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The Composition of Minerals with Addition of Pectinase in young wines *cv. Isabel* produced both in Handmade and Full Production

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***Abstract*— *Minerals are part of the wines' ashes, take part in the clarification and stabilization process and contribute to their characterization. This work aimed at describing the dry red wine (cv. Isabel) produced in two canteens (A: handmade one and B: industrial one), regarding the possible differences in their mineral composition by adding pectinase. The wine produced in the canteens had the following treatments: HCVwtE (handmade canteen vinified without enzyme), HCMwtE (handmade canteen microvinified without enzyme), HCMwE (handmade canteen microvinified with enzyme), ICVwtE (industrial canteen vinified without enzyme) ICMwtE (industrial canteen microvinified without enzyme) and ICMwE (industrial canteen microvinified with enzyme). The P content was assessed calorimetrically; the Ca, Mg, Mn, Fe, Cu and Zn contents were assessed by atomic absorption; and the K and Na contents were assessed by flame emission. The CRD in factorial 2 x 3 (canteen x enzyme) with four repetitions was used. Data were tested with ANOVA, Tukey's test and Principal Component Analysis (PCA). The canteen modified the contents of K and Zn, and the enzyme influenced the content of Mg. The contents of P, Mn and Cu of the wine produced in the canteens weren't altered by the process of vinification with pectinase. The PCA applied to the contents of Zn, Ca, Fe, P and K distinguished the wines of the ICMwtE and ICMwE from all the others. However, it was not possible to separate the wines produced in the canteens with pectinase.*

Keywords*— *Canteens, Clarification, Industry, Artisan.

Composição de Minerais Com Adição de Enzima pectinase em Vinhos Jovens *cv. Isabel* Produzida em Escala Artesanal e Industrial

***Resumo*— *Os minerais representam uma parte das cinzas dos vinhos, participa do processo de clarificação, estabilização e contribuem para sua caracterização. Objetivou-se caracterizar o vinho tinto seco de mesa (cv. Isabel) produzido em duas cantinas (A: artesanal e B: industrial) podendo se diferenciar em sua composição mineral com adição de pectinase. Os vinhos produzidos nas cantinas constituíram nos seguintes tratamentos: CAVsE (cantina artesanal vinificado sem enzima), CAMsE (cantina artesanal microvinificado sem enzima), CAMcE (cantina artesanal microvinificado com enzima), CIVsE (cantina industrial vinificado sem enzima), CIMsE (cantina industrial microvinificado sem enzima), e CIMcE (cantina industrial microvinificado com enzima). O teor de P foi determinado por calorimetria. Os teores de Ca, Mg, Mn, Fe, Cu e Zn foram feitas por absorção atômica, enquanto K e Na por emissão de chama. O delineamento utilizado foi DIC em fatorial 2 x 3* *(cantina x enzima) com quatro repetições. Os dados foram submetidos à análise de variância, teste de Tukey e a Análise de Componentes Principais (ACP). A cantina modificou os teores de K e Zn, e a enzima influenciou no teor de Mg. Os teores de P, Mn e Cu dos vinhos produzidos nas cantinas não foram alterados pela introdução ao processo de vinificação de enzima pectinase. A ACP aplicada aos teores de Zn, Ca, Fe, P e K diferenciou os vinhos da cantina industrial microvinificado sem enzima e da cantina industrial microvinificado com enzima dos demais, no entanto, não foi capaz de separar os vinhos produzidos nas cantinas com a introdução de enzima pectinase.*

***Palavras-chave*— Cantinas, Clarificação, Industria, Artesanal.**

I. INTRODUCTION

The Vineyard area with 82.507 hectares produces 1.456 thousand tons of grapes annually. In 2012, approximately 43% of total production was commercialized for fresh consumption and 57% was processed into wine and grape juice (MELLO, 2013).

The Espírito Santo State produced 1.810 tons of grapes, and the city of Santa Teresa is the greatest producer, with 33,6% of the State's production (IBGE, 2012).

The preparation of wine begins with the vintage and the quality of the wine depends on the quality of the grape, essentially (variety, heath and proper maturation). Such quality is assessed by the physicochemical properties of the wine, which allows the visualization of the wine's balance, also either identifying or not the effective control of the winemaking process steps (GUERRA, 2009).

The use of specific enological technologies contributes to the increase or decrease of the content of minerals in wines. The maceration of red wines and the peculiar maceration of white wines contribute to the increase in contents of minerals in the wine. The mineral in greater concentration is K, followed by Ca and Mg. Among other cations found in smaller quantity are Na, Mn, Fe, Cu, Zn, Li and Rb (RIBÉREAU-GAYON et al., 2003).

The pectic enzymes can be used in the vinification process, since they have the advantage of facilitating the extraction of poliphenols, enhancing the flavor, favoring the pressing, increasing the yield in wine and favoring the clarification / filtration of the wine (Amorim *et al*., 2006). The use of pectinase provide a greater extraction of the coloring matter and the chemicals in general (DUCRET; GLORIES, 2002).

Considering the importance of the wine sector for the city of Santa Teresa and the little access to information on wine, this work aimed at assessing the young wines *cv. Isabel* produced both in handmade and full production, aiming at identifying the differences in their mineral compositions with the addition of pectinase.

II. MATERIALS AND METHODS

Initially, the Grape and Wine Producers of Santa Teresa Assossiation (Associação dos Produtores de Uva e Vinho Teresense – APRUVIT) was contacted. Then, two designated canteens were selected: i) handmade canteen (HC): a premise with an adapted structure (facilities and equipment), producing 5000 L/ year of red wine, with vinification and microvinification held in polypropylene tanks; and ii) industrial canteen (IC): a premise with a technified structure (facilities and equipments), producing 27000 L / year of red wine, with vinification held in steel tanks and microvinification in polypropilene tanks.

The wine produced in two canteens, a handmade one and an industrial one, constitutes six treatments, with three repetitions each, adding up to 18 experimental units, with the following treatments (Painting 1).

Painting 1 – Description of the treatments applied to the experiment

Treatments	Description
1	HCVwtE (handmade canteen vinified without enzyme)
2	HCMwtE (handmade canteen microvinified without enzyme)
3	HCMwE (handmade canteen microvinified with enzyme)
4	ICVwtE (industrial canteen vinified without enzyme)
5	ICMwtE (industrial canteen microvinified without enzyme)
6	ICMwE (industrial canteen microvinified with enzyme)

The dry red wine produced in both handmade canteen and industrial canteen comes from commercial plantation. (harvest: summer of 2012) of *Vitis labrusca* L.

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(*cv. Isabel*) located in Santa Teresa/ES, coordinates 19º 59' 20" S and 40º 34' 44" W, altitude 155 m (INCAPER, 2011).

The vinification in red wine applied was the classic method, which was modified since the references Rosier (1995), Rizzon et al. (2003) (Figure 1). The grape was transported to the canteens (handmade and industrial) in polyethylene boxes with capacity of 20 kg. The grapes were weighed in platform scales branded 'Cauduro', model 118PL. The berries were separated from the rachis and smashed in an inox crusher-destemmer branded 'Japa', model DZ-35 (3000 kg h^{-1}) with coupled pump. A must sample was transferred to a 500 mL measuring cylinder and it was determined that, with mustmeter ºBabo, the amount of sugar in grams in 100 g must.

In the vinification of the HCVwtE and ICVwtE treatments, polypropylene and stainless steel fermentation tanks (varying volumes) were used, respectively. In the microvinification of the HCMwtE, HCMwE, ICMwtE and ICMwE treatments, 50L propylene tanks were used. The period of maceration (fermentation: tumultuous phase) lasted from 5 days (industrial canteen) to 7 days (handmade canteen), with daily pumping. During this stage, 20g potassium metabisulfite per hL^{-1} must. This was inoculated with active dry yeast (*Saccharomyces cerevisiae*) Maurivin™ – UCD 522, produced by *AB Mauri*, in quantity of 20 $g.hL^{-1}$ must, adding to the HCMwE and ICMwE the recommended dose of 3 ml hL^{-1} must.

Following the removal of the cap and the pressing, the musts were chaptalized with crystal sugar (5.4 kg sugar per hL must). Afterward, the fermented musts were collected and transferred to six fermentation tanks of varying volumes, which three of them, made of polypropylene, held the HCVwtE treatments and the other three, made of stainless steel, held the ICVwtE treatment. In microvinification, the fermented musts of each treatment (HCMwtE, HCMwE, ICMwtE and ICMwE) were transferred to the three 30L polypropylene tanks, all of them with hydraulic bung.

The fermentation, in its slow phase, lasted 20 days with the first racking being realized 15 days by the end of the slow phase. The second racking was realized 30 days by the end of the first racking, and it was added to it 8g potassium metabisulfite per hL must. The third racking was realized 30 days by the end of the second one, and after each racking the casks were tapped and topped up.

The wine was bottled in the canteens by using semi automatic filling machines branded JAPA. The wines were poured into 750 ml new dark bottles, sealed with cork and labeled according to their respective treatments. The bottles were stored in horizontal position, in a dry, airy and light-protected place , under the temperature of 25±1ºC.

The experimental design applied was the Completely Randomized Design (CRD) with 2X3 factorial treatment combinations (canteen factor X enzyme factor) in 3 repetitions. The mineral results (P, Ca, Mg, K, Na, Mn, Fe, Cu, e Zn) were submitted to the Analysis of variance, the Tukey Test (enzyme) under 5% probability. Besides, the Principal Component Analysis (PCA) was also applied. Regarding the statistical analysis, procedures of the statistical program SAS (*Statistical Analysis System – SAS* Institute Inc., North Carolina, USA, 1992) version 9.2, licensed for the Universidade Federal de Viçosa/UFV – MG, were applied.

The minerals were determined at the Forest Soil Laboratory at Federal University of Viçosa - Minas Gerais State. The content of phosphorus (P) was determined through the Bel-1105 photocolorimeter, following the methodology proposed by Perkin-Elmer (2000). The contents of calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn) were analyzed through a Varian atomic absorption spectrophotometer, model Spectra 220-FS, following the methodology in Perkin-Elmer (2000). The determinations of potassium (K) and sodium (Na) were calculated using a Corning-400 flame photometer, according to Ough and Amerine's methodology (1988), and the results were expressed in mg L^{-1}.

Eight 750ml bottles of dry red wine were collected randomly from both handmade and industrial canteens and identified according to each treatment (HCVwtE, HCMwtE, HCMwE, ICVwtE, ICMwtE and ICMwE) in three repetitions, summing up 144 bottles. The bottles of wine were stored in the canteens in polypropylene boxes in the horizontal position and transported to their respectively laboratories in paper boxes with 12 bottles each, under 25±1 °C permanently until the beginning of the analysis in October 2012.

The Principal Components Analysis (PCA) was applied to the analytical characteristics of the actual alcohol content, total acidity, volatile acidity, pH, total dry extract, reduced dry extract, total sugars, total sulfur dioxide, total chlorides, ashes, alcohol in weight / reduced dry extract, methyl alcohol, anthocyanins, total polyphenols, color index and tonality.

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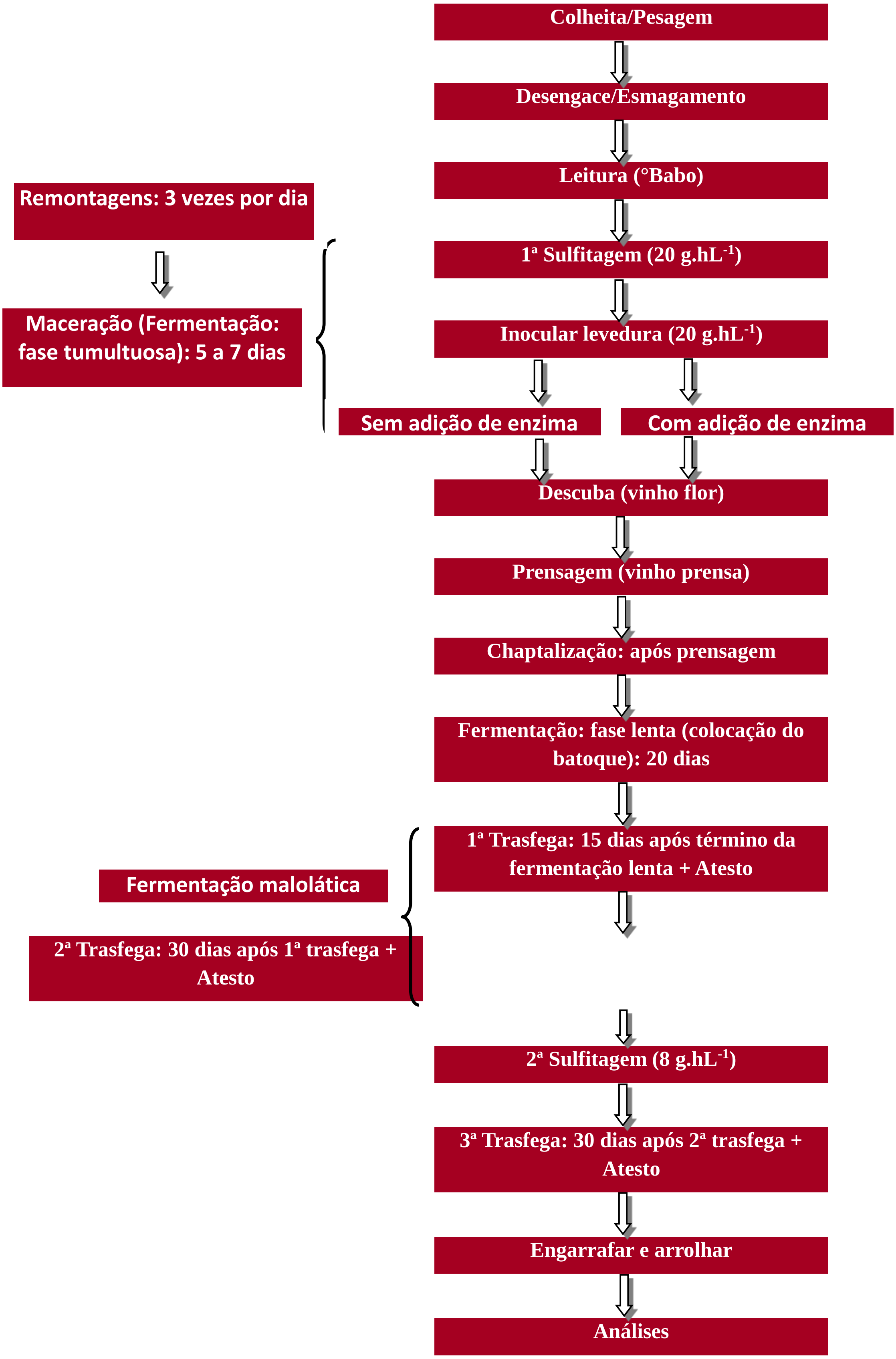

Fig.1 - Operations that were carried out for the elaboration of dry red table wines (Source: modified from Rosier, 1995; Rizzon, Meneguzzo and Manfroi, 2003).

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III. RESULTS AND DISCUSSION

The variance analysis of K, Na, Ca, Mg, Zn and Fe did not detect significant effect ($p > 0,05$) regarding the interaction canteen*enzyme. Significant effects were considered ($p < 0,05$) for K and Zn (canteens) and Mg (enzymes). The average contents of Na, Ca, Mg and Fe equaled ($p > 0.05$) in both handmade canteen (HC) and industrial canteen (IC) wines. Also the contents of K, Na, Ca, Zn and Fe equaled ($p > 0,05$) in wines either added or not enzymes to them (VwtE: vinified without enzyme; MwtE: microvinified without enzyme; and MwE: microvinified with enzyme). However, the average contents of K and Zn in the canteens were different (Table 1) and Mg in wines which either or not used enzymes (Table 2) were different ($p < 0,05$). The content of minerals in the wines depends on several factors, such as the presence of these elements in soil, enological practices, processing conditions, industrial development and contact with materials that may have such compounds during the wine production and conservation stages (RIBÉREAU-GAYON et al., 2003).

The average contents in the handmade and industrial canteens for K were, respectively, 1041.32 mg L^{-1} and 1120.97 mg L^{-1} (Table 1), such results are superior to the ones found in wines from Serra Gaúcha, by Rizzon, Miele and Meneguzzo (2000); Rizzon and Miele (2002; 2005; 2006; 2011); Rizzon, Salvador and Miele (2008). The content of K was greater compared to the composition of Serra Gaúcha wines, probably due to the time of maceration applied to the vinification. Almost of of the potassium in the wine comes from the pellicle and the seed of the product, extracted from the maceration process (Rizzon, Salvador and Miele, 2008). The K is the most important mineral in musts and wines. Its presence is paramount for determining and stabilizing the Potassium bitartrate.

The average content of Zn (Table 1) found in the handmade canteen was 0.07 mg.L^{-1} and in the industrial canteen 0.13 mg L^{-1}, these numbers were inferior to the ones observed by Rizzon, Miele and Meneguzzo (2000); Rizzon and Miele (2002; 2005; 2006; 2011); Rizzon, Salvador and Miele (2008) for table red wine, and uncer the maximum limit established by Brasil (1965) and OIV (2012). According to Ribéreau-Gayon *et al.* (2003), wines from more prolonged macerations present a higher concentration of Zn. However, despite the greater time of maceration taken in the handmade canteen, it was observed in this work a greater concentration of Zn in the industrial canteen compared to the handmade one.

Table 1 – Average contents and mineral standard deviation of dry red wines (cv. Isabel) of Santa Teresa – ES.

Components	Canteens	
	Handmade (HC)	Industrial (IC)
Potassium (mg.L^{-1})	1041.32 ± 99.57	1120.97 ± 11.52
Zinc (mg.L^{-1})	0.07 ± 0.07	0.13 ± 0.05

The average Mg contents in the VwtE, MwtE and MwE treatments (Table 2) were, respectively, 56.98 mg L^{-1}, 65.78 mg L^{-1}, and 62.06 mg L^{-1}. The MwE and MwtE treatments did not differ ($p < 0,05$) from one another, but differed ($p > 0,05$) from the VwtE treatment; these MG contents were smaller than the ones found by Rizzon, Miele and Meneguzzo (2000); Rizzon and Miele (2005, 2006 and 2011); Rizzon, Salvador and Miele (2008). For Rizzon (2010) The Mg concentration found in wines varies between 50 and 90 mg.L^{-1}. The Mg average concentrations in microvinified wines (MwtE and MwE) were greater (Table 2), since, according to Daudt, Dal Piva and Rizzon (1992) these wines go through a differing maceration due to the relatin between the surface of the contact / volume of the wine. On the other hand, wines non microvinified show a smaller relation between the surface of contact and the volume during maceration.

Table 2 – Average contents and mineral standard deviations in dry red wines (cv. Isabel) of Santa Teresa – ES, submitted to different treatments

Components	Enzymes		
	VwtE	MwtE	MwE
Magnesium (mg.L^{-1})	56.98 ± 3.66 B	65.78 ± 0.27 A	62.06 ± 5.09 AB

Averages followed by the same letter in the lines, for each characteristic, do not differ from one another according to the Tukey Test ($p < 0.05$).

VwtE (vinificated without enzyme); MwtE (microvinificated without enzyme); MwE (microvinificated with enzyme).

The average contents of phosphorus (P), manganese (Mn), and copper (Cu) were significantly ($p > 0,05$) affected by the interaction canteen*enzyme. The consequences of the interaction is represented on Table 3. The phosphorus (P) is naturally present in wines, both in mineral and organic forms. This element plays an important role (mainly when

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the contents are elevated) in the composition of precipitated ferric phosphate, causing turbidity in wine (Rizzon, 2010). According to Table 3, the contents of P from the analyzed wines vary between 25.47 mg L^{-1} (HWMwE: handmade wine/microvinified with enzyme) and 63.59.05 mg L^{-1} (IWMwtE: industrial wine/microvinified without enzyme); the numbers are below the ones found by Rizzon and Miele (2005 and 2011); but approximate to the ones found by Rizzon and Miele (2006); Rizzon, Miele and Meneguzzo (2000).

The average contents of P in wines do not differ significantly ($p < 0{,}05$) between the ICVwtE (industrial canteen/vinified without enzyme), ICMwtE (industrial canteen/microvinified without enzyme) and ICMwE (industrial cantee/microvinified with enzyme) treatments (Table 3). Manfroi and Rizzon (1996) showed that the time of maceration is directly related to the amount of phosphorus extracted from the pellicle. In this study, however, the time of maceration resulted into fewer extractions of P from the pellicle in the HCVwtE, HCMwtE and HCMwE treatments when compared to the ICVwtE, ICMwtE and ICMwE, despite the handmade canteen wines being macerated for longer.

The average content of Mn was superior and differed significantly ($p > 0{,}05$) in the HCVwtE treatment when compared to all the other ones (Table 3). In red wine vinification the fermentation (tumultuous phase) is carried out with the berry (bark, pulp, seed) and, since in the handmade canteen the time and maceration are greater than in the industrial canteen, the seed will be in contact with the must for longer.

The red wines present greater contents of those minerals, since they are found in greater amount in the seeds. Some phytosanitary products used for disease control in vines can increase their concentration in wines. The content of Mn found in the wine varies between 0.5 and 3.5 mg. L^{-1} (RIBÉREAU-GAYON et al., 2003; RIZZON, 2010). The concentration of this cation found by Rizzon, Miele and Meneguzzo (2000); Rizzon and Miele (2005; 2006 e 2011); Rizzon, Salvador and Miele (2008) were greater than the ones found in this work (Table 3).

Table 3 – Average contents and mineral standard deviation in dry red wines (cv. Isabel) of Santa Teresa – ES, submitted to different treatments

Components		Enzymes		
		VwtE	MwtE	MwE
Phosphorus (mg.L^{-1})	HC	25.47 ± 0.62 B	44.36 ± 0.11 A	44.37 ± 0.26 A
	IC	59.33 ± 6.09 A	63.59 ± 6.42 A	55.72 ± 0.09 A
Manganese (mg.L^{-1})	HC	0.15 ± 0.02 A	0.11 ± 0.01 B	0.05 ± 0.02 C
	IC	0.09 ± 0.02 A	0.11 ± 0.01 A	0.12 ± 0.01 A
Copper (mg.L^{-1})	HC	0.03 ± 0.01 B	0.05 ± 0.01 A	0.02 ± 0.01 B
	IC	0.02 ± 0.00 A	0.02 ± 0.01 A	0.02 ± 0.00 A

Averages followed by the same capital letter in lines, for each characteristic, do not differ from one another according to the Tukey Test ($p < 0{,}05$) in the HC, IC, VwtE, MwtE and MwE.

Table 3 presents the average contents of Cu (between 0.02 and 0,05 mg.L^{-1}) which were lower than the ones found by Rizzon, Miele and Meneguzzo (2000); Rizzon and Miele (2005; 2006); Rizzon, Salvador and Miele (2008), and lower than the limits established by Brasil (1965) and OIV (2012) regarding its toxicity. It was observed that (Table 3) the HCMwtE treatment presented a greater content of Cu, differing significantly ($p > 0{,}05$) from all the other treatments. Probably, the grape used in the handmade canteen (HC) vinification went through a greater pulverization (cupric treatment) for mildew control, what can result in a greater concentration of this cation in the wine. According to Rizzon (2010), Cu joins the processes of turbidity and oxidation of the wines. Its concentration depends on the phytosanitary treatments carried out (mildew control) or the wine contact with other materials and containers which have Cu. The content of Cu found in wine varies between traces and 5 mg L^{-1}.

This way, it was applied the Principal Component Analysis (PCA) to the contents of the minerals in dry red wines of Santa Teresa – ES in order to verify whether the mineral characteristics could differ them under the following treatments: HCVwtE, HCMwtE, HCMwE, ICVwtE, ICMwtE and ICMwE (Figure 2).

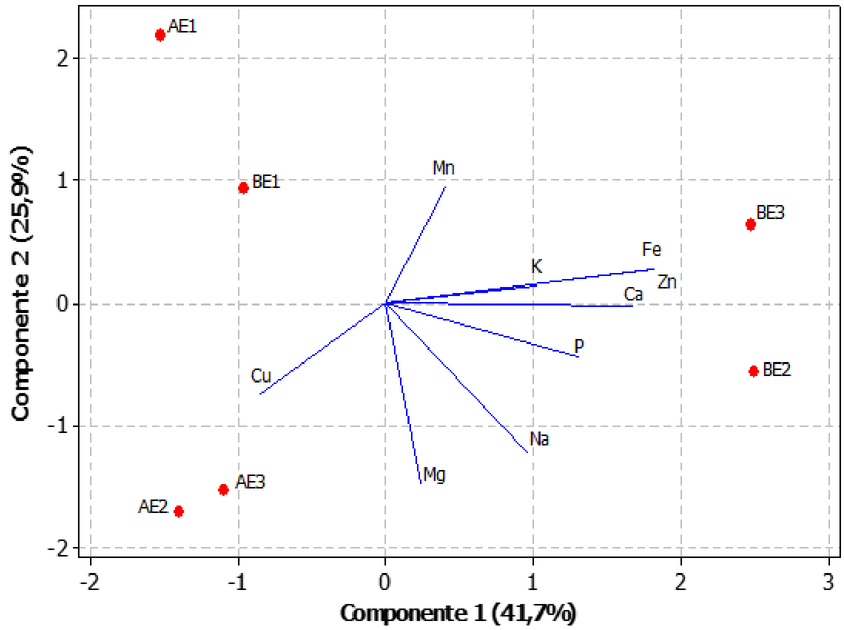

Fig.2 – Disposition of the mineral characteristics and treatments in relation to the first two main components. Symbols: P – phosphorus; K – potassium; Ca – calcium; Mg – magnesium; Fe – iron; Cu – copper; Zn – zinc; Mn – manganese e Na – sodium.

Rizzon, Salvador and Miele (2008) applied the PCA aiming at determining the concentration of the main cations (K, Na, Ca, Mg, Mn, Fe, Cu, Zn, Rb, Li) of the Serra Gaúcha wines, so that they could describe their features. In this work, such multivariate analysis technique allowed to differentiate the wines according to their color – red, rosé, white – as well as their type – fine wine and table wine.

According to PCA, it was possible to verify that the first two main components (CP1 *versus* CP2) are responsible for 67,6% of the variability in data distribution: CP1 is responsible for 41,7% of the variation and CP2 justified 25,9% of the variation between the samples. The spatial separation of the six treatments (HCVwtE, HCMwtE, HCMwE, ICVwtE, ICMwtE e ICMwE) indicates the formation of three different groups, each of them made of the following treatments: a) HCVwtE with ICVwtE; b) HCMwtE with HCMwE; c) ICMwtE with ICMwE (Figure 2).

The mineral contents are represented by vectors. The positive area of axis 1 (CP1: explains 41,7 % of the sample variability) is associated to the content of Fe, Zn, Ca, P, K, Na, Mn and Mg, while the negative area of axis 1 (CP1) is associated to the content of Cu. So, the further to the right of treatments (HCVwtE, HCMwtE, HCMwE, ICVwtE, ICMwtE and ICMwE) they are locate in this axis, the greater their contents of Fe, Zn, Ca, P, K, Na, Mn and Mg will be; and, the further to the left, the greater the content of Cu will be. The axis 2 (CP2) of the positive areas is associated to the cations Mn, Fe, Zn, K, Ca and the negative area to the cations Mg, Na, Cu and P.

The discrimination of the treatments can be verified according to the size of the vector which represents the cation, that is, the larger the vector, the greater the importance of discriminating the treatments is. The proximity of all dry red wine treatments in relation to the vectors indicates the cation in greater content in the treatment.

Figure 2 suggests that the contents of Zn, Ca, Fe, P, and K (positive correlations with the first main component CP1) are present in greater concentration in the ICMwtE and ICMwE treatments, since these are located to the right (positive area of the horizontal axis). On the other hand, the HCVwtE, HCMwtE, HCMwE and ICVwtE treatments, located to the left area of the horizontal axis (negative area) have such contents in less concentration. The CP2, otherwise, can separate the ICMwtE and ICMwE treatments, mainly through the Mn (correlação positiva com CP2), Mg e Na (negative correlation with CP2).

The PCA, applied to the mineral contents was able to discriminate the ICMwtE and ICMwE treatments from the other ones. However, the PCA applied to the contents of Zn, Ca, Fe, P e K, despite the satisfactory discrimination of ICMwtE and ICMwE treatments from the other, it was not able to separate the dry red wines produced in the handmade canteens(HC) from the ones produced in the industrial canteens(IC) after the introduction of the vinification process of the pectinolytic enzyme. Possibly, this is due to the fact that the concentrations of minerals in wines reflects not only the edaphoclimatic conditions, variety and the must composition, but it is also related to the use of phytosanitary products and enological techniques (RIZZON, 2010).

IV. CONCLUSION

The canteen modified the contents of K and Zn, and the enzyme influenced the contents of Mg. The contents of P, Mn and Cu of the wines produced in the canteens were not altered by the introduction of the process of vinification of the pectinase. The PCA applied to the contents of Zn, Ca, Fe, P and K differed the wines from the industrial/microvinified without enzyme treatment from the others, however, it was not able to separate the wines produced in the canteens with the introduction of the pectinase.

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