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Comparative study of the behaviour of different wines after remaining for long periods of time in two different bottles (Beronesa and Bordelesa)

1. BACKGROUND OF THE BERONESA BOTTLE PROJECT

In the last 200 years, the bottle as a container for the transport of a liquid content has been subjected to some cosmetic changes, but functional changes were never contemplated. The Beronesa bottle intends to prove, through a scientifically demonstrable and measurable study, the actual advantages it contributes to the favorable evolution of bottled wine. It has been conceived and developed to become the most efficient protection against the potential damages the bottled wine may confront, from the moment it leaves the wine cellar until it fills our cup. It is a completely new milestone in regard to its technical contribution, which makes it "something more than a plain bottle", due to its design that optimizes and preserves an already optimized elaboration. Apart from other considerations, this is possible because it allows the cork to be in permanent contact with the wine, no matter the position of the bottle, even in vertical position, which is the most frequent and dangerous during the transport and storage. It is then when the most frequent damages to the bottle wine (inappropriate temperature and incorrect position of the bottles) happen. When the above two factors occur, the outcome is a slow and progressive damage of the ideal conditions for the preservation of wine, leading to a loss of aromatic and expressive potential. This means an irreparable loss of value and image of the product to the detriment of the consumer's expectations. We intend to prove that the Beronesa bottle extends and preserves all the properties and singularity of every wine, due to its controlled reductive evolution in bottle, and all of this due to its innovative design, which ensures appropriate and favorable physical-chemical reactions during all the time the wine is in the bottle.

2. SCIENTIFIC OBJECTIV

The aim of this study is to carry out a comparative study about the behavior of different wines when remaining for long periods of time in 2 different bottles (Beronesa and Bordelesa).

In order to approach such a comparative study, we conceived to carry out an accelerated ageing in oven (35 ºC) covering all the delivered samples, as well as to maintain, under the above conditions, a period of time as long as possible, taking into account the limitation of time previously fixed by the Company's people, who required to have results available in a short term. Working under these conditions means to accelerate somewhat the processes occurred in the wine along the time in bottle. According to previous publications, the behavior of the wine after 60 days at 35ºC may be equal to its behavior after 1 year at room temperature. So we decided to use an incubation time of 60 days with measurements every fortnight.

The parameters to be measured, in order to make a follow-up in regard to time, included both parameters related to eventual reduction problems and parameters related to oxidation problems. Both processes may appear in wines as long as they are in the bottle.
In order to evaluate the reduction, we measured the levels of free volatile sulfur compounds (hydrogen sulfide, methanothiol, ethanothiol and dimethylsulfide). However, in this case, this family of compounds is also useful to evaluate the behavior of the aromatic compounds with –mercapto group. This family of compounds has special significance, as they are considered variety aroma compounds contributing tropical nuances, box, to wines such as Sauvignon Blanc. This is the case of 4-mercapto-4-methyl-2-pentanona, 3-mercaptohexanol or 3-mercaptohexyl acetate.
In order to evaluate the level of protection of the wines in the different periods of time, the levels of free and total sulfur dioxide (SO_2) were also measured.
On the other hand, we decided to evaluate the levels of Strecker aldehydes and total acetaldehyde, since these data provide with information about the tendency of a wine to oxidize.

3. MATERIAL AND METHODS

Analyzed samples

The Company delivered us 4 wines coming from the ICVV of La Rioja and from the Bodega Institucional La Grajera, of the Government of La Rioja, which were bottled in the same Bodega La Grajera.

The incoming date was the 7th of February of 2018.

The wines included in the study were the following:

- Verdejo white wine (Beronesa bottle, Bordelesa bottle)
- Rosé (Beronesa bottle, Bordelesa bottle)
- Tempranillo aged (vintage) wine (Beronesa bottle, Bordelesa bottle)
- Tempranillo reserva (extra vintage) wine (Beronesa bottle, Bordelesa bottle)

Analytical methodologies

- The analysis of free sulfur compounds in wine was carried out according to the technique developed by our research team, as published in the following article: “Quantitative analysis of free and bonded forms of volatile sulfur compounds in wine. Basic methodologies and evidences showing the existence of reversible cation-complexed forms”, Journal of Chromatography A, 2014, 1359, pages 8 – 15.

It is based on a determination through head space with injection in a chromatographic system of gazes with photometric flame pulse detector (HS-GC-PFPD). This detector is used to quantify sulfur compounds, due to its high selectivity.

- The analysis of free and total sulfur dioxide, SO_2, was carried out through the Ranking method.
- The analysis of total acetaldehyde was performed through HPLC (so far unpublished).
- Strecker aldehydes were determined through a methodology based on SBSE-GC-MS (so far unpublished).

Date of realization of tests: March-May 2018.

4. RESULTS

a. Determination of volatile sulfur compounds

The following tables show the levels of free sulfur compounds found in the 4 wines in the different periods of time of analysis. In the case of positively identified compounds, its concentration is given in µg/L.

The first column shows the officially accepted olfaction threshold for every analyzed compound. This parameter is defined as the minimum concentration that can be perceived in a determined matrix. As far as hydrogen sulfide and methanothiol are concerned, two values are given. The first one corresponds in both cases to the estimated threshold in red wine, and the second one refers to white wine. The intermediate columns show the concentration levels found along with their standard deviations. In case the analytic targets are not detected, this is indicated by nd. The last column provides with information about the limits of detection for every analytic target, according to the applied methodology.

Tabla 1.a. White wine - Bordelesa bottle

		t 0		15 days		30 days		45 days		60 days		
Odor threshold (µg/L)	**Compound**	average	s	average	s	average	s	average	s	average	s	**LD (µg/L)**
1.1 – 1.6	H_2S	0.65	0.04	4.10	0.05	2.63	0.04	0.68	0.01	0.94	0.01	0.65
1.8 – 3.1	MeSH	< LD		< LD		< LD		< LD		< LD		0.50
1.1	EtSH	< LD		< LD		< LD		< LD		< LD		0.50
25	DMS	2.21	0.06	2.93	0.14	5.60	0.05	6.64	0.11	7.29	0.05	1.00

H_2S: Hydrogen sulfide, **MeSH:** methanothiol, **EtSH:** ethanothiol, **DMS**: dimethylsulfide, **nd:** no determined; **LD**: limite of detection

Tabla 1.b. White wine – Beronesa bottle

		t 0		15 days		30 days		45 days		60 days		
Odor threshold (µg/L)	**Compound**	average	s	average	s	average	s	average	s	average	s	**LD (µg/L)**
1.1 – 1.6	H_2S	0.65	0.03	3.62	0.01	4.13	0.14	3.88	0.09	4.73	0.23	0.65
1.8 – 3.1	MeSH	< LD		< LD		0.67	0.03	0.72	0.02	0.81	0.04	0.50
1.1	EtSH	< LD		< LD		< LD		< LD		< LD		0.50
25	DMS	1.96	0.27	1.60	0.25	5.69	0.04	7.16	0.27	7.99	0.01	1.00

H_2S: Hydrogen sulfide, **MeSH:** methanothiol, **EtSH:** ethanothiol, **DMS**: dimethylsulfide, **nd:** no determined; **LD**: limite of detection

Tabla 2.a. Rosé wine – Bordelesa wine

		t 0		15 days		30 days		45 days		60 days		
Odor threshold (µg/L)	**Compound**	average	s	average	s	average	s	average	s	average	S	**LD (µg/L)**
1.1 – 1.6	H_2S	< LD		2.56	0.07	4.42	0.20	4.81	0.10	< LD		0.65
1.8 – 3.1	MeSH	< LD		< LD		< LD		< LD		< LD		0.50
1.1	EtSH	< LD		< LD		< LD		< LD		< LD		0.50
25	DMS	2.81	0.02	5.34	0.09	6.44	0.14	8.29	0.24	8.08	0.03	1.00

H_2S: Hydrogen sulfide, **MeSH:** methanothiol, **EtSH:** ethanothiol, **DMS**: dimethylsulfide, **nd:** no determined; **LD**: limite of detection

Tabla 2.b. Rosé wine – Beronesa bottle

		t 0		15 days		30 days		45 days		60 days		
Odor threshold (µg/L)	**Compound**	average	s	average	s	average	s	average	s	average	s	**LD (µg/L)**
1.1 – 1.6	H_2S	< LD		< LD		0.46	0.01	3.85	0.02	< LD		0.65
1.8 – 3.1	MeSH	< LD		< LD		< LD		< LD		< LD		0.50
1.1	EtSH	< LD		< LD		< LD		< LD		< LD		0.50
25	DMS	8.08		0.72	0.01	0.72	0.01	8.21	0.12	6.66	0.09	1.00

H_2S: Hydrogen sulfide, **MeSH:** methanothiol, **EtSH:** ethanothiol, **DMS**: dimethylsulfide, **nd:** no determined; **LD**: limite of detection

Tabla 3.a. Aged red wine – Bordelesa wine

		t 0		15 days		30 days		45 days		60 days		
Odor threshold (µg/L)	**Compound**	average	s	average	s	average	s	average	s	average	s	**LD (µg/L)**
1.1 – 1.6	H_2S	< LD		< LD		0.72	0.02	0.84	0.01	< LD		0.65
1.8 – 3.1	MeSH	< LD		< LD		< LD		< LD		< LD		0.50
1.1	EtSH	< LD		< LD		< LD		< LD		< LD		0.50
25	DMS	4.13	0.17	7.96	0.11	9.19	0.15	12.1	0.01	11.1	0.07	1.00

H_2S: Hydrogen sulfide, **MeSH:** methanothiol, **EtSH:** ethanothiol, **DMS**: dimethylsulfide, **nd:** no determined; **LD**: limite of detection

Tabla 3.b. Aged red wine – Beronesa wine

		t 0		15 days		30 days		45 days		60 days		
Odor threshold (µg/L)	**Compound**	average	s	average	s	average	s	average	s	average	s	**LD (µg/L)**
1.1 – 1.6	H_2S	< LD		< LD		0.77	0.04	0.88	0.12	1.18	0.07	0.65
1.8 – 3.1	MeSH	< LD		< LD		< LD		< LD		< LD		0.50
1.1	EtSH	< LD		< LD		< LD		< LD		< LD		0.50
25	DMS	4.69	0.20	6.86	0.44	8.52	0.30	10.7	0.06	11.6	0.27	1.00

H_2S: Hydrogen sulfide, **MeSH:** methanothiol, **EtSH:** ethanothiol, **DMS**: dimethylsulfide, **nd:** no determined; **LD**: limite of detection

Tabla 4.a. Reserva wine – Bordelesa wine

		t 0		15 days		30 days		45 days		60 days		
Odor threshold (µg/L)	**Compound**	average	s	average	s	average	s	average	s	average	s	**LD (µg/L)**
1.1 – 1.6	H_2S	< LD		< LD		< LD		< LD		0.68	0.02	0.65
1.8 – 3.1	MeSH	< LD		< LD		< LD		< LD		< LD		0.50
1.1	EtSH	< LD		< LD		< LD		< LD		< LD		0.50
25	DMS	3.79	0.19	5.84	0.06	6.42	0.04	6.20	0.15	9.37	0.08	1.00

H_2S: Hydrogen sulfide, **MeSH:** methanothiol, **EtSH:** ethanothiol, **DMS**: dimethylsulfide, **nd:** no determined; **LD**: limite of detection

Tabla 4.b. Reserva wine – Beronesa wine

		t 0		15 days		30 days		45 days		60 days		
Odor threshold (µg/L)	**Compound**	average	s	average	s	average	s	average	s	average	s	**LD (µg/L)**
1.1 – 1.6	H_2S	< LD		< LD		< LD		< LD		0.72	0.03	0.65
1.8 – 3.1	MeSH	< LD		< LD		< LD		< LD		< LD		0.50
1.1	EtSH	< LD		< LD		< LD		< LD		< LD		0.50
25	DMS	3.70	0.16	6.10	0.28	7.06	0.14	9.32	0.01	9.62	0.10	1.00

H_2S: Hydrogen sulfide, **MeSH:** methanothiol, **EtSH:** ethanothiol, **DMS**: dimethylsulfide, **nd:** no determined; **LD**: limite of detection

b. Determination of free and total sulfur dioxide SO_2

Free and total sulfur dioxide, SO_2, was analyzed according to the Ranking method in the established days. The following table shows the results.

Table 5. Levels of SO_2 in different periods of time

	t 0		15 days		30 days		45 days		60 days	
Sample (mg/L)	Free SO_2	Total SO_2	Free SO_2	Total SO_2	Free SO_2	Total SO_2	Free SO_2	Total SO_2	Free SO_2	Total SO_2
White Bordelesa	1.6	42.4	1.6	41.6	1.6	40.0	3.2	35.2	0.0	33.6
White Beronesa	1.6	41.6	1.6	40.0	0.0	35.2	1.6	35.2	1.6	40
Rosé Bordelesa	3.2	17.6	3.2	17.6	1.6	12.8	2.4	13.6	1.6	16
Rosé Beronesa	3.2	15.8	3.2	8.8	1.6	9.6	1.6	11.2	1.6	8
Aged red Bordelesa	1.6	17.6	0.0	11.5	0.0	9.6	2.4	13.6	1.6	14.4
Aged red Beronesa	4.0	17.6	4.8	18.4	1.6	14.4	6.4	16.0	6.4	24
Reserve Bordelesa	4.8	~~4.8~~	3.2	16.0	4.8	20.8	7.2	21.4	7.2	24.8
Reserve Beronesa	4.0	16.8	4.8	18.4	4.8	19.2	6.4	16.8	7.2	19.2

c. Determination of total acetaldehyde

After carrying out the analysis of total acetaldehyde at the different established times (initial time, 15 days, 30 days, 45 days and 60 days), the following results were obtained, which are shown hereunder in graphic form, according to the type of wine. The measurements were duplicated and the deviation is shown on error bar. The wines contained in Bordelesa bottle are designed as “control”.

Figure 1. Evolution of the level of total acetaldehyde (mg/L) in white wine bottled in Beronesa and Bordelesa bottle

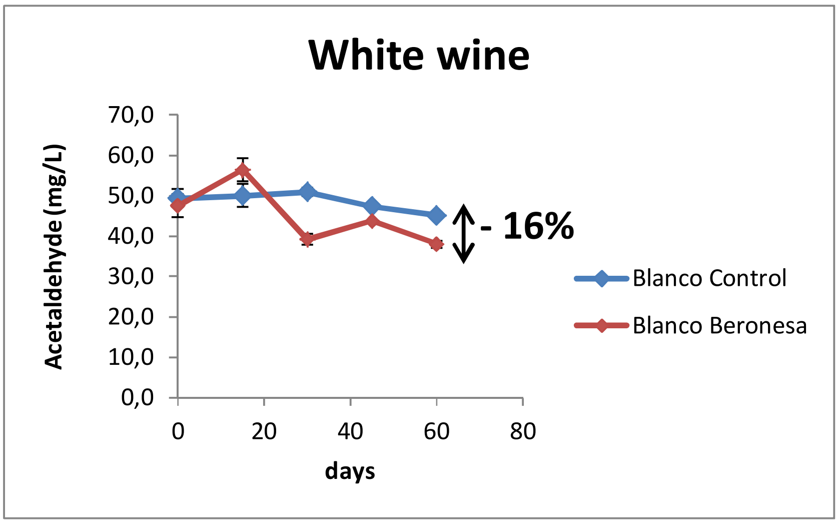


Figure 2. Evolution of the level of total acetaldehyde (mg/L) in rosé wine bottled in Beronesa and Bordelesa bottle

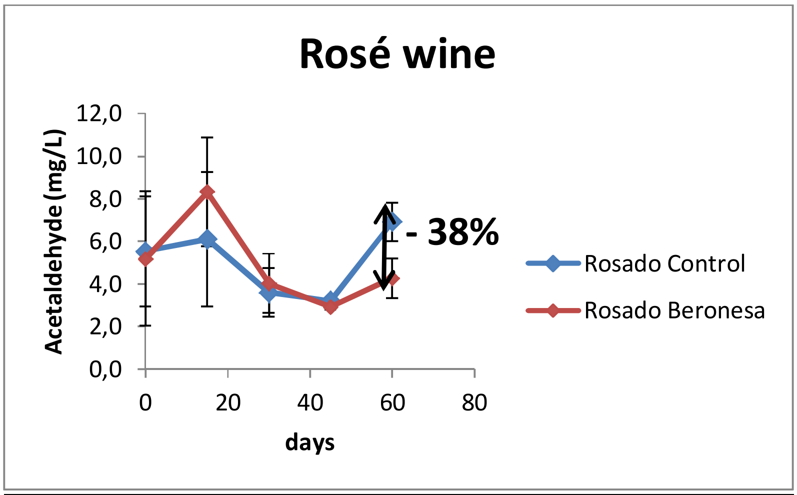


Figure 3. Evolution of the level of total acetaldehyde (mg/L) in aged red wine bottled in Beronesa and Bordelesa bottle

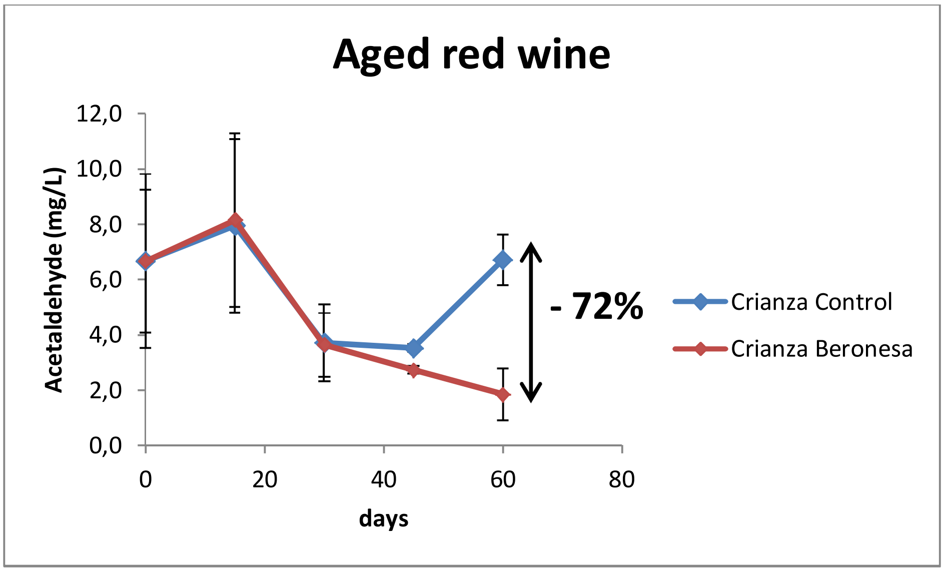
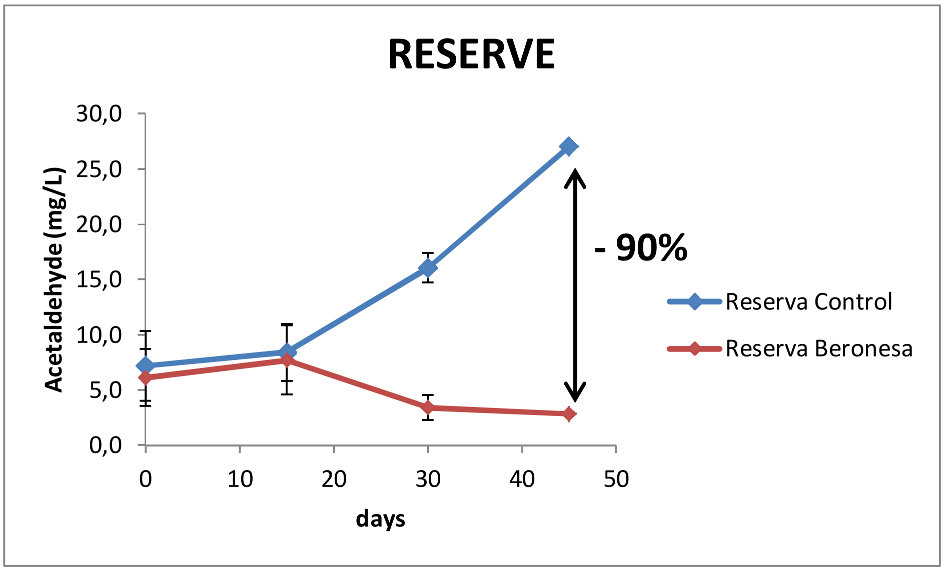


Figura 4. Evolution of the level of total acetaldehyde (mg/L) in reserve wine bottled in Beronesa and Bordelesa bottle



d. Determination of Strecker aldehydes

The following tables show the found levels of the five aldehydes analyzed, indicated as relative areas. Concentration units are not given because calibration straight lines are not available at the moment. Anyway, the relative areas allow us to carry out a comparative study including the different bottles and to evaluate the tendency of every wine in regard to time.

Table 6. Levels of Strecker aldehydes, expressed as units of relative area, estimated in white wine

White wine	Bordelesa t0	Beronesa t0	Bordelesa t15	Beronesa t 15	Bordelesa t 30	Beronesa t 30	Bordelesa t 45	Beronesa t 45	Bordelesa t 60	Beronesa t 60
Isobutyraldehyde	0.76	0.91	0.88	1.31	1.03	1.41	2.11	1.54	1.49	1.04
3-Methylbutanal	8.92	7.12	5.95	6.29	9.73	10.15	11.45	8.56	10.72	8.09
Diacetyl	6.03	4.25	8.01	11.17	4.50	6.77	7.96	6.46	4.22	3.26
Methional	0.34	0.54	0.36	0.30	0.29	0.31	0.79	0.53	0.39	0.22
Phenylacetaldehyde	63.37	72.86	33.74	26.31	31.26	31.54	46.92	37.17	48.83	33.31

Table 7. Levels of Strecker aldehydes, expressed as units of relative area, estimated in rosé wine

Rosé wine	Bordelesa t0	Beronesa t0	Bordelesa t15	Beronesa t 15	Bordelesa t 30	Beronesa t 30	Bordelesa t 60	Beronesa t 60
Isobutyraldehyde	0.51	0.48	0.69	0.50	2.40	0.64	1.38	1.22
3-Methylbutanal	4.36	4.82	6.31	3.67	4.62	4.82	7.31	7.42
Diacetyl	8.24	9.39	12.20	8.53	18.16	8.08	8.17	11.77
Methional	0.27	0.26	0.45	0.27	0.38	1.24	0.40	0.28
Phenylacetaldehyde	31.13	22.61	25.33	25.55	18.51	31.65	43.82	30.52

Table 8. Levels of Strecker aldehydes, expressed as units of relative area, estimated in aged red wine

Aged red wine	**Bordelesa t0**	**Beronesa t0**	**Bordelesa t15**	**Beronesa t 15**	**Bordelesa t 30**	**Beronesa t 30**	**Bordelesa t 45**	**Beronesa t 45**	**Bordelesa t 60**	**Beronesa t 60**
Isobutyraldehyde	1.19	1.85	0.94	0.85	1.03	1.28	1.13	1.31	1.53	1.57
3-Methylbutanal	6.53	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01
Diacetyl	22.76	11.73	6.21	9.38	6.80	10.29	6.47	7.74	5.62	7.75
Methional	0.39	0.37	0.21	0.29	0.19	0.25	0.24	0.27	0.17	0.26
Phenylacetaldehyde	46.99	40.88	33.31	35.85	29.20	32.93	33.78	32.51	31.51	34.44

Table 9. Levels of Strecker aldehydes, expressed as units of relative area, estimated in reserve wine

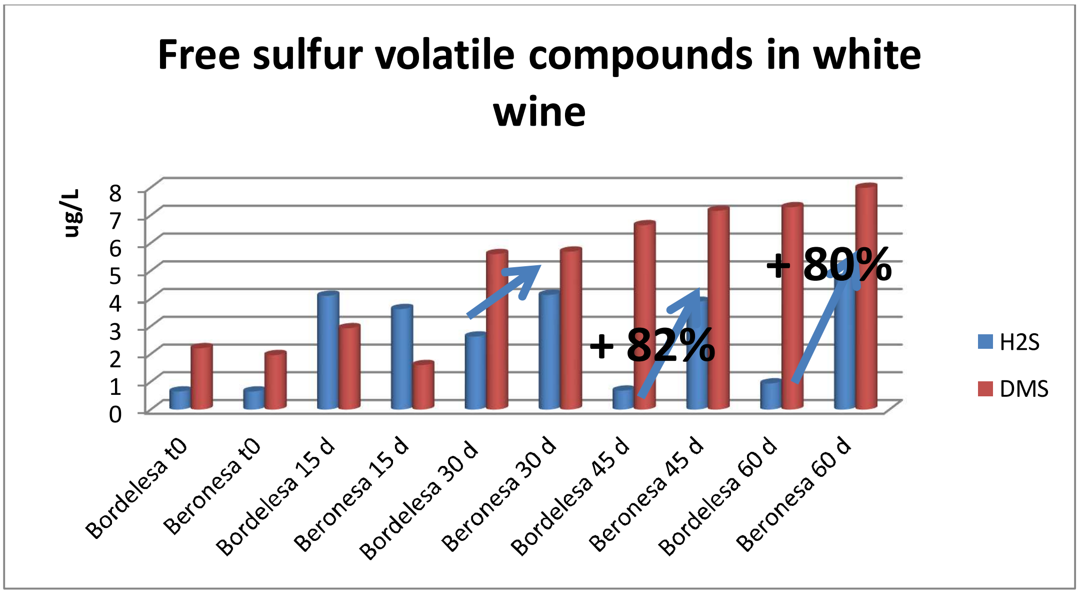
Reserve wine	**Bordelesa t0**	**Beronesa t0**	**Bordelesa t15**	**Beronesa t 15**	**Bordelesa t 30**	**Beronesa t 30**	**Bordelesa t 45**	**Beronesa t 45**	**Bordelesa t 60**	**Beronesa t 60**
Isobutyraldehyde	0.99	1.17	1.06	1.41	1.21	1.31	1.94	1.14	1.32	1.68
3-Methylbutanal	4.09	4.09	4.06	4.29	5.68	4.45	7.15	3.75	4.03	4.40
Diacetyl	6.68	6.15	7.99	8.09	6.85	6.88	15.13	7.72	8.29	5.63
Methional	0.21	0.23	0.22	0.20	0.47	0.47	0.26	0.22	0.22	0.21
Phenylacetaldehyde	38.23	39.46	28.62	31.88	46.76	36.64	50.64	29.66	31.91	25.78

5. INTERPRETATION OF RESULTS

Due to experimental restrictions, the time of observation of this experiment has been limited to 60 days only, working at a temperature of 35ºC. This period of time is too short to make evident some changes related to oxidation. Despite of it, we could observe some consistent tendencies and behaviors which allow making the following assertions:

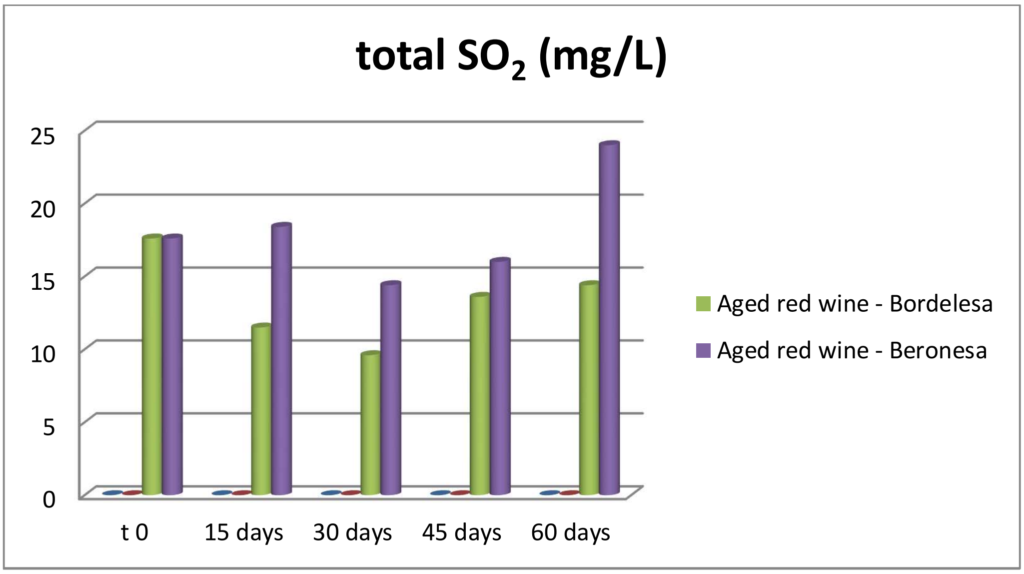
- **The Beronesa bottle has a far higher capacity to avoid the oxidation of the mercapto groups.** This higher capacity is clearly shown by the **significantly higher level of hydrogen sulfide (H_2S)** obtained in white wine in Beronesa bottle, after 30 days of incubation or longer. As shown in Fig. 5, whereas the level of this molecule in wine bottled in Bordelesa bottles decreases, as a first consequence of the oxidation, this is not so in the sample bottled in Beronesa bottle. The H_2S can be considered as a marker of aromatic molecules with the mercapto group. Therefore, these results suggest that the design of the Beronesa bottle achieves the protection of the mercapto function of aromatic molecules. This group is characteristic of variety bouquets as relevant as 4-mercapto-4-methyl-2-pentanona, 3-mercaptohexanol or acetate of 3-mercaptohexyl.

Figure 5. Levels of H_2S and DMS in white wine



- **The Beronesa bottle avoids the loss of SO_2 in unstable wines.** This can be seen in vintage wine, that is the only one in which a significant decrease of SO_2 can be observed in the short period of time of the study. The results show a behavior in favor of the Beronesa bottle, as shown in Fig. 6.

Figure 6. Levels of total SO_2 (mg/L) in aged red wine



- **The Beronesa bottle avoids the accumulation of acetaldehyde in all cases.** In this case, the results are consistent and involve all wines. We can note that the wines in Beronesa bottle present lower levels than those stored in Bordelaise bottle (see Fig. 1-4). Therefore, this supports the theory suggesting that the Beronesa bottle is able to protect the aromatic compounds of wine better than the Bordelaise design.

- The Beronesa bottle hinders the accumulation of **Strecker aldehydes**. Considering the short period of time of our sampling, it seems that the necessary level of oxidation at which these molecules can accumulate could not be reached, except in the cases of white wine and rosé, which, according to Fig. 7-9, show lower levels of phenylacetaldehyde after storage in Beronesa bottle.

Figure 7. Levels of phenylacetaldehyde, expressed in areas

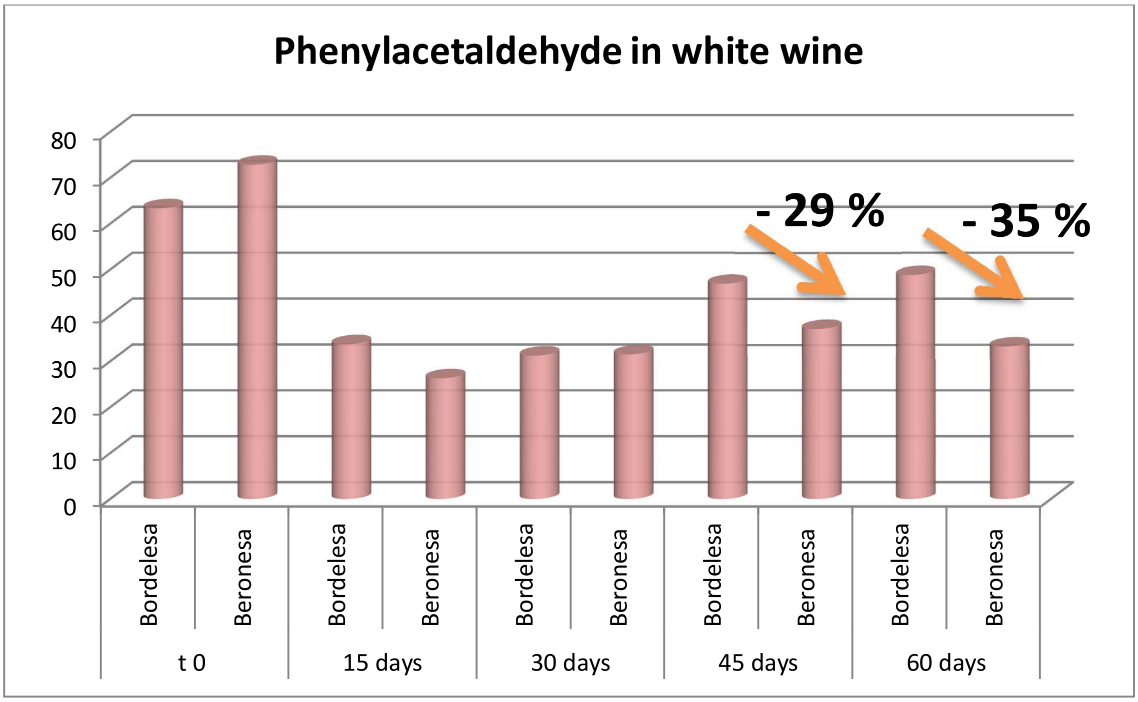


Figure 8. Levels of 3-methylbutanal, expressed as areas

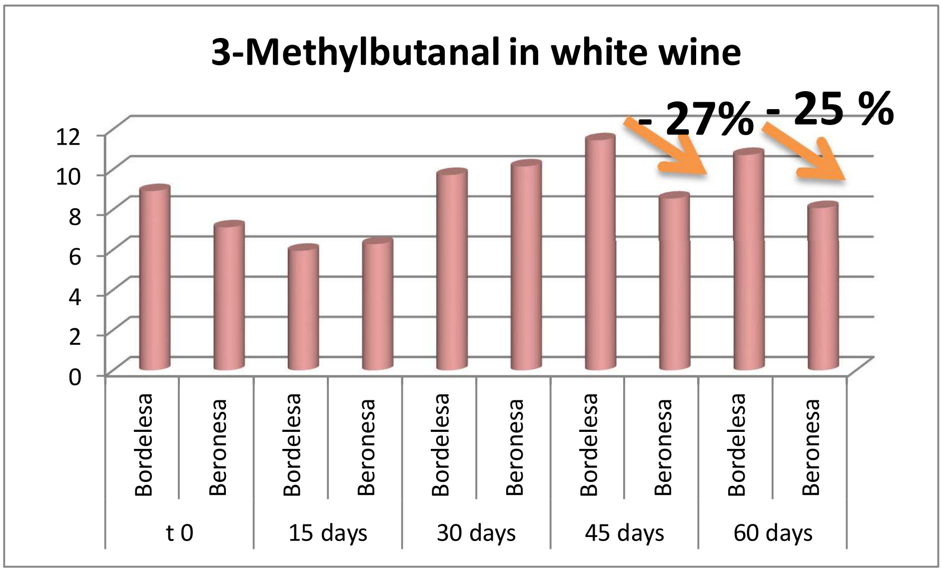
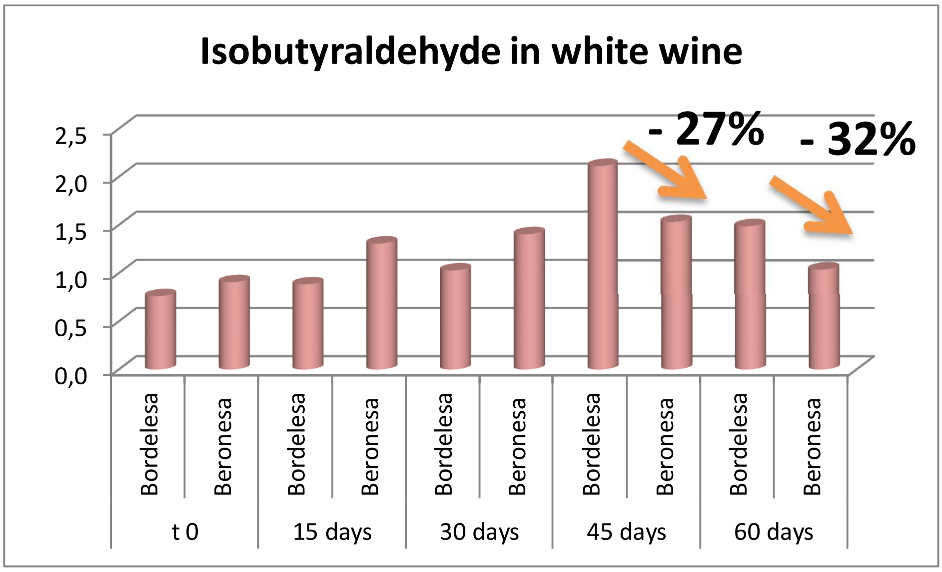


Figure 9. Levels of isobutyraldehyde, expressed as areas



FINAL CONCLUSIONS

Very revealing conclusions have been extracted about the potencial of the Beronesa bottle's desing. This study reveals that Beronesa bottle is able to protect the lábiles bouquets more efficiently than the Bordelesa bottle.

In this study SH2 (hydrogen sulfide) is used as a molecule group-SH indicator, which are varietal bouquets that with the time tend to oxidize, and their presence is good for the wine.

This is why, what really matters is that one the SH2 is formed, it can be observed how it decreases in the Bordelesa bottle, while it can be seen how it presence maintains, or it even increases in the Beronesa bottle's. This is the reason why we say that the design Beronesa of the Beronesa bottle's is able to protect the bouquet of the wine.

The Beronesa bottle permits that the SO2 endures longer and therefore it endures the wine's life and it improves it's synthetic and microbiologic stability during a long period of time.

If the amount sulphites is reduced, the Beronesa bottle can contribute to make the oxidative stability equivalent, but it cannot contribute -initially- to improve the microbiological stability. However, if the bottle is employed in a microbiologicaly secure context, with sterile microbic filters, it must allow to reduce the sulphites with an equivalent protection level.