octanoate, butyric acid and octanoic acid. The higher alcohols, volatile fatty acids and esters form, quantitatively and qualitatively, the largest group in the fraction of volatile aroma of alcohol, higher alcohols, being the most abundant alcohol (BERRY, 1995). Classes identified at higher concentrations were alcohols, volatile fatty acids, and esters, corroborating studies by Garde-Cerdan *et al.* (2008) with red wine and by Vilanova *et al.* (2010) with white wine. The analysis of variance levels of Acetaldehyde, 1-Propanol, 3-Methyl-1-pentanol, 2-Phenylethanol, Isoamyl acetate, Ethyl butyrate, Diethyl succinate, Ethyl lactate, Mono ethyl succinate, Benzoic acid, Phenylethyl acetate, Geraniol, Linalool, Menthol, α-Terpeniol, b-Citronellol, and Trans-3-hexen-1-ol detected no significant effect ($p > 0.05$) from the cellar*enzyme interaction. Levels of 2-phenylethanol, Mono ethyl succinate, Linalool, and Trans-3-hexen-1-ol were considered significant effects for the cellar ($p<0.05$), and for enzyme: Ethyl lactate and Phenylethyl acetate. We conclude that the cellar did not influence the levels of Acetaldehyde, 1-Propanol, 3-Methyl-1-pentanol, Isoamyl acetate, Ethyl butyrate, Diethyl succinate, Ethyl lactate, Benzoic acid, Phenylethyl acetate, Geraniol, Menthol, α-Terpeniol, and b-Citronellol; the enzyme did not affect the levels of Acetaldehyde, 1-Propanol, 3-Methyl-1-pentanol, 2-Phenylethanol, Isoamyl acetate, Ethyl butyrate, Diethyl succinate, Mono ethyl succinate, Benzoic acid, Geraniol, Linalool, Menthol, α-Terpeniol b-Citronellol and Trans-3-hexen-1-ol. The cellar ($p<0.05$) influenced in the levels of 2-Phenyl-ethanol, Mono ethyl succinate, Linalool, and Trans-3-hexen-1-ol; and the enzyme ($p <0.05$) influenced in the concentrations of Ethyl lactate and Phenylethyl acetate. The effect of cellars in volatile compounds of dry red table wines (cv. Isabel.) Santa Teresa – ES in summer crop of 2012 are listed in Table 1.

Table 1. Mean contents and standard deviation of the cellar in volatile compounds of dry red table wines (cv. Isabel) of Santa Teresa – ES in the summer 2012 crop

	Cellars	
	A	**B**
2-Phenyl-ethanol (μg.L^{-1})	1137,51±283,90	764,44±261,16
Ethyl monosuccinate (μg.L^{-1})	7,98±6,03	2,63±1,13
Linalool (μg.L^{-1})	11,34±4,24	5,11±3,66
Trans-3-hexen-1-ol (μg.L^{-1})	3,64±1,41	5,80±1,70

Table 2. Mean contents and standard deviation of the pectinolytic enzyme in volatile compounds of dry red table wines (cv. Isabel) of Santa Teresa – ES in the summer 2012 crop

	Enzymes		
	E1	E2	E3
Ethyl lactate (μg.L^{-1})	70,40±14,26b	72,26±10,79b	99,33±28,54a
Phenylethyl acetate (μg.L^{-1})	49,82±22,95ab	69,99±19,70a	43,37±15,58b

Means followed by the same letter within rows, for each characteristic, do not differ by Tukey test (p<0.05).

Table 3. Mean contents of the pectinolytic enzyme in volatile compounds of dry red table wines (cv. Isabel) of Santa Teresa – ES

(μg L^{-1})		Enzymes		
		E1	E2	E3
2-Methyl-1-Propanol	Cellar A	9904,9A	5345,3B	4924,2B
	Cellar B	3888,9A	4906,3A	5308,0A
1-Butanol	Cellar A	7,6A	88,3A	74,7A
	Cellar B	185,0A	139,9AB	56,0B
2-Methyl-1-Butanol	Cellar A	36752,6A	30205,3A	33122,1A
	Cellar B	25207,1B	28865,6B	41963,3A
Ethyl octanoate	Cellar A	500,6A	417,3A	459,1A
	Cellar B	693,2A	577,6A	226,0B
Isobutyric acid	Cellar A	23,3B	43,0A	28,4B
	Cellar B	22,6A	20,8A	23,4A
Butyric acid	Cellar A	426,3A	187,4B	315,3B
	Cellar B	315,6A	256,9AB	125,1B
Hexanoic acid	Cellar A	29,4A	74,8A	61,9A
	Cellar B	104,5A	86,0A	22,8B
Octanoic acid	Cellar A	232,3A	388,0A	389,7A
	Cellar B	808,9A	651,2A	138,8B
Decanoic acid	Cellar A	172,5A	150,7A	218,1A
	Cellar B	319,5A	278,5A	66,9B
1-Hexanol	Cellar A	234,3C	362,2B	455,7A
	Cellar B	389,6B	469,4A	398,6B

Means followed by the same letter within lines, for each characteristic, do not differ by Tukey test (p<0.05).

Table 4. Mean scores and standard deviation for flavor descriptors (bitterness, persistence, and astringency) of dry red table wines (cv. Isabel) of Santa Teresa – ES in the summer crop of 2012

	Cellars	
	A	B
Flavor/bitterness	3,34±1,71	2,92±1,72
Flavor/persistence	5,48±1,22	5,19±1,27
Flavor/astringency	3,25±1,25	2,73±1,05

Table 5. Mean scores and standard deviation of the addition of pectinolytic enzyme in the characteristics of the sensory profile of dry red table wines (cv. Isabel) of Santa Teresa – ES in the crop of summer 2012

		Enzymes		
		E1	**E2**	**E3**
Appearance / clarity	Cellar A	8,11±0,48A	8,08±0,51A	4,30±1,71B
	Cellar B	8,49±0,69A	8,65±0,37A	8,50±0,49A
Appearance / full intensity	Cellar A	6,56±0,95B	7,91±0,66A	5,33±1,20C
	Cellar B	6,03±1,38A	5,85±1,33A	6,21±1,31A
Appearance / violet hue	Cellar A	3,32±1,49B	5,39±1,40A	2,79±1,09B
	Cellar B	3,32±1,40A	2,94±1,48A	3,48±1,68A
Aroma / Full intensity	Cellar A	6,70±0,93AB	7,14±0,67A	6,47±0,90B
	Cellar B	6,52±0,70A	6,55±0,93A	6,91±0,69A
Unwanted odor	Cellar A	1,58±0,73B	2,86±1,64A	3,12±1,46A
	Cellar B	1,91±1,48A	2,47±1,16A	1,59±0,87A
Flavor / full intensity	Cellar A	6,56±0,87A	6,53±0,99A	5,97±1,02B
	Cellar B	5,85±1,40A	5,64±1,21A	6,12±1,08A
Flavor / acidity	Cellar A	6,71±0,97A	7,26±1,11A	7,45±0,91A
	Cellar B	6,58±1,48A	6,52±1,31A	5,85±1,28B
Flavor / Isabel typicality	Cellar A	6,03±1,30A	5,80±1,59A	5,17±1,31B
	Cellar B	5,67±1,33A	5,47±1,25A	5,98±1,28A
Texture/body – structure	Cellar A	5,29±1,16A	5,54±1,16A	4,43±1,17B
	Cellar B	4,64±1,41A	4,64±1,20A	5,06±1,07A

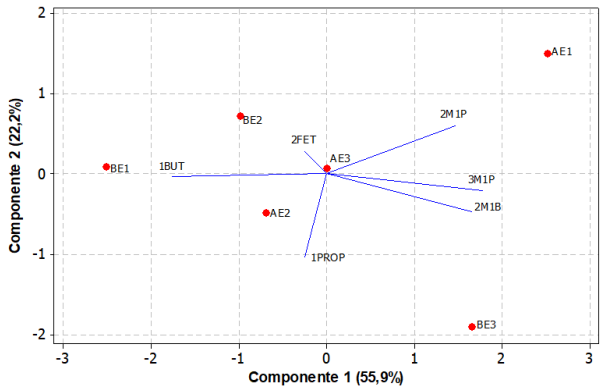
Means followed by the same uppercase letter within lines, for each characteristic, do not differ by Tukey test (p<0.05).

The mean values of 2-Phenyl-ethanol, Ethyl monosuccinate, Linalool for cellars (A and B) are different. In the content of 2-Phenylethanol, the greater value is cellar A (artisan, 1137, 51 μg.L^{-1}), indicating a greater aeration of the must, i.e., longer maceration.

The excessive fermentation favors aeration and, as a consequence, higher consumption of nitrogenous compounds, which are the precursors of higher alcohols (RIBÉREAU-GAYON *et al*., 2000; GUERRA, 2010).

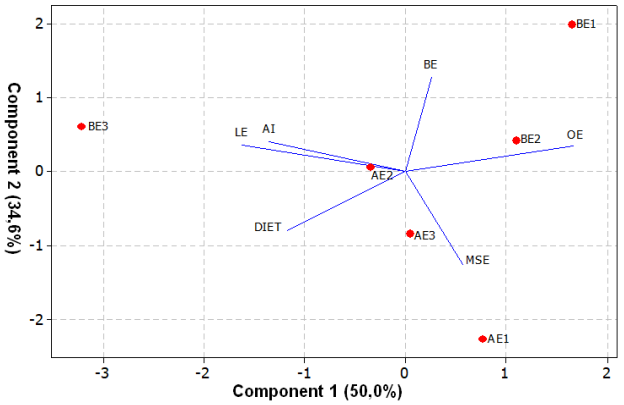
Regarding Ethyl monosuccinate (Table 1), cellar A (artisanal) obtained the highest concentration (7,98 µg.L^{-1}), probably the largest due to the must aeration. In this sense, there are controversial results. Valero *et al.* (2002) found that the presence of oxygen favors the formation of esters, however, Moio *et al.* (2004) observed that in the absence of oxygen, the formation of these compounds were higher. Concentrations of Linalool (cellar A: 11.34 µg.L^{-1}) and Trans-3-exen-1-ol (cellar B: 5.80 µg.L^{-1}) were affected by the cellar factor. Many of these volatile compounds are often found in wine and come from grapes, fermentation and winemaking process (CLIFF *et al.*, 2002). Marcon *et al.* (2011) concluded in their studies that the highest concentration of terpenes is achieved with 6 days of maceration, with high concentration of citronellol, followed by linalool, α-terpeniol and geraniol. Table 2 shows the results of the average concentrations of volatile compounds of enzyme effect of red wines dry table (cv. Isabel) of Santa Teresa – ES in the summer 2012 crop. The effect of the enzyme in the production of dry red wine table ($p<0.05$) influenced in the concentrations of Ethyl lactate and Acetate phenylethyl, and in enzyme E3 (microvinificated with enzyme), wine had the highest concentration of Ethyl lactate (99,33 µg.L^{-1}) and the lowest of Acetate phenylethyl (43.37 µg.L^{-1}). One purpose of adding enzyme in red wines is aromatic characterization, however, under the conditions of this study, increase in the concentration of Phenylethyl acetate (Table 2) was not observed, i.e., the effect of enzyme seems to be dependent on many variables. In the variance analysis, 2-Methyl-1-propanol, 1-Butanol, 2-Methyl-1-Butanol, Ethyl octanoate, Isibutyric acid, Butyric acid, Hexanoic acid, Octanoic acid, Decanoic acid, and 1-Hexanol ($p> 0.05$) were significantly affected by the cellar*enzyme interaction. The unfolding of the interaction is shown in Table 3. In Table 3, the volatile belonging to the class of alcohols that showed the highest levels of 2-Methyl-1-butanol (41963.3 µg.L^{-1}) was the wine of treatment BE3 (industrial cellar / microvinificated with enzyme), which differed significantly ($p<0.05$) from treatments BE1 and BE2.

The highest content of 2-Methyl-1-propanol (9904.9 microg.µg.L^{-1}) was treatment AE1 (artisanal cellar/without enzyme), which differed significantly ($p<0.05$) from the other treatments. The content of 1-Butanol (185.0 µg.L^{-1}) in wine of treatments BE1 (industrial cellar / no enzyme) differed significantly ($p> 0.05$) from treatment BE3 (industrial cellar / with enzyme) and did not differ ($p> 0.05$) from treatment BE2 (industrial cellar / microvinificated without enzyme). In the concentration of ester, the Ethyl octanoate compound (226.0 µg.L^{-1}) was lower in treatment BE3 (industrial cellar / microvinificated with enzyme), differing significantly ($p> 0.05$) from treatments BE1 (industrial cellar / without enzyme) and B2 (industrial cellar / microvinificated without enzyme). There was no significant difference ($p> 0.05$) for Ethyl octanoate between treatments AE1 (artisanal cellar / without enzyme), AE2 (artisanal cellar / microvinificated without enzyme), and AE3 (artisanal cellar / microvinificated with enzyme) (Table 3). The addition of enzyme did not influence the concentrations of higher alcohols and esters in treatments AE3 (artisanal cellar / microvinificated with enzyme) and BE3 (industrial cellar / microvinificated with enzyme) treatments, except in the higher alcohol content of 2-Methyl-1-butanol. The concentrations obtained in the fatty acids class (Table 3), and the concentrations of Isobutyric acid (43.0 µg.L^{-1}) and Butyric acid (426.3 µg.L^{-1}) were higher and differed significantly ($p<0.05$) in treatment AE2 (artisanal cellar / microvinificated without enzyme) and in treatment AE1 (artisanal cellar / without enzyme), respectively. The Hexanoic (22.8 µg.L^{-1}), Octanoic (138.8 µg.L^{-1}) and Decanoic acids (66.9 µg.L^{-1}) were lower in treatment BE3 (industrial cellar / microvinificated with enzyme), differing significantly ($p<0.05$) from other treatments. The addition of enzyme did not influence the levels of fatty acids in wine from treatment BE3 (industrial cellar / microvinificated with enzyme). In the volatile compound of class C_6, the 1-Hexanol (469.4 µg.L^{-1}) compound was significantly different ($p<0.05$) in treatment BE2 (industrial cellar / microvinificated without enzyme) from the other treatments. The results of this study demonstrate that the concentrations of volatiles in treatments AE3 (artisan cellar / microvinificated with enzyme) and BE3 (industrial cellar / microvinificated with enzyme) were lower, with exception of



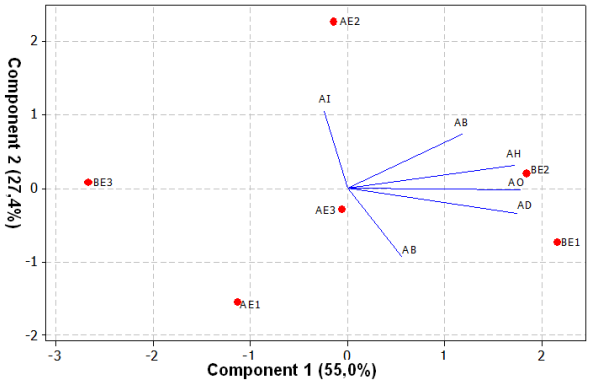
Caption: 1PROP - 1-propanol; 2M1P - 2-methyl-1-propanol; 1BUT - 1-butanol; 2M1B - 2-methyl-1-butanol; 3M1P - 3-methyl-1-propanol and 2FET - 2-Phenyl ethanol

Figure 3. Arrangement of volatile compounds (alcohols class) and treatments in relation to the first two principal components



Legend: AI - isoamyl acetate; BE - ethyl butyrate; DIET - diethyl succinate; LE - ethyl lactate; MSE - Monosuccinate ethyl, and OE - ethyl octanoate

Figure 4. Arrangement of volatile compounds (alcohols class) and treatments in relation to the first two principal components



Caption: ABE - benzoic acid; ABU - butyric acid; AI - isobutyric acid; HA - hexanoic acid; AO - octanoic acid, and AD - decanoic acid.

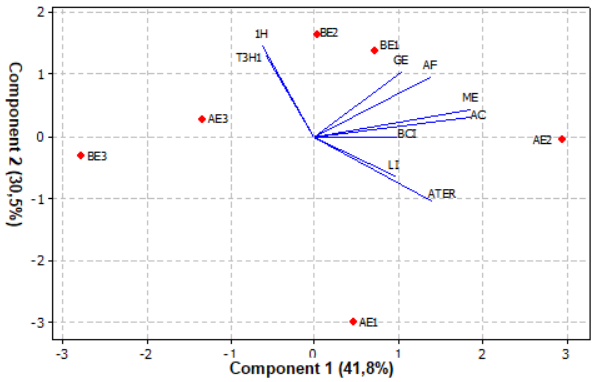
Figure 5. Arrangement of volatile compounds (fatty acids class) and treatments in relation to the first two principal components

the volatile 2-Methyl-1-butanol and, for this reason, the effect of the cellars (A and B) and the addition or non-addition of the enzyme appears to be dependent on several variables. In Figures 3, 4, 5, and 6, the graphs of the Principal Component Analysis (PCA) of volatile compounds generated from the classes of alcohols, esters, volatile fatty acids, and aldehyde + acetate + monoterpene + C_6 compounds are shown, respectively. In this type of chart, levels of volatiles are represented by vectors. The importance of the compounds in the discrimination of treatments is directly related to the size of the vector and its proximity with the treatment. The axes explain the percentage

of variation among treatments (AE1, AE2, AE3, BE1, BE2, and BE3). According to the PCA of the alcohols (1-Propanol, 2-Methyl-1-propanol, 1-Butanol, 2-Methyl-1butanol, 3-Methyl-1-propanol, and 2-Phenylethanol), it was possible to observe that the first two principal components (CP1 *versus* CPPC2) accounted for 78.1% of the variability in the data, with CP1 accounting for 55.9% of the variation occurred, and CP2 explained 22.2% of the variation among treatments (Figure 3). The discrimination of treatments can be verified by the size of the vector representing each volatile, that is, the higher the vector, the more important it is to discriminate between treatments. The principal component 1 (PC1) managed to explain 55.9% of the variation between treatments and 3-Methyl-1-propanol, 2-Methyl-1-butanol, and 2-Methyl-1-propanol volatile compounds along with the positive region of CP1, and 1-Butanol with the negative region of CP1. The principal component 2 (PC2) explained 22.2% of the variation between treatments and is associated with 2-Phenylethanol in the positive region and with 1-Propanol in the negative region of the axis. Thus, the rightmost treatments are located on this axis, the greater the concentration of 3-Methyl-1-propanol, 2-Methyl-1-butanol, and 2-Methyl-1-propanol and, more to the left, the greater the concentration of 1-Butanol (Figure 2). Axis 2 (CP2) of the positive sides is associated with volatile 2-Phenylethanol, and the negative to volatile 1-Propanol.

The smaller size of the vector 2-Phenylethanol suggests that this volatile has a minor contribution to discriminate the treatments. Treatment BE1 (industrial cellar/without enzyme) is located furthest to the left of the negative region of CP1, suggesting a higher concentration of alcohol added to this region of the axis, namely 1-Butanol. Treatment BE2 (industrial cellar/microvinificated without enzyme) is located in the same quadrant, showing the same profile. To Brerenton (2000), PCA employs a mathematical procedure that transforms a set of correlated response variables into a new set of uncorrelated variables called principal components (PCs). PCA can be used to reduce the number of original variables into a smaller number of variables, or PCs, keeping the largest and most important CPs. The positive region of CP1 is associated with 3-Methyl-1-propanol, 2-Methyl-1-butanol, and 2-Methyl-1-propanol volatile alcohols. These alcohols are in greater concentration in treatments AE1 (artisanal cellar / without enzyme) and BE3 (industrial cellar / microvinificated with enzyme); on the other hand, it presents low concentrations of 1-Butanol. Further to the right, in the quadrant between the positive parts of axis 1 and axis 2, is treatment AE1 (artisan cellar / no enzyme), which has a high concentration of 2-Methyl-1-propanol. The proximity of the vectors means that treatment AE2 (artisanal cellar / microvinificated without enzyme) showed high concentration of 1-Propanol when compared to treatment AE1 (artisanal cellar / without enzyme). The PCA of esters (Figure 4) was able to explain 84.6% of the variation between samples. The principal component 1 (PC1) explained 50.0% of the variations and had its positive portion aggregated to Ethyl octanoate, and the negative to Ethyl lactate, Isoamyl acetate and Diethyl succinate. The principal component 2 (PC2) explained 34.6% of this variation and is related to Ethyl butyrate and Ethyl Monosuccinate in the positive and negative region of the axis, respectively. Treatment BE3 (cellar B / microvinificated with enzyme) differed from the other treatments (AE1, AE2, AE3, BE1 and BE2) by the higher concentration of Ethyl lactate and Isoamyl acetate.

The wines of treatments AE1 (artisanal cellar / without enzyme) and E3 (artisanal cellar / microvinificated with enzyme) are close to vector of Ethyl Monosuccinate and distant from Isoamyl acetate and Ethyl lactate. Ethyl octanoate characterized the treatment BE1 (industrial cellar / without enzyme) and BE2 (industrial cellar / microvinificated without enzyme) located in the positive portion of CP1. However, Diethyl succinate in the negative quadrant (CP1 and CP2) provided low concentration in these treatments (Figure 4). The PCA of the class belonging to volatile fatty acids (Figure 5) explained 82.4% of the differences among treatments. The positive part of axis 1 (PC1: 55.0%) is determined by the parameters of Octanoic acid, Decanoic acid, Hexanoic acid and Butyric acid; the more treatments to the right, the higher the values of these parameters. Thus, axis 1 suggests that treatments BE1 (industrial cellar / no enzyme) and BE2 (industrial cellar / microvinificated without enzyme) that ranged far right of the graph and next to vectors Octanoic acid, Decanoic acid, Hexanoic acid and Butyric acid, had higher values for these parameters. The principal component 2 (PC2) explained 27.4% of this variation and is associated with Butyric acid and Isobutyric acid in the positive and negative regions of the axis, respectively (Figure 4). CP2 can separate treatments AE1 (artisanal cellar / no enzyme) and AE2 (artisanal cellar / microvinificated without enzyme), mostly by concentrations of Butyric Acid (positive correlation with CP2) and Isobutyric acid (negative correlation with CP2). Treatments AE1 (artisanal cellar / without enzyme) and BE3 (industrial cellar / microvinificated with enzyme) apparently were not influenced by volatile fatty acids studied, due to their low concentrations. Figure 6 shows the PCA for aldehyde + acetate + monoterpene + compounds C_6, the first two axes (PC1 vs. PC2) explain 72.3% of the differences among treatments. This figure shows the graph of scores of CP1 (with 41.8% of the variance) versus PC2 (30.5% variance). The positive part of the axis 1 (CP1: 41.8%) is determined by the Acetaldehyde, Menthol, α-Terpeniol, Fenietil acetate, Geraniol And Linalool parameters. The positive part of axis 2 (CP2: 30.5%) is determined by the 1-Hexanol and Trans-3-hexen-1-ol parameters. Thus, axis 1 suggests that treatment E2 (artisan cellar / microvinificated without enzyme) were amounted rightmost in the graphic and close to the Acetaldehyde, Menthol, α-Terpeniol, Fenietil acetate, Geraniol, Linalool, and b-Citronellol vectors, showing the highest values for these parameters. Treatments AE3 (artisanal cellar / microvinificated with enzyme) and BE2 (industrial cellar / microvinificated without enzyme) showed higher concentrations of 1-Hexanol and Trans-3-hexen-1-ol, however, treatments AE1 (artisanal cellar / no enzyme) showed the lowest concentrations of Acetaldehyde, Menthol, Fenietil acetate and Geraniol, and BE3 (industrial cellar / microvinificated with enzyme) of Acetaldehyde, Menthol, α-Terpeniol, Fenietil acetate, Geraniol, Linalool, and b-Citronellol (Figure 6).



Caption: AC - Acetaldehyde; AF - Phenylethyl acetate; GE - Geraniol; LI - Linalool; ME - Menthol; ATER - α-Terpeniol; BCI - b-Citronellol; 1H - 1-Hexanol and T3H1 - Trans-3-hexen-1-ol.

Figure 6. Arrangement of volatile compounds (aldehyde + acetate + monotherpenic + C_6 compounds class) and treatments in relation to the first two principal components

Sensory profile of red wines dry table: Two sessions for the development of descriptive terminology, proposition and testing of the references and making of the record of the descriptive analysis (Figure 2) were necessary, with structured scales (every half point) of 15 cm, with terms of intensity anchored at their ends, and the minimum being on the left (not intense: 1) and the maximum on the right (very intense: 9), therefore a scale from 1 to 9 points. The appearance (visual aspect) of the samples was described with the terms of clarity, full intensity and violet hue. As to aroma (olfactory aspect), the descriptors used were full strength, foxy (labrusca), fruity (strawberry/raspberry), floral and undesirable odor. The flavor (gustatory aspect) was described with terms of full intensity, sweetness, acidity, astringency, bitterness, persistence, Isabel typicality (labrusca). For the texture (mouth sensations), the body/frame descriptor was used, totaling 16 sensory attributes.

Variance analysis of aroma descriptors (foxy, fruity and floral), and Flavor (sweet, bitter, persistent, and astringency) did not detect significant effect (p>0.05) from the cellar*enzyme interaction. Statistically significant effects (p <0.05) were considered for the cellar factor: flavor (bitterness, persistence, and astringency). We conclude that the cellar did not influence the aroma descriptors (foxy, fruity and floral), and flavor (sweetness); and the enzyme did not influence the aroma descriptors (foxy, fruity and floral), flavor (sweetness, bitterness, persistence, and astringency). Mean scores and standard deviation for flavor descriptors (bitterness, persistence, and astringency) of dry red table wines (cv. Isabel) of Santa Teresa – ES in the crop of summer 2012 are listed in Table 4. The mean scores of flavor descriptors (bitterness, persistence, and astringency) were different between cellars A (artisanal) and B (industrial). In the variance analysis of Appearance scores (clarity, overall intensity and violet hue), Aroma (full intensity), Unwanted odor, Flavor (full intensity, acidity, and Isabel typicality), and Texture (body/structure) were significantly affected (p>0.05) by the cellar*enzyme interaction. The unfolding of the interaction is shown in Table 5. The wines from artisan cellar / microvinificated without enzyme (E2) were considered as better perceived in appearance (full intensity and violet hue), Aroma (full intensity), and the wine from artisanal cellar / microvinificated with enzyme (E3) had worst intensity perceived in appearance (clarity), differing significantly (p>0.05) from each other, as evidenced in Table 5. The undesirable odor attribute showed a significant difference (p<0.05) between treatments AE3 (artisanal cellar / without enzyme) and BE3 (industrial cellar / microvinificated with enzyme), and was statistically similar (not different: p<0.05) in all treatments. Treatments AE1 (artisanal cellar / no enzyme) and AE2 (artisanal cellar / microvinificated without enzyme) did not differ between themselves (p<0.05), but differed significantly from the others (p> 0.05) showing a better-perceived intensity in flavor (full intensity) and texture (body – structure) of wines (Table 5). The wine from treatment BE3 (industrial cellar / microvinificated with enzyme) appeared poorly intense in flavor (acidity and Isabel typicality), differing statistically (p> 0.05) from other treatments. The sensory attributes judged on the Descriptive Analysis Sheet (Figure 7) are represented by vectors in the PCA, whose decomposed resulting in each axis explain the segmentation of treatments (AE1, AE2, Ae3, BE1, BE2, and BE3) with respect to the axes (CP1 and CP2). The higher the result of a given vector (attribute) in a given axis, the most important attribute to segment treatments in that axis. The PCA (Figure 6) was applied to all treatments of dry table red wine in Santa Teresa/ES in order to get a better view in relation to the addition of pectinolytic enzyme.

Figure 6 shows PCA performed on the data in which the first two principal components (PC1 and PC2) are responsible for 82.9% of the variation between treatments. It is found that 47.6% of the variation seen between treatments were explained as CP1, while CP2 explained 35.3% of the variability of treatments. The descriptors that most contributed to the first positive axis (PC1) were Flavor (Isabel typicality, persistence, sweetness, and full intensity), Aroma (fruity, foxy, floral, and full intensity), Appearance (clarity, overall intensity and hue violet), and Texture (body/structure), the further to the right the treatments are, the higher the values of these parameters. The negative part of axis 1 (CP1) is determined by the following descriptors: Odor (unwanted) and Flavor (acidity, astringency and bitterness), and the more to the left, the higher the values of these parameters. The positive part of axis 2 (CP2) is determined by the following parameters: Aroma (floral and fruity), Flavor (sweetness and Isabel typicality) parameters, and Appearance (clarity). The negative part of axis 2 (CP2) is determined by the following descriptors: Appearance (clarity and full intensity), Aroma (full intensity and foxy), Smell (unwanted), Flavor (acidity, astringency, full intensity, persistence, and bitter) and Texture (body/structure) (Figure 7).

Treatments situated to the left of axis 1 (Figure 7), such as AE3 wines (artisanal cellar / microvinificated with enzyme) differed from wines located to the right of axis 1, for presenting higher intensity of attributes with major determinants decomposed in the left side of axis 1, which are: Odor (undesirable), Flavor (acidity, bitterness, and astringency). The more to the left of the axis I is located the sample, such as occurs with wine AE3, the greater intensity of attributes in this wine, compared to the samples situated to the right of axis I, such as treatments AE1, AE2 and BE3.

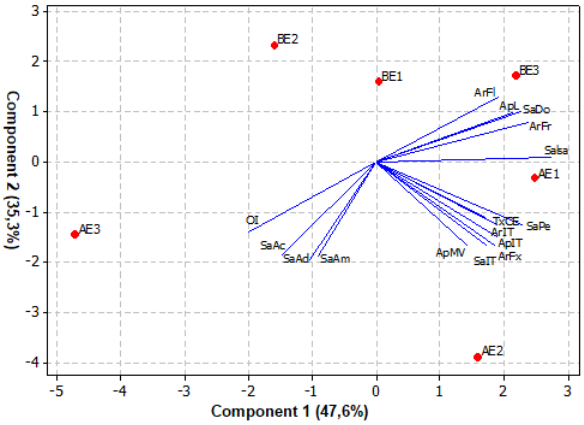


Figure 7. Arrangement of the sensory profile and treatments in relation to the first two principal components. Caption: ApL - Appearance / clarity; ApTI - Appearance / full intensity; ApMV - Appearance / violet hue; ArIT - Aroma / full intensity; ArFx - Aroma / foxy; ArFr - Aroma / fruity; ArFl - Aroma / floral; OI - unwanted odor; SaIT - Flavor / full intensity; SaDo - Flavor / sweetness; SaAC - Flavor / acidity; SaAm - Flavor / bitter; SaPe - Flavor / persistence; Salsa - Flavor / Isabel typicality ; SaAd - Flavor / astringency and TxCE - Texture / body - structure

Figure 7 also suggests that the wines located to the right of axis 1, notably treatments AE1, AE2, and BE3 have a higher intensity of the attributes that cast greater determinants on the right side of axis 1, which are: Flavor (Isabel typicality, persistence, sweetness, and full intensity), Aroma (fruity, foxy, floral and full intensity), Appearance (clarity, overall intensity and violet hue), and Texture (body/structure), showing higher values for these parameters. Treatments AE1 and AE2 showed the highest intensities of Appearance (full intensity and violet hue), Aroma (full intensity and foxy), Flavor (full intensity and persistence) and Texture attributes (body – structure). BE1 and BE2 had lower intensities of the attributes of Appearance (full intensity and violet hue), Aroma (full intensity and foxy), Smell (unwanted), Flavor (full intensity, acidity, bitterness, persistence, and astringency) and Texture (body/structure), while AE3 treatments showed the highest attributes of Odor (undesirable) and Flavor (acidity, bitterness, and astringency), and BE3 was characterized by lower levels of Appearance (clarity), Aroma (fruity and floral) and Flavor (sweetness and Isabel typicality) (Figure 5). Based on these results (Figure 7), we can state that the PCA applied to the intensity of sensory taxes failed to discriminate the wines produced in cellar A (artisanal) of cellar B (industrial), after the introduction of the winemaking process of pectinolytic enzyme.

CONCLUSION

Among the volatiles separated and detected in the wines, 27 were identified as forming 7 classes (alcohols, esters, volatile fatty acids, monoterpene, C_6 compounds, aldehyde and acetate). Cellar A (artisanal) showed higher concentration of 2-Phenyl-ethanol (alcohol), Ethyl monosuccinate (esters), Linalool, and lower concentration of Trans-3-hexen-1-ol. The wine microvinificated with enzyme (E3) had the highest concentration of Ethyl lactate and the lowest of Phenylethyl acetate. The results show that concentrations of volatiles in the artisanal cellar / microvinificated with enzyme (E3) and industrial cellar / microvinificated with enzyme (BE3) were lower than the other wines, with the exception of the volatile 2-Methyl-1-butanol. The PCA applied to volatiles of the alcohol, ether, aldehyde + acetate + monoterpene class discriminated the industrial cellar /

microvinificated with enzyme (BE3), and when applied to the C_6 class, it discriminated the artisanal cellar / microvinificated with enzyme (AE3). The cellar did not influence the scores of aroma (foxy, fruity and floral) and flavor (sweetness); and the enzyme did not influence the scores of aroma (foxy, fruity and floral) and flavor (sweetness, bitterness, persistence, and astringency). The PCA applied to the intensity of sensory taxes managed to discriminate the wines produced in cellar A (artisanal) of cellar B (industrial), after the introduction of the winemaking process of pectinolytic enzyme. The wine from the artisanal cellar / microvinificated with enzyme (AE3) showed higher intensity of scores for Odor (undesirable), Flavor (acidity, bitterness, and astringency) and was worse in appearance (clarity). The wine from the industrial cellar / microvinificated with enzyme (BE3) was characterized by lowest scores for Appearance (clarity), Aroma (fruity and floral) and Flavor (sweetness and Isabel typicality).

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