Phenolic composition and antioxidant activity in sparkling wines: Modulation by the ageing on lees

C.A. Stefenon a,b,*, C. De M. Bonesi b, V. Marzarotto b,c, D. Barnabé c, F.R. Spinelli d, V. Webber d, R. Vanderlinde a,d

a Instituto de Biotecnologia, Universidade de Caxias do Sul, Caxias do Sul, RS, Brazil
b Biotecsl Tecnologia em Alimentos Ltda, Caxias do Sul, RS, Brazil
c Laboratório Randon Ltda, Caxias do Sul, RS, Brazil
d Laboratório de Referência Enológica, Caxias do Sul, RS, Brazil

A R T I C L E   I N F O

Article history:
Received 26 March 2013
Received in revised form 9 August 2013
Accepted 16 August 2013
Available online 29 August 2013

Keywords:
Sparkling wine
Charmat
Champenoise
β-Glucosidase
Ageing on lees
Polyphenols

A B S T R A C T

Sparkling wines (SW) have a special biological ageing on lees that is performed using two distinct methods: in the bottle (Champenoise) or in isobaric tanks (Charmat method). The objective of this study was to compare the levels of phenolic compounds, β-Glucosidase and antioxidant activity during the ageing on lees, in samples of SW produced at industrial scale by both methods. The β-Glucosidase activity has been constant over time, showing a close relationship with all the polyphenols studied (resveratrol, piceid, tyrosol, gallic, caffeic and ferulic acids), which were affected by the sur lie time. With these cross-reactions, the biological properties of the SW were also modulated. The results showed that the long period of ageing decreased the antioxidant potential in all samples. This work demonstrates that the sur lie is more important than the production method itself, due to its ability to modulate the necessary changes to achieve the specific objective.

1. Introduction

The elaboration of SW consists of two phases. In the first one, the base wine (BW) is obtained after applying white vinification. The second phase is conducted through the Champenoise or Charmat methods. The principal differences between these methods are the conversion of glucose in ethanol by yeasts (second fermentation) and ageing on lees (sur lie) that can take place in the same bottle or in isobaric tanks. During this time of contact, the exchanges between the components present in the medium (wine) and in the yeast cells will serve as the substratum for the chemical and enzymatic reaction forming different biochemical profiles (Buxaderas & López-Tamames, 2012; Gallardo-Chacón Vichi, Urpi, López-Tamames, & Buxaderas, 2010; Pozo-Bayón, Martínez-Rodríguez, Puyo, Moreno-Arribas, 2009; Torrens, Riu-Aumatell, Vichi, López-Tamames, & Buxaderas, 2010; Bosch-Fusté et al., 2009). Thus, as those reactions are modulated by the technology used, the sensorial and biological characteristics of each one of the products are directly related to the microorganism employed, and the chemical composition of the BW, resulting in unique profiles with many points of interest for the scientific, as well as for the economic and technical communities.

The Saccharomyces cerevisiae yeast in dried and active form is widely used in wineries, because it can ensure a homogeneous fermentation, resulting in high quality wines (Buxaderas & López-Tamames, 2012; Valero, Moyano, Millan, Medina, & Ortega, 2002). Reactions of hydrolysis during the winemaking are caused by enzymes of the grapes themselves or from the microorganisms taking part in the process, as the β-Glucosidases. The influence in the wine composition has been studied, mainly because these enzymes are also capable of hydrolysing non-volatile wine compounds (Hernández, Espinosa, Fernández-González, & Briones, 2003). Polyphenols are a wide range of biological molecules which play a protective role in plants and are daily found in many types of foods and beverages (Leopoldini, Russo, & Toscano, 2011; Prokop, Abrman, Seligson, & Sovak, 2006). The chemical structure of the polyphenols determines their physiological actions, including the antioxidant activity, protection against heart diseases, cancer and neuronal disorders (Stefenon et al., 2012a; Fukui, Choi, & Zhu, 2010; Leopoldini et al., 2011). Resveratrol and its derivatives glucosylated, tyrosol and phenolic acids are cited, between others activities, as neuroprotective and anticancer agents (Fukui et al., 2010; Rodrigo, Miranda, & Vergara, 2011; Vauzour, Corona, & Spencer, 2010). To the best of our knowledge, there are few reports...
about β-Glucosidase performance and about the role of phenolic compounds, especially during ageing on lees in SW, both regarding their capacity to help in human health maintenance as well as in improving the quality of products (Gallardo-Chacón et al., 2010; Stefenon et al., 2010b).

In this context, the goal of this study was to show, for the first time, a comparison between the levels of these phenolic compounds, the enzymatic (especially β-Glucosidase) and antioxidant activities during ageing on lees, in samples of SW produced at industrial scale using the Champenoise and Charmat methods in the South of Brazil. Furthermore, due to the worldwide increase in sales of these products at, the bonds between the aspects cited above, the general quality of the SW and the differences between both methods were also discussed.

2. Material and methods

2.1. Samples

The samples were elaborated in industrial scale in the companies Möet Henessy do Brazil – Vinhos e Destilados Ltda and Cave Geisse Ltda, using the Charmat and Champenoise methods (Fig. 1) and divided into three groups: (A) 7000 bottles of Champenoise 100% Chardonnay (CHC; base wine – BW1); (B) 7000 bottles of Champenoise Assemblage with 48% Chardonnay + 42% Italic Riesling + 10% Pinot Noir (CHA – BW2) and (C) 21,000 bottles of Charmat Assemblage (BW2 too). Groups A and B were split in pupitres with a capacity of 120 bottles each one. As for Group C, from three tanks with a capacity of 53,000 litres each one, three other blocks of 7000 bottles were separated. The yeast used in both methods was the S. cerevisiae EC1118 and the procedures of filtration, tartaric and protein stabilization were performed before the SW elaboration.

2.2. Chemical reagents

Reagents for the enological assays and DPPH* (2,2-diphenyl-1-picrylhydrazyl) were acquired from E. Merck, Darmstadt, Germany, while the reagents for the high-performance phenolic liquid chromatography (HPLC) and enzyme analyzes were acquired from Sigma–Aldrich (except for piceid HPLC grade, which was acquired from Polyphenols Laboratories AS, Sandnes, Norway). All other reagents were acquired from Extrasynthese, Gennay, France.

2.3. Enological analysis

The alcohol content, total acidity, pressure, volatile acidity, pH, free and total SO₂, dry extract and reduced dry extract, concentration of glucose and ascorbic acid were determined using the methods described by Zoecklein, Fugelsang, Gump, and Nury (2000, chap. 7). For each group of samples, those analyzes were performed in six bottles randomly chosen (twice in each one).

2.4. Determination of polyphenols by UV spectrophotometry

Total polyphenols (TP) and total hydroxycinnamates (THC) were quantified by measuring the absorbance at 280 and 320 nm (Shimadzu UV-1700 spectrophotometer), respectively. TP were expressed as mg/L of catechin and THC as mg/L of caffeic acid. The total flavonoids (TF) were calculated using the following formula, as described by Iland, Ewar, Sitters, Markides, and Bruer (2000), and expressed in mg/L of catechin. TF = [A_{280} – 4] – 0.66) × (A_{320} – 1.4).

To determine the total amount of oligomeric procyanidins (OPC), first an acid hydrolysis was performed and then the absorbance at 520 nm was measured in a spectrophotometer (Fukui & Nakahara, 2006). The results were expressed in mg/L of OPC. For each group of samples, those analyzes were performed in six bottles randomly chosen (twice in each one).

2.5. Evaluation of antioxidant activity in vitro

The scavenging capacity of the free radical DPPH* was measured adding a Tris–HCl buffer solution (100 mM, pH 7.0) containing...
250 μM of DPPH* dissolved in ethanol to the SW pure or diluted in distilled water [0.1; 1.0; 10 and 50% (v/v)]. In the control tube, sterilized distilled water was used instead of SW. The tubes were kept in the dark for 20 min and the absorbance was measured at 517 nm (Shimadzu UV-1700 spectrophotometer) (Yamaguchi, Takamori, Matoba, & Terao, 1998). The results were expressed in values of IC50 (SW needed to reduce 50% of DPPH* free radical), calculated by polynomial regression graphs (Mensor, Menezes, Leitão, Reis, Dos Santos, & Coube, 2001), using the average of triplicates. For each group of samples, those analyzes were performed in six bottles randomly chosen (three times in each one).

2.6. Evaluation of enzymatic activity in vitro

The β-Glucosidase assay was based on the procedures described by (Riou, Salmon, Vallier, Günsta, & Barre, 1998) and Dhake and Patil (2005) by mixing 0.1 mL of 5 mM P-nitrophenyl β-D-glucopyranoside (pNPG) and 0.4 mL of 0.1 M sodium acetate buffer at pH 5.5. After incubation for 10 min at 50 °C, the reaction was interrupted by adding 2 mL of 1 M sodium carbonate, and the p-nitro phenol released was monitored at 420 nm. One unit of β-Glucosidase activity corresponds to the release of 1 μmol of p-nitro phenol/min under the assay conditions. For each group of samples, those analyzes were performed in six bottles randomly chosen (three times in each one).

2.7. Determination of polyphenols by HPLC

Determination of tyrosol, caffeic acid and ferulic acid. The determination of phenolic acids was made in HPLC (Agilent Technologies 1100 Series) with UV detector according to the methodology adapted from Roggero and Archier (1989). The column used was a Zorbax SB-C18 (5a, 4.6 × 250 mm) from Agilent Technologies. The mobile phases were composed of pure water/acetic acid (95:5 v/v) and acetic acid/Milli-Q water/ethanol (5:65:30). The samples were filtered and detections were performed in wavelengths of 280 nm for tyrosol, 294 nm for caffeic and ferulic acids. The samples were filtered with a membrane of 0.2 μm. The injection volume was 20 μL, the column was maintained at 25 °C and the analysis flow was 0.8 mL/min.

Determination of resveratrol and piceid. The determination of resveratrol and piceid was made in HPLC (Agilent Technologies 1100 Series) with DAD detector (diode array detector) according to the methodology adapted from McMurtrey, Minn, Pobanz, and Schultz (1994). The column used was a Zorbax SB-C18 (5a, 4.6 × 250 mm) from Agilent Technologies preceded by a guard column LiChrospher 100RP-18 (5 mm, 4 mm × 4 mm). The mobile phase consisted of water Milli-Q/acetonitrile (50:50 v/v) pH 3, adjusted with orthophosphoric acid and filtered through membrane of 0.45 μm. The detection was performed in wavelength of 254 at 316 nm for resveratrol and piceid. The samples were filtered with a membrane of 0.2 μm. The injection volume was 20 mL, the column was maintained at 25 °C and the analysis flow was 0.8 mL/min.

Determination of gallic acid. The determination of gallic acid was made in HPLC (Agilent Technologies 1100 Series) with DAD detector according to the methodology adapted from Lamuela-Raventós and Waterhouse (1994). The column used was a Zorbax SB-C18 (5a, 4.6 × 250 mm) from Agilent Technologies preceded by a guard column Zorbax 300SB-C18 (5 mm, 12 mm × 4.6 mm). The mobile phases were composed of: Solvent A: NH4-H2PO4 solution of 50 mmol/L pH 2.6, adjusted with orthophosphoric acid; Solvent B: acetonitrile/solvent A (80:20 v/v); Solvent C: orthophosphoric acid solution 0.2 mol/L pH 1.5, adjusted with ammonium hydroxide. The detection was performed at a wavelength of 204 nm. The samples were filtered with a membrane of 0.2 μm. The injection volume was 5 μL, the column was maintained at 25 °C and the analysis flow was 0.5 mL/min. For each group of samples, those analyzes were performed in six bottles randomly chosen (three times in each one).

3. Results and discussion

3.1. Enological analysis

Total acidity of the SW varied from 4.1–7.33 g/L of tartaric acid. The average levels of pressure, volatile acidity, and pH were 5.6 ± 0.2 atm, 0.41 ± 0.01 g/L of acetic acid and 3.50 ± 0.03, respectively. The average concentrations of free SO2 were 22.50 ± 0.58 mg/L and of total SO2 were 95.67 ± 6.08 mg/L. Analysis of the dry extract and reduced dry extract showed, respectively, values (expressed in g/L) of 22.70 ± 0.50 and 17.23 ± 0.15 for CHC, 21.90 ± 3.67 and 16.33 ± 0.38 for CTA and 27.10 ± 0.70 and 18.53 ± 0.06 for CHA. The increase in the concentration of glucose and alcohol in the SW in relation with its BW is a natural consequence of the second fermentation; the small variations in the analysis results over time were not significant and both cases occurred independently of the elaboration method (data not shown). These results show that the grapes were healthy, appropriate vinification practices were used and the values are in the average of the contents normally found worldwide (Pozo-Bayón et al., 2009; Torrens et al., 2010). The presence of L-ascorbic acid into SW and its relationship with many factors such as yeast metabolism, offer of sunlight on the wine, grape variety and maturation degree reported by colleagues were discussed by our group (Stefanon et al., 2010a). In this study the results obtained were similar and the levels of this compound had no significant differences in all SW analysed (data not shown). However, our results suggest that the Chardonnay variety can have more vitamin C than Pinot Noir and Italic Riesling, because BW1 showed 82.76% more L-ascorbic acid than BW2. This grape variety is used in SW production around the world and is considered as responsible for the structure and pleasant citrus aromas that can be found in them (Buxaderas & López-Tamames, 2012; D’Incecco et al., 2004; Sánchez, Díaz-Maroto, Hidalgo, González-Vinás, & Pérez-Coello, 2005). Moreover, taking into account that this acid is strongly reactive, we can suggest that the presence of this compound has an important role in the maintenance of aromatic characteristics in these products, due to the changes in the production of higher alcohols and esters by S. cerevisiae (Valero et al., 2002).

3.2. Determination of polyphenols by UV spectrophotometry

Table 1 shows a decrease in the levels of TP and TF in both methods, while THC content remained without significant differences. The oxidation reactions taking place in the first steps of the process have strong affinity by small molecules such as the THC, while larger molecules tend to react along the time (Bosch-Fusté et al., 2009; Pozo-Bayón et al., 2009; Stefanon et al., 2010a). The content of OPC shows an increase into CHA and CTA samples, whereas on the CHC, no significant differences were found. This is probably due to the red grape employed, because it is rich in phenolic compounds (Stefanon et al., 2010a). Then, regarding generic phenolic groups, we can assume that the ageing on lees and grape variety were variables with more influence than the production method. In addition, a negative correlation was observed between TP and OPC (R = −0.687; p = 0.01) as well as between TF and OPC (R = −0.710; p = 0.01) only for the Assemblage SW (both CHA and CTA). Pozo-Bayón et al. (2009) reported many factors involved in the chemical composition of SW, such as: grape variety, vineyard yield, quality of the base wine and yeast strain for
second fermentation; they agree that the second fermentation and the ageing on lees are the key factors used to explain the quality since both events are involved in the distinctive character of each SW.

3.3. Evaluation of antioxidant activity in vitro

Beyond the general quality of the SW, another points of view are the beneficial effects of these compounds in the human health (Gallardo-Chacón et al., 2010; Stefenon et al., 2010b; Vauzour et al., 2010). It is relevant to remember that the pharmacological, medicinal and biochemical properties of polyphenols were extensively studied in recent reviews (Leopoldini et al., 2011; Rodrigo et al., 2011). However, to the best of our knowledge, this is the first comparison between the generic phenolic groups profile related with the methods Charmat and Champenoise in controlled samples. Table 1 shows an increase on IC50 values along the time, i.e., the older the SW is, the lower the antioxidant activity will be. Our results show a greater influence of the ageing over the Champenoise than over Charmat ones, because the loss of this capacity was 91.12% to CHC, 22.81% to CHA and 15.17% to CTA sparkling wines. Nevertheless, when young, CHC was more antioxidant than the others at the same point of the sur lie, around the 120 days. But in the middle of the ageing period studied, this SW was less effective than the Assemblage SW in both production methods. In accordance with what was discussed above, these results can be linked with the higher content in ascorbic acid into CHC due to the presence of Pinot Noir grapes into CHA and CTA samples. These responses are modulated by many factors and Table 2 shows the correlations (negative or positive) between some important variables and the antioxidant activity of SW. Many phenols can be grouped into TP, TF, THC and OPC (Ilind et al., 2000) and it becomes difficult to assess how and which changes occur in each method. In an attempt to clarify some of these aspects, we discuss below about some important polyphenols, as the resveratrol, to improve the management of SW production.

3.4. Determination of enzymatic activity in vitro

Table 1 shows for the first time the levels of β-Glucosidase (β-G) DPPH*, total polyphenols (TP), total flavonoids (TF), total hydroxycinnamates (THC) and oligomer procyanidins (OPC) in SW in different periods of sur lie (aging on lees).
period of up to 360 days. Earlier studies by our group showed similar data in commercial samples of SW acquired in supermarkets and wine stores (Stefenon et al., 2010b). Yeast autolysis represents an enzymatic self-degradation of cell components that begins at the end of the stationary growth phase of alcoholic fermentation and is associated with cell death, resulting in the release of cellular components into the wine and their interaction with the wine constituents (Buxaderas & López-Tamames, 2012). The yeast cell wall can also act as an absorptive surface agent, but the β-Glucosidase activity seems to have not been influenced by these aspects, because no changes from the base wine until the end of the second fermentation were verified (data not shown) and the levels remained unchanged over time both in Champenoise and in Charmat ones. Since the β-Glucosidase integrates the pool of yeast enzymes (Hernández et al., 2003), the demonstration that it remains active during the ageing on lees opens new research possibilities and other experiments are being conducted by our group on this subject. Hence, during the sur lie, the method used seems to be less important than the employed varieties, because the CHC showed 28.6% more β-Glucosidase activity than CHA and CTA. This characteristic can be related, at least partially, to the high acceptance of products produced with chardonnay grapes (Buxaderas & López-Tamames, 2012), because this enzyme is linked with an aromatic profile and can explain the changes occurred in them over time (D’Incecco et al., 2004; Sánchez et al., 2005).

3.5. Determination of polyphenols by HPLC

In this study, we investigated the connection between the β-Glucosidase activity with the possible changes on the phenolic profile and its relationship with the antioxidant potential of SW, especially about the balance of resveratrol and piceid levels. Furthermore, SW contains relatively high concentrations of phenolic acids and phenolic alcohols (D’Incecco et al., 2004; Vauzour et al., 2010). The beneficial effects of the caffeic acid and tyrosol in the human vascular system and in the neuroprotective capacity, as well as the therapeutic use of ferulic and gallic acids against oxidative stress and its complications (asthma, coronary diseases, diabetes, etc.) have been investigated (Leopoldini et al., 2011; Rodrigo et al., 2011; Vauzour et al., 2010). However, assessing the role of these compounds on the biochemical and sensorial profile of the SW in order to offer, at the same time, products of high quality and with bioactive useful to maintain of human health is still necessary.

3.5.1. Determination of resveratrol

The first research was about the levels of resveratrol and its glycosylated derivative during the sur lie. CHC (Fig. 2a) shows a decrease on piceid and an increase in resveratrol contents probably due to the β-Glucosidase activity, which was larger in these samples. The positive correlation observed between this enzymatic property and the level of this phenolic compound (R = 0.412, p = 0.05) for this group of samples corroborate this hypothesis. For the CHA and CTA samples (Fig. 2b and c), the effect of glucose concentration (10.41 g/L ± 0.58) is important too, because in these samples, the content of glucose was on average 45% higher than in CHC (6.88 g/L ± 0.65) and constant levels of piceid and a decrease on the resveratrol concentration were observed. In the first case, the stability can be explained by the occurrence of the reverse reaction, when an aglycone is released it returns to the form of its glucosylated derivative as described by Medina et al., 2010. The negative correlation observed between the glucose and resveratrol levels (R = −0.454, p = 0.05) reinforce this idea. The reduction in resveratrol contents may be due to photosomeration and other enzymatic reactions, such as those mediated by phenoloxidases present in the medium or in which cofactors (e.g. metals) are involved in the formation of derivatives previously identified in wine (Prokop et al., 2006; Stefenon et al., 2012). Furthermore, the concentration of both compounds mediated by the presence of β-Glucosidase can have a strong influence in the antioxidant activity as demonstrated by the clear relation between them (Table 2).

3.5.2. Determination of tyrosol

The tyrosol is a compound commonly found in chardonnay grapes (D’Incecco et al., 2004) and in the CHC samples the content was initially high (Fig. 3a). Our data were similar to those found in Champagne samples (Vauzour et al., 2010). Regarding the Champenoise method (varietal/CHC or assemblage/CHA) we can consider the level of tyrosol to be constant, because at the end of the ageing period studied, the level is similar to the one of the two analysed blocks. And the slight increase observed in CHC samples until
120 days can explain the larger antioxidant activity in these SW. The influence of tyrosol over the IC50 is clear (Table 2). Regarding the Charmat samples, a gradual increase was observed. As it is known, the complex array of aroma and flavour found in SW is largely originated from the grapes, yeast metabolism during the alcoholic fermentations and the ageing on lees (D’Incecco et al., 2004; Torrens et al., 2010). In this case, the most important variable seems to be the elaboration method, because the correlation between the tyrosol content and sur lie was opposite: Charmat (R = 0.917, p = 0.01) and Champenoise (R = −0.519, p = 0.01). Since tyrosol is formed from tyrosine by a sequence of three reactions (deamination, decarboxylation and reduction), the reductive ambient in a larger volume (Charmat process) can explain this assumption. Furthermore, as discussed previously, the β-Glucosidases, in the presence of glucose on a rich medium, as the wine, are able to modulate the response of many compounds, such as, the transference of the glucose molecule to the tyrosol to form salidroside. On the other hand, salidroside may be degraded into tyrosol and glucose (Ling-Ling, Zhu, Petrovic, & Gonsalves, 2007). More studies will be performed to corroborate this hypothesis, because to our knowledge, the salidroside in wines has not been demonstrated until now.

3.5.3. Determination of gallic acid

The contents of gallic acid (Fig. 3b) into CHC and CHA samples showed a tendency to increase during the sur lie. This possibility can be related with the enzymes released during yeast autolysis that could be involved in the hydrolysis of tannins polymers (Pozo-Bayón et al., 2009). This result is reinforced by the positive correlation observed between the sur lie and gallic acid (CHC: R = 0.659, p = 0.01; CHA: R = 0.603, p = 0.01). The content was similar and constant for the three analysed groups. This aspect is very important, because the browning increase is due to the formation of brown macromolecules coming from the polymerisation of phenols; the decrease in the main hydroxycinnamic acids present in SW is also related with these reactions and can affect the overall quality (Bosch-Fusté et al., 2009). Moreover, the caffeic acid associate with proteins creates an initially soluble molecule, but with the growth, the complex becomes insoluble, generating turbidity into wines (Esteruelas et al., 2011). Additionally, the degree of insolubility is affected by the nature of the sugars present in the medium and in these samples, negative correlation between caffeic acid and glucose was observed (CHC: R = −0.446, p = 0.05; CHA: R = −0.477, p = 0.05; CTA: R = −0.772, p = 0.01). As this compound positively affects the antioxidant activity (Table 2), actions to promote the

![Graphs showing the evolution of phenol alcohol and phenol acids](data:image/png;base64,iVBORw0KGgoAAAANSUhEUgAAAwAAAAHjCAYAAABKfW1xAAAABGdiamVU///8AAABVwtJxg79+Pj4AAAAAElFTlGAAAAfAkrbbuAAAAABJRU5ErkJggg==)

**Fig. 3.** Evolution of phenol alcohol (a) and phenol acids (b–d) during the sur lie to Champenoise Chardonnay SW (CHC), Champenoise Assemblage SW (CHA) and Charmat Assemblage SW (CTA).
balance between concentration and ageing of SW are very important. The same situation was found between ferulic acid and glucose contents (CHA: \( R = -0.667, p = 0.01 \); CTA: \( R = -0.885, p = 0.01 \)). Regarding this compound (Fig. 3d), the varieties are also important, because their performance was similar to the one of caffeic acid, including the minimal decrease due to the precipitation linked to natural proteins (Esteruelas et al., 2011). Bosch-Fusté et al. (2009) studied the development of Cava sparkling wine during its ageing in contact with lees and the caffeic acid showed content similar to the one of Champaigne Chardonnay 100%, whereas the typical Brazilian assemblage (Chardonnay, Italic Riesling and Pinot Noir) showed higher concentration of this phenolic acid, in both methods. The sur lie should be accurately monitored because in those last mentioned samples, we found a negative correlation between ferulic acid and ageing on lees (CHA: \( R = -0.525, p = 0.01 \); CTA: \( R = -0.638, p = 0.01 \)). Changes on the chemical structure of the phenols and the reactions over time may result in easily oxidizable derivatives (Leopoldini et al., 2011), such as the eugenol, which have carnaton aroma and can depreciate the sensorial profile of SW.

Finally, the Principal Components of Analysis (PCA) show the sur lie as the first component extracted. This variable explains more than 40% of variance for all cases. Together with resveratrol, \( \beta \)-Glucosidase, caffeic acid and tyrosol, more than 80% of the variance was also explained (Fig. 4). This finding is remarkable, because it clearly shows that the ageing on lees is able to modulate many compounds in the medium. The samples used in this work were produced under controlled and equal conditions and the results found were similar to the ones observed in commercial SW previously studied by our group. This fact is important because the compounds discussed above have many enological and biological properties and this statement can result in an approximation of the scientific evidences and its innovations related with the industrial realities and markets demands. Hence, the target of winemakers worldwide is to certify the quality of the product to the consumer and to offer new technologies to improve the enological practices, aiming at the production of SW of increasingly high quality.

To summarise, this work has provided a comparison between the two principal production methods of sparkling wines. Primarily, it is known that Champaigne and Charmat have important differences, since in the first one, the long period of sur lie is associated with sensorial characteristics, such as: more structure, body, marked flavours and aromatic complexity. In Charmat ones, freshness and elegance with delicate aromas are usually found, and this is directly related with the short sur lie, but these aspects cannot determine the quality level. Yeast autolysis is a slow process that involves the interaction between components released by dead yeast cells and the wine and through this study we can conclude that the volume of wine in contact with the lees surface (bottle or tanks) can affect the sequential reactions involved in the whole process, since the compounds showed different curves to each method, such as the tyrosol and gallic acid ones.

Secondly, the grapes are the matrices of the SW profile and we showed that the chardonnay grape has more \( \beta \)-Glucosidase activity than the assemblage used. The metabolism is triggered by enzymes and we proved that this activity not only exists into SW, but also that it remains unchanged while the ageing happens. Therefore, we can conclude that the \( \beta \)-Glucosidase activity is stable in the wine conditions. This is important because the reactions that involve this enzyme, the levels of resveratrol and piceid plus the glucose concentration, may be able to maintain or improve the SW antioxidant capacity. Besides, caffeic and ferulic acids play significant roles in this context and are also affected by the glucose levels in the medium, acting in this way on the overall quality of the SW.

Our results showed that the older the SW is, the smaller the antioxidant activity is too. As white and red wines can act against the oxidative stress in distinct ways, the choice for a short or long ageing on lees will determine the response of the SW, because the sur lie is able to modulate the necessary changes to achieve a specific objective. Therefore, we can conclude that the ageing on lees becomes more important than the production methods of SW due to, mainly, its close relationship with the phenolic profile.

**Acknowledgements**

The authors are grateful to CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior); UCS (Universidade de Caxias do Sul), ABE (Associação Brasileira de Enologia), Moët Hennessy do Brasil – Vinhos e Destilados Ltda, Vinícola Geisse Ltda, and Prof. Abel Prezzi Neto for his assistance in the view of English.

**References**


