PHYSICOCHEMICAL COMPOSITION OF CABERNET-SAUVIGNON WINE MADE FROM GRAPES AFFECTED BY GRAPE RIPE ROT

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Abstract

Aim: In the last years, grape ripe rot has been one of the most important diseases of the Serra Gaúcha vineyards, RS, Brazil. In order to determine its influence on wine quality, we studied the physicochemical composition of must and wine made from affected Cabernet-Sauvignon grapes.

Methods and results: The experimental design was a randomized complete block, with six treatments - musts containing 0, 2.5, 5, 7.5, 10, and 20 % of affected grapes - and three replications. Grape musts were extracted from ripe fruits and wines were made in 20-L glass recipients. Polynomial regression analysis showed that ripe rot significantly increased the ºBrix, density, pH, and ºBrix/total acidity ratio of grape must. In wine, increases were observed for most variables evaluated except for density, absorbance at 520 nm, color intensity, and anthocyanins, which decreased.

Conclusions: These results show that grape ripe rot affects must and wine composition. The effect is more striking in wine, where it significantly reduces color. In this way, grape ripe rot should be prevented or controlled in the vineyards.

Significance and impact of the study: These results are important because they show the negative effect of grape ripe rot on wine composition and quality.

Key words: *Colletotrichum* spp., fungal disease, viticulture, enology

Résumé

Objectif: Dans les dernières années, le 'ripe rot' du raisin a été une de plus importantes maladies des vignobles de la Serra Gaúcha, RS, Brésil. Concerné par cette situation et ayant l'objectif de déterminer son effet négatif sur la qualité du vin, un essai a été conduit pour établir l'effet de cette maladie sur la composition physico-chimique du moût et du vin de Cabernet-Sauvignon.

Méthodes et résultats: La configuration de l'essai a été en bloc aléatoire complet avec six traitements - 0, 2,5, 5, 7,5, 10 et 20 % de raisins affectés par le 'ripe rot' - et trois répétitions. Les moûts ont été extraits des raisins mûrs et les vins faits dans des récipients en verre de 20 L. L'analyse de régression polynomiale a montré que le 'ripe rot' du raisin a causé une augmentation significative du °Brix, de la densité, du pH et du rapport ºBrix/acidité totale dans le moût. Dans le vin, on a observé l'augmentation de la plupart des paramètres étudiés. Néanmoins, il y a eu diminution de la densité, de l'absorbance à 520 nm, de l'intensité de couleur et des anthocyanes.

Conclusions: Ces résultats montrent que le 'ripe rot' du raisin affecte la composition du moût et du vin. L'effet sur le vin est plus considérable parce qu'il diminue significativement sa couleur. Pour cette raison, l'incidence de 'ripe rot' doit être évitée ou contrôlée dans les vignobles.

Signification et impact de l'étude: Ces résultats sont importants parce qu'ils montrent l'influence négative du 'ripe rot' sur la composition et la qualité du vin.

Mots clés: *Colletotrichum* spp., maladie cryptogamique, viticulture, œnologie

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INTRODUCTION

Serra Gaúcha is the most important Brazilian viticultural region, accounting for about 90 % of the total grapes crushed in the country. Today, this corresponds to about 700 thousand tons per year ; most of it goes to the wine and grape juice industry.

This region is characterized by small farms of about 2.5 hectares each. Such small vineyards may cause economic and social problems, which are mainly due to increasing production costs. Besides, the climatic conditions are not ideal for growing certain grape cultivars, because the normal rainfall in this region is 1,736 mm a year. This tends to prevent complete grape maturation through leave photosynthesis and favor the development of some diseases. The most important ones have been the gray bunch rot and the sour bunch rot, because they lead to lower fruit yield and poorer wine quality. Grape ripe rot has been reported in the Brazilian vineyards since at least the 1980s (ABRAHÃO *et al*., 1993) ; however, the number of severe infection has increased in the last decade (MIELE *et al*., 2005). Grape ripe rot is also present in other viticultural regions of the world, such as Australia (SAMUELIAN *et al*., 2012 ; WHITELAW-WECKERT *et al*., 2007), Japan (YAMAMOTO *et al*., 1999), Korea (JANG *et al*., 2011), and the United States of America (DAYKIN and MILHOLLAND, 1984 ; KUMMUANG *et al*., 1996). In general, the level of grape ripe rot infection varies according to factors such as climate (STEEL and GREER, 2008), locality (MEUNIER and STEEL, 2009), and grape cultivar (JANG *et al*., 2011 ; SHIRAISHI *et al*., 2007 ; STEEL *et al*., 2011 ; SUZAKI, 2011). The level of infection is also dependent on fungicide sensitivity since both pathogens responsible for grape ripe rot, *Colletotrichum acutatum* and *Colletotrichum gloeosporioides*, have different infection behavior and respond differently to fungicide applications (GREER *et al*., 2011 ; SHIRAISHI *et al*., 2007).

In warm and humid conditions, mainly at bloom and veraison stages, the pathogenic agent spreads very rapidly on the berry surface, from the top of the clusters to the tip, attacking the entire cluster which later may shrive. Today, considering the climatic conditions of the Serra Gaúcha this disease is not entirely controlled and is frequently associated to yield losses (not yet assessed) and wine composition and quality defects.

Colletotrichum have been found on shoots, pea sized and mature berries, mummified clusters, spurs, and canes during growing and dormant seasons. This means that the fungus remains in the dormant tissues during the wintertime, probably acting as the most important source of primary inoculum for the next growing season (SAMUELIAN *et al*., 2012).

Therefore, given the lack of information regarding the effect of grape ripe rot on the Brazilian wine composition and its negative impact on the grape and wine supply chain, the objective of this work was to establish the influence of grapes affected with this disease on the physicochemical composition of Cabernet-Sauvignon must and wine.

MATERIALS AND METHODS

1. Vineyard

A Cabernet-Sauvignon vineyard at Embrapa Uva e Vinho, located in a Brazilian subtropical region named Serra Gaúcha, was used as source of healthy and ripe rot-affected grapes. Grapevines were pergola trellised, head trained, cane pruned, and grafted on 1103 Paulsen rootstock. Distances were 2.5 m between rows and 1.5 m between vines in the row. During the 2003/2004 vegetative cycle, the grapevines were spray-treated to control different diseases but not grape ripe rot.

2. Grape sampling

Grape status (healthy vs. affected) and severity of ripe rot infection were assessed visually. Healthy grape clusters (60 kg) were sampled in February 2004 and placed in 20-kg-capacity plastic boxes ; following this, 300 kg of grape clusters affected by ripe rot were collected and placed in the same kind of plastic boxes. These clusters were sampled when 90 % to 100 % of the berries showed ripe rot symptoms. The grapes were immediately transported to Embrapa's Microvinification Laboratory, which is located near the vineyard. There, both sets of grapes, healthy and diseased, were 'homogenized' separately to form six treatments : 0, 2.5, 5, 7.5, 10, and 20 %, by weight, of grapes showing ripe rot symptoms. This means that treatment 0 % was constituted by 18 kg of healthy grapes ; 2.5 % by 17.55 kg of healthy grapes and 0.45 kg of ripe rot grapes ; and so on. This procedure was done in triplicate. Hence, there were six treatments and three replicates, which formed 18 plots disposed in a completely randomized design.

3. Winemaking

The grapes were crushed, destemmed and transferred into 20-L glass recipients, and 50 mg L^{-1} of SO_2 were added to each recipient. Later on, 0.20 g L^{-1} of active dry wine yeast (*Saccharomyces cerevisiae*) were added and glass recipients were fitted with rubber

stoppers and water-filled airlocks. After eight days of alcoholic fermentation, the wines were pressed off the skins and transferred to 9-L glass recipients also fitted with rubber stoppers and water-filled airlocks. These recipients were kept at 24 ºC±1ºC until sugar concentration was less than 4.0 g L^{-1}. Malolactic fermentation was naturally processed, and then total SO_2 was adjusted to 50 mg L^{-1}. When this fermentation ended (as confirmed by paper chromatography), the wines were racked and transferred to 750-mL bottles, sealed with cork, and stored at 15 ºC in a temperature-controlled room.

4. Must and wine analysis

Before the beginning of alcoholic fermentation, 80-mL samples of free run musts were collected from replicates of each treatment for must analysis. Five variables were measured in must − ºBrix, density, pH, titratable acidity, and ºBrix/titratable acidity ratio − according to the methodology of RIBÉREAU-GAYON *et al*. (1982)**.** The following variables were measured in wine: density, ethanol, titratable acidity, pH, dry extract, reduced dry extract, reducing sugars, alcohol in weight/reduced dry extract ratio, ashes, alkalinity of ashes, absorbance − 420 nm, 520 nm, and 620 nm −, color intensity, hue, anthocyanins, total polyphenols index, tannins, tartaric and malic acid, volatile compounds − acetaldehyde, ethyl acetate, methanol, 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and sum of higher alcohols −, and minerals (N, P, K, Ca, Mg, Na, Mn, Fe, Zn, and Rb).

The classical variables were determined by physicochemical methods (RIBÉREAU-GAYON *et al*., 1982); anthocyanins by pH difference; tannins by acid hydrolysis; absorbance at 420 nm and 520 nm by UV/VIS spectrophotometry using a 1-mm path length cell and at 620 nm using a 10-mm path length cell (RIBÉREAU-GAYON and STONESTREET, 1965, 1966).

Tartaric and malic acids were analyzed by using a Perkin Elmer HPLC in isocratic conditions, equipped with a 20-µL Rheodyne injector and a Diode Array 235C detector (AUGUSTE, 1979). The two organic acids were separated by coupling reversed-phase and a 4.6 mm i.d. x 15 cm column (Varian MCH-NCAP-5), both acids being measured at a wavelength of 212 nm. Ultrapure water acidified to pH 2.5 with phosphoric acid was used as eluent. Concentrations were established using an internal standard.

Volatile compounds were determined by using a Perkin Elmer GS AutoSystem XL gas chromatograph with flame ionization detection, equipped with a 60 m length capillary column, polyethylene glycol WAX stationary phase (N9316406). Wine samples (3 µL) were directly injected into the chromatograph. The internal standard was a 10 % solution of 4-methyl-2-pentanol at 1 g L^{-1} (BERTRAND, 1975).

Mineral contents were determined by using a Perkin Elmer model 2380 atomic absorption spectrophotometer with flame ionization detection. The minerals were analyzed without previous sample treatment but were diluted in ultrapure deionized water (Milli-Q) when necessary. The mineral concentration of each element was determined according to a calibration curve using Merck standard solutions. The elements were determined as follows: K, Na, and Rb using flame emission, and Ca, Mg, Mn, Fe, and Zn using atomic absorption; P, with the ammonium molybdate (PERKIN ELMER, 2000); and N, according to the methodology of TEDESCO *et al*. (1995).

The data were subjected to polynomial regression analysis at a 5 % significance level.

RESULTS AND DISCUSSION

Results of the Cabernet-Sauvignon must and their regression analyses are in Table 1 and Figure 1. They show that ºBrix, density, pH, and ºBrix/titratable acidity ratio linearly increased with increasing percentages of grape ripe rot, whereas titratable acidity was not affected. ºBrix increased by 14.7 %, density by 1.1 %, pH by 4.1 %, and ºBrix/titratable acidity ratio by 23.6 %. The low percentages of density and pH were due to the relatively low difference values of these variables, but they can be important in winemaking. The increase in ºBrix, density, pH, and °Brix/titratable acidity ratio may be attributed to the dehydration of affected grapes and consequent concentration of the grape must and to an acceleration of the grape ripening process. The increase in pH could be also due to the increase of K concentration in the grape juice. Indeed, K is a cation present in high concentration in grapes, especially in the skin of Cabernet-Sauvignon. As the grape skins are degraded by *Colletotrichum*, K is released into the juice of the pulp cells where it reacts with the tartaric acid – a salification process – and increases the pH. Data related to vineyard productivity show that compared with healthy grapes, affected grapes plots had lower values of weight/cluster (-45.5 %), juice yield/cluster (-50.6 %), and fruit yield/vine (-44.7 %). The results related to ºBrix and density are in accordance with those obtained in Australia (MEUNIER and STEEL, 2009) but differ with respect to titratable acidity and pH.

Table 1 - Effect of grapes affected by ripe rot on the physicochemical composition of Cabernet-Sauvignon grape must and regression analysis data.

Variable	Grapes with ripe rot (%)						Regression analysis		
	0	2.5	5	7.5	10	20	Equation	Prob. > F	R^2
ºBrix	19.1	19.9	19.3	20.4	20.9	21.9	y = 19.196 + 0.139X	0.00009	0.89
Density (g mL^{-1})	1.0816	1.0851	1.0831	1.088	1.0896	1.0937	y = 1.089 + 0.0006X	0.00002	0.88
Titratable acidity (meq L^{-1})	103	92	98	97	101	96	.-.-.	NS	.-.-.
pH	3.20	3.28	3.22	3.25	3.29	3.33	y = 3.220 + 0.006X	0.00103	0.7
ºBrix/Titratable acidity	24.6	29	26.3	28.1	27.6	30.4	y = 26.072 + 0.217X	0.00585	0.55

Table 2 - Effect of grapes affected by ripe rot on the physicochemical composition of Cabernet-Sauvignon wine and regression analysis data.

Variable	Grapes with ripe rot (%)						Regression analysis		
	0	2.5	5	7.5	10	20	Equation	Prob. > F	R^2
Density (g mL^{-1})	0.9935	0.9928	0.9931	0.9929	0.9931	0.9918	y = 0.99343 – 0.720X	0.00042	0.78
Ethanol (% v/v)	11.30	11.70	11.58	11.92	12.21	14.15	y = 11.091 + 0.141X	0.00001	0.93
Titratable acidity (meq L^{-1})	75	70	74	73	73	62	- -	NS	.-.-.
pH	3.51	3.57	3.48	3.50	3.65	3.91	y = 3.45 + 0.021X	0.00003	0.79
Dry extract (g L^{-1})	18.40	17.90	17.90	18.50	19.40	22.00	y = 17.512 + 0.207X	0.00114	0.86
Reduced dry extract (g L^{-1})	16.90	16.80	16.80	17.50	18.30	20.90	y = 16.301 + 0.215X	0.00022	0.92
Reducing sugars (g L^{-1})	2.52	2.03	2.06	2.04	2.08	2.16	.-.-.	NS	.-.-.
Alcohol in weight/Reduced dry extract	5.35	5.54	5.49	5.47	5.31	5.24	.-.-.	NS	.-.-.
Ashes (g L^{-1})	2.03	1.95	1.71	1.75	2.01	2.70	y = 1.742 + 0.038X	0.00121	0.57
Alkalinity of ashes (meq L^{-1})	20.50	20.83	19.33	19.50	21.00	26.67	y = 18.918 + 0.318X	0.002	0.69
Absorbance at 420 nm	0.236	0.235	0.24	0.229	0.209	0.217	.-.-.	NS	.-.-.
Absorbance at 520 nm	0.351	0.345	0.369	0.327	0.279	0.248	y = 0.365 – 0.006X	0.00109	0.81
Absorbance at 620 nm	0.077	0.079	0.081	0.074	0.067	0.067	.-.-.	NS	.-.-.
Color intensity	0.665	0.659	0.691	0.63	0.555	0.523	y = 0.681 – 0.008X	0.00511	0.76
Hue	0.673	0.683	0.65	0.703	0.746	0.87	y = 0.641 + 0.011X	0.00001	0.88
Anthocyanins (mg L^{-1})	408	435	428	417	400	346	y = 435.194 – 3.889X	0.00147	0.75
Total polyphenols index	29.90	31.00	30.80	30.20	29.5	29.80	.-.-.	NS	.-.-.
Tannins (g L^{-1})	1.25	1.34	1.25	1.22	1.11	1.05	.-.-.	NS	.-.-.
Tartaric acid (g L^{-1})	2.25	2.21	2.83	2.46	1.90	2.23	.-.-.	NS	.-.-.
Malic acid (g L^{-1})	0.10	0.15	0.10	0.10	0.10	0.10	.-.-.	NS	.-.-.

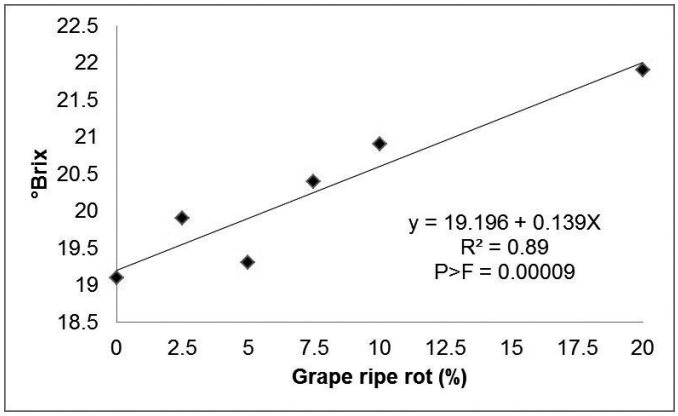

Figure 1 - Linear relationship between °Brix of Cabernet-Sauvignon must and percentage of grape ripe rot.

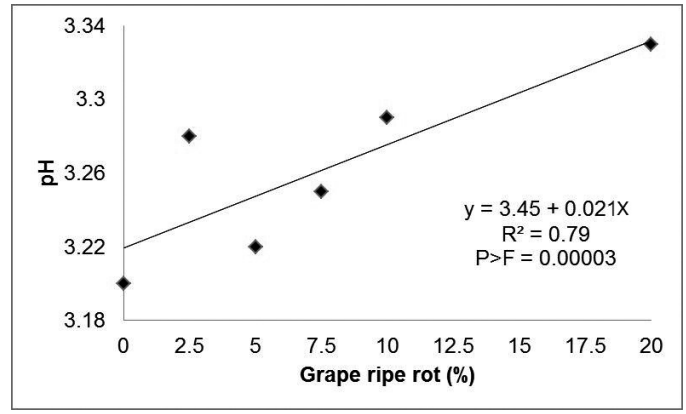

Figure 2 - Linear relationship between pH of Cabernet-Sauvignon wine and percentage of grape ripe rot.

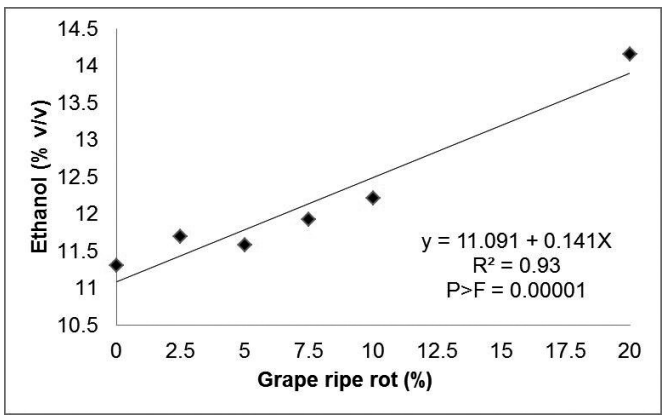

Figure 3 - Linear relationship between ethanol (% v/v) of Cabernet-Sauvignon wine and percentage of grape ripe rot.

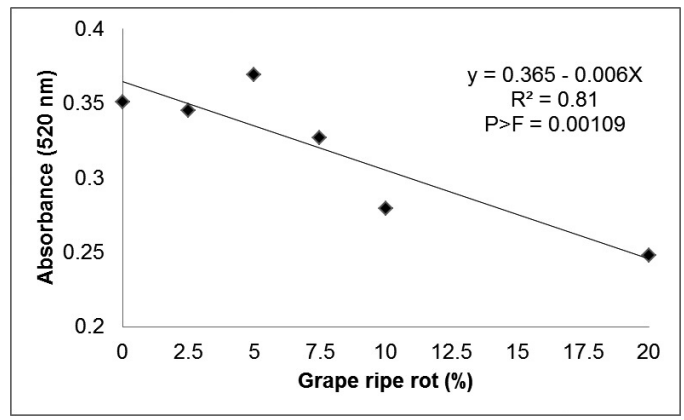

Figure 4 – Linear relationship between absorbance at 520 nm of Cabernet-Sauvignon wine and percentage of grape ripe rot.

Table 3 - Effect of grapes affected by ripe rot on the volatile compounds of Cabernet-Sauvignon wine and regression analysis data.

Variable (mg L^{-1})	Grapes with ripe rot (%)						Regression analysis		
	0	2.5	5	7.5	10	20	Equation	Prob. > F	R^2
Acetaldehyde	2.13	0.10	0.10	0.10	4.26	1.76	.-.-.	NS	.-.-.
Ethyl acetate	148	124	141	155	147	137	.-.-.	NS	.-.-.
Methanol	57	63	57	74	81	83	y = 59.072 + 1.417X	0.00039	0.74
1-Propanol	15.20	24.90	21.50	21.10	24.60	25.70	.-.-.	NS	.-.-.
2-Methyl-1-propanol	137	153	144	155	174	203	y = 136.976 + 3.269X	0.00003	0.93
2-Methyl-1-butanol	82	90	82	88	88	99	y = 82.989 + 0.744X	0.00109	0.69
3-Methyl-1-butanol	384	419	384	404	413	467	y = 384.175 + 3.727X	0.00351	0.74
Sum of higher alcohols	618	687	632	668	700	794	y = 623.6 + 8.1X	0.00011	0.83

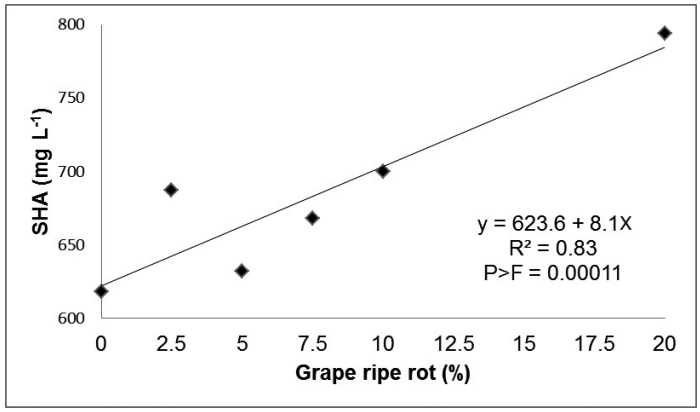

Figure 5 – Linear relationship between the sum of higher alcohols (SHA) (mg L^{-1}) of Cabernet-Sauvignon wine and percentage of grape ripe rot.

Results of wine analysis show that classical variables were differently affected by grape ripe rot (Table 2 and Figures 2, 3, and 4). Some increased with increasing percentage of ripe rot-affected grape-ethanol, pH, dry extract, reduced dry extract, ashes, alkalinity of ashes, and hue ; others decreased-density, absorbance at 520 nm, color intensity, and anthocyanins ; and a third group was not affected-titratable acidity, reducing sugars, alcohol in weight/reduced dry extract, absorbance at 420 nm, absorbance at 620 nm, total polyphenols index, tannins, tartaric acid, and malic acid.

The partial shrivel of ripe rot-affected grape berries changes the skin-to-pulp (solid-to-liquid phases) ratio. In addition, the action of the enzymes present in the pathogen softens the berry skin and increases the release of substances to the liquid phase during maceration. However, the enzymes released by this fungus to the grape juice have not been well studied ; further, possible toxins synthesized by these enzymes also deserve more attention in future researches. In this way, the values of ethanol, dry extract, reduced dry extract, ashes, and alkalinity of ashes increased. The behavior of pH was mainly due to the increasing K concentration in wine. However, one of the most striking results is related to the wine color. Indeed, the anthocyanin contents decreased as the percentages of grape ripe rot increased, which is supported by the decrease of the absorbance at 520 nm, and led to the decrease in color intensity. Color hue increased because absorbance at 420 nm was not affected by ripe rot and absorbance at 520 nm decreased. It is interesting to emphasize, however, that among the phenolic compounds studied there was no effect on the absorbance at 420 nm, and hence on tannins. So, the total polyphenols index, despite the results related to the red pigments, followed those of tannins. The decrease in wine density was expected because the ºBrix of the grape must increased, which is in accordance with the increase in ethanol content. Regarding wine acidity, there was no effect of grape ripe rot on the tartaric and malic acid concentrations. Malic acid was not affected because malolactic fermentation was naturally processed. Titratable acidity was also not affected by grape ripe rot.

However, volatile acidity increased from 0.57 to 1.06 g L^{-1} (+86 %) in wines made with 3 % of grapes affected by ripe rot (MEUNIER and STEEL, 2009), which can be particularly problematic to wine quality. Does ripe rot alone cause acetic acid biosynthesis or are there other organisms involved, such as the *Acetobacter* and *Gluconobacter* bacteria, which could be present on the surface of grapes in the vineyards? It is a matter to be considered. It should also be

Table 4 - Effect of grapes affected by ripe rot on the minerals of Cabernet-Sauvignon wine and regression analysis data.

Variable (mg L^{-1})	Grapes with ripe rot (%)						Regression analysis		
	0	2.5	5.0	7.5	10.0	20.0	Equation	Prob. > F	R^2
N	81	81	67	87	90	148	y = 65.951 + 3.589X	0.00008	0.79
P	109	114	111	128	126	168	y = 103.829 + 3.023X	0.00006	0.93
K	822	785	716	728	859	1021	y = 731.497 + 12.127X	0.00064	0.59
Ca	51	52	53	58	56	61	y = 52.045 + 0.498X	0.00088	0.86
Mg	81	85	83	88	92	101	y = 81.261 + 1.020X	0.00001	0.95
Na	5.26	5.30	6.30	5.20	5.46	6.20	.-.-.	NS	.-.-.
Mn	1.36	1.96	1.80	1.93	2.00	1.96	y = 1.688 + 0.020X	0.01916	0.34
Fe	1.23	1.30	1.46	1.50	1.40	1.43	.-.-.	NS	.-.-.
Zn	0.20	0.46	0.23	0.46	0.50	0.83	y = 0.232 + 0.029X	0.00006	0.81
Rb	5.00	5.93	5.13	5.46	5.93	6.26	y = 5.227 + 0.053X	0.00121	0.55

mentioned that ripe rot promotes gluconic acid, a characteristic substance of fungal contamination, and glycerol synthesis (MEUNIER and STEEL, 2009).

With respect to volatile compounds (Table 3 and Figure 5), Cabernet-Sauvignon wine made from grapes affected by ripe rot had higher concentrations of methanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and the sum of higher alcohols. However, grape ripe rot had no significant effect on acetaldehyde, ethyl acetate, and 1-propanol concentrations. The increase in most volatile substances could be a function of the increase in ethanol synthesis, but this does not explain why other substances were not affected by increasing proportions of grape ripe rot. The increase in the concentration of higher alcohols could be due to yeasts too, either directly from sugars or indirectly from amino acids (Ehrlich reaction). Different mechanisms seem to affect the synthesis of these compounds. For instance, the increase in methanol could be due to the increase in the amount of pectins hydrolyzed by pectic enzymes, such as pectin methylesterase. Indeed, methanol, as well as acidic pectins, are synthesized by the catalysis of the demethylesterification of homogalacturonans by that enzyme (MICHELI, 2001). It should also be mentioned that the methanol concentration was increased by 45.6 % in wine made with 20 % of ripe rot-affected grapes compared to the one made with healthy grapes ; nonetheless, it is in accordance with the Brazilian legislation.

With respect to minerals (Table 4), all minerals studied (i. e., N, P, K, Ca, Mg, Mn, Zn, and Rb) except Na and Fe linearly increased their concentration with increasing percentages of affected grapes. This mineral behavior seems to be due to the concentration of the grape must by berry shrivel.

Besides affecting the wine physicochemical composition, grape ripe rot also modifies its sensory characteristics. It was found that wines made from affected grapes had off-flavor described as a musty aroma similar to hessian sack, and bitterness (MEUNIER and STEEL, 2009).

Despite the fact that grape ripe rot was described more than 100 years ago, the literature related to the effect of this disease on wine is scarce. To our knowledge, only one article considering the effect of this disease on wine has been published (MEUNIER and STEEL, 2009), and the results of that study do not entirely match with the results of the present study. The limited number of works on the subject is mainly due to the fact that grape ripe rot only occurs under certain climatic conditions, as was already mentioned.

Thus, it should be emphasized that grape ripe rot is a serious fungal disease in warm and humid climate regions, such as those prevailing in Serra Gaúcha, and can be influenced by a series of factors and affect vineyard yield, wine composition, and wine quality in different ways and to different degrees.

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