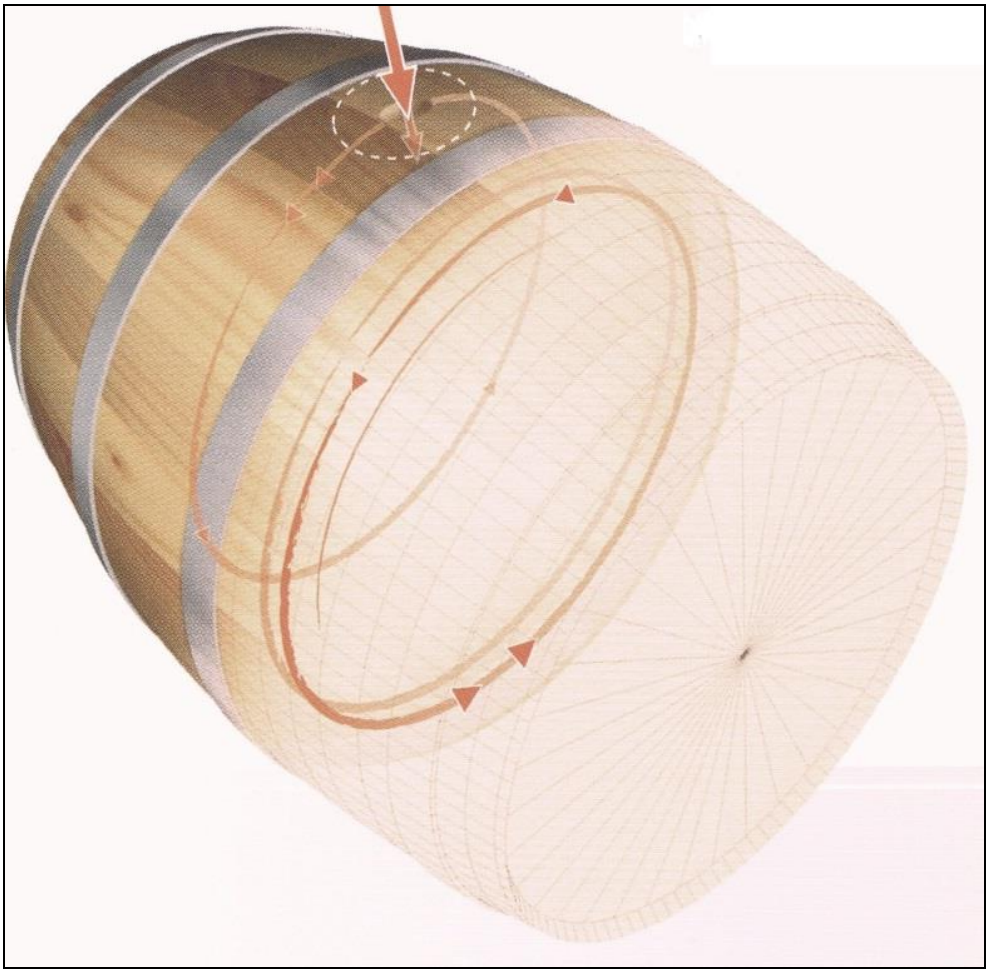

STUDIES CONDUCTED ON THE EFFECT ON WINE WOOD AROMAS AND MICROBIOLOGY IN REGENARATED BARRELS USING THE BARENA® METHOD

PhD Antonio Palacios (Laboratorios Excell Ibérica S.L.)
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INTRODUCTION

The regeneration method of barrels used with the Barena® method consists of the following processes:

- Initial physical-mechanical stripping away by blasting with jets of quartz particles. The pressure is regulated depending on the state of the barrel. The stripping away process is effective on a surface thickness of between 0.2 and 0.4 millimeters.
- Removal of stripping residues by rinsing with previously dechlorinated water.
- Application of dry steam at above 100º for a few minutes to obtain a high level of microbiological sanitization.
- Rinsing, drying and putting bungs in the barrels.
- Subsequently, gaseous sulfur is injected to keep the barrel in a state of optimum preservation while being shipped to the receiving winery.

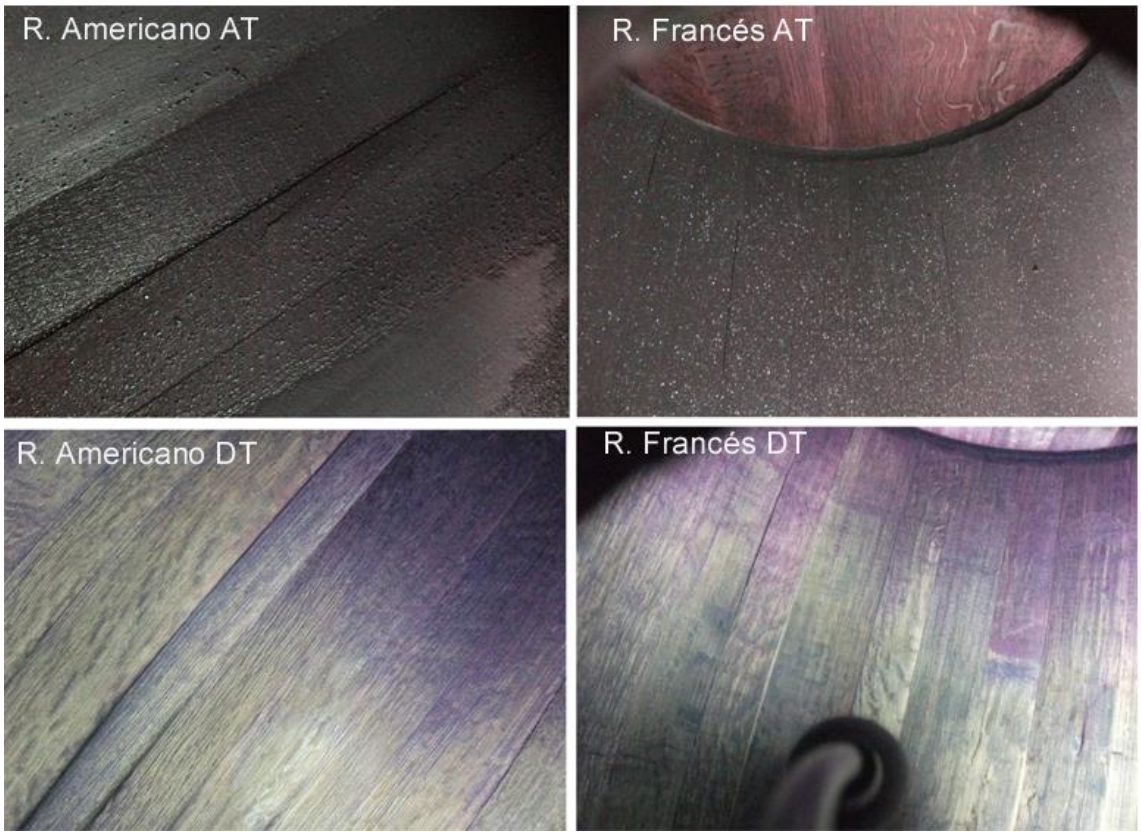

Figure 1. *Photograph of the interior of untreated barrels (AT) and after the Barena® treatment (DT).*

INDUSTRIAL WINERY TESTS. AROMATIC BENEFITS OF OAK:

1-. Tests on several wines of the Tempranillo variety in French oak (Rioja):

This is a study of several wines from the same winery, spread over a homogeneous barrel room with different treatments. The first three samples come from the same wine in three different barrels:

- The first is a crianza wine from the year 2011, which has spent 15 months in 6-year-old unregenerated barrels, and then 3 months in a regenerated barrel.
- The control barrel is the second graphic representation, with the wine spending 18 months in unregenerated barrels (6 years old).
- The third sample is a wine aged in regenerated barrels (6 years old) for 18 months.
- The fourth sample is a 2012 vintage wine with 18 months of aging in regenerated barrels (6 years old).
- The fifth one is a wine from the 2012 vintage with 11 months of aging in regenerated barrels (6 years old).

It can be seen from these results (figure 1) that there is a transfer of positive aromatic characteristics from the wood to the wine. In this way, it can be said that the regenerated barrels continue to release said aromas after the regeneration treatment of spraying quartz particles followed by steam disinfection. This can be seen in the level of eugenol and the corresponding lactones, demonstrating that there is not a loss of these aromas in the regenerative process, but quite the opposite. The wines with the longest time in barrels from 2011 have more wood aromas than those from 2012. *(Analysis carried out by Laboratorios Excell Ibérica).*

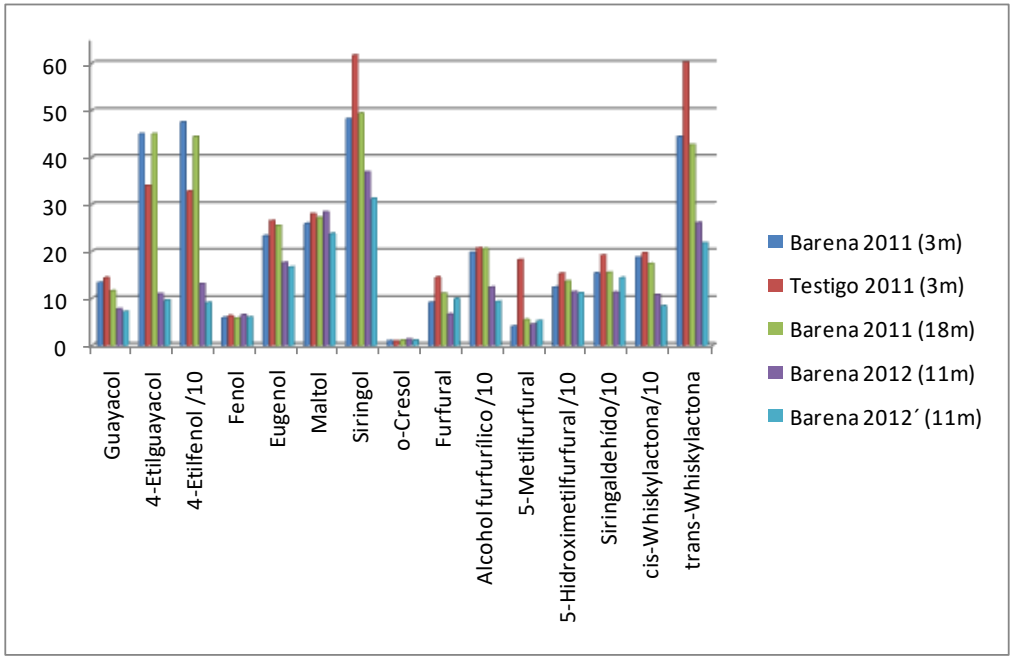

***Figure 2**: Graph showing aromas from oak wood in different wines from the same cellar in regenerated and unregenerated barrels (concentrations in µg / L).*

2-. Test on a wine of the Tempranillo variety in new, regenerated and shaved stave barrels (Rioja):

In this other study, the first three samples come from the same wine aged for 18 months in new barrels. The first two are from American oak, and the third is from French oak.

The fourth sample comes from the contents of a Barena® regenerated barrel (8 years old) with the same wine. The fifth one is from a shaved stave barrel that has not been retoasted.

In this analysis of the samples presented above, it can be seen that there is a strip of residual toasted wood left in the Barena® regenerated barrels, as can be seen by the levels of the presence of the 5-methylfurfural compound, an aroma that comes specifically from wood which has been toasted by fire. Another interesting result is that the concentration of whiskey lactone is much higher in new American barrels, which is normal, but almost at the same level in the new, French and regenerated barrels. A for the other compounds, there are no more significant differences. *(Analysis carried out by CEAD Laboratories).*

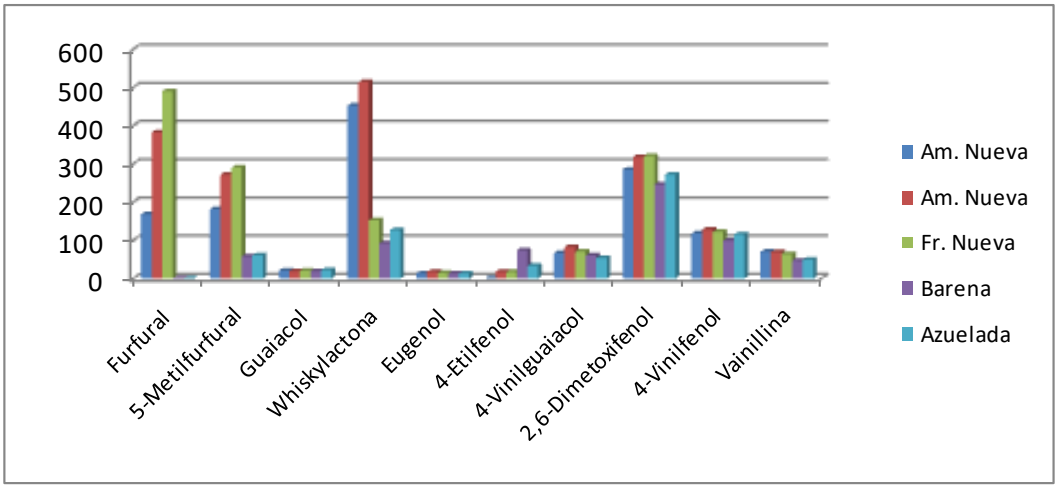

***Figure 3**: Graph showing the aromatic wood characteristics of the same wine from the tempranillo variety preserved in different types of new, regenerated and shaved stave barrels, (concentrations in µg / l).*

3-. Test on a wine of the Tempranillo variety, showing aromatic evolution over time (Rioja):

In this cellar trial, the evolution of wood aromas in the control wine which has been conserved in a stainless steel tank without contact with wood, and the same wine at 4 and 6 months of aging in barrels have been studied (Barrel from 2005, regenerated in 2012).

As can be seen (figure 4), during the first 6 months there continues to be a continuous increase in the concentration of typical wood aromas, mainly with respect to syringaldehyde, 5-hydroxymethyl furfural, cis-whiskey lactone, furfural and vanillin, which have constantly increased during 6 months of aging. This shows that both the typical aromas of wood toasting

and of the wood itself, continue to give sensory value to the wine aged in regenerated Barena® barrels. *(Analysis carried out by Laboratorios Excell Ibérica).*

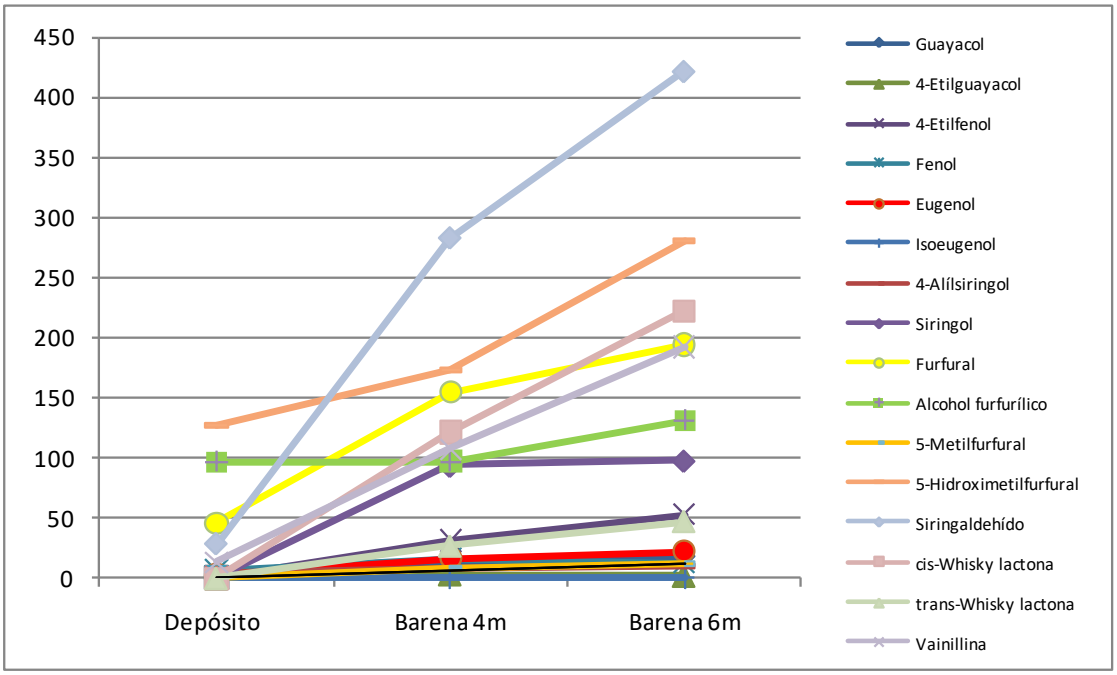

***Figure 4**: Graph showing the evolution of oak wood aromas for 6 months in regenerated Barena® barrels, (concentrations in µg / L).*

4-. Test on a wine of the Tempranillo variety in barrels with one and two regenerations (Rioja):

In this test, which was also performed at an industrial level, the aromatic profile of wood in the same wine can be compared.

The starting wine is a sample of wine in 11-year-old standard-type barrels from the winery.

The Barena® 11aR7 sample is the same 11-year-old barrel wine (2002) that has been regenerated in its seventh year (2009).

The Barena® 12aR7R9 sample is the same wine in 11-year-old barrels (2002) regenerated twice, in its seventh year (2009) and then in 2013.

This study is very interesting, since as it can be seen, only maltol and furfural are higher in the barrel considered as standard. However, compounds such as 5-methylfurfural ,, furfuryl alcohol and syringaldehyde are higher in regenerated barrels , being equivalent both in the first regeneration and in the second. Consequently, it is estimated that this second treatment continues to respect part of the area of toasted wood. This effect on the positive toasted aromas can also be verified in other aromas that are also extremely desirable in barrel-aged wine, such as eugenol, cis-whiskeylactone, trans-whiskeylactone and vanillin.All aromas which are typical in quality oak. *(Analysis carried out by Laboratorios Excell Ibérica).*

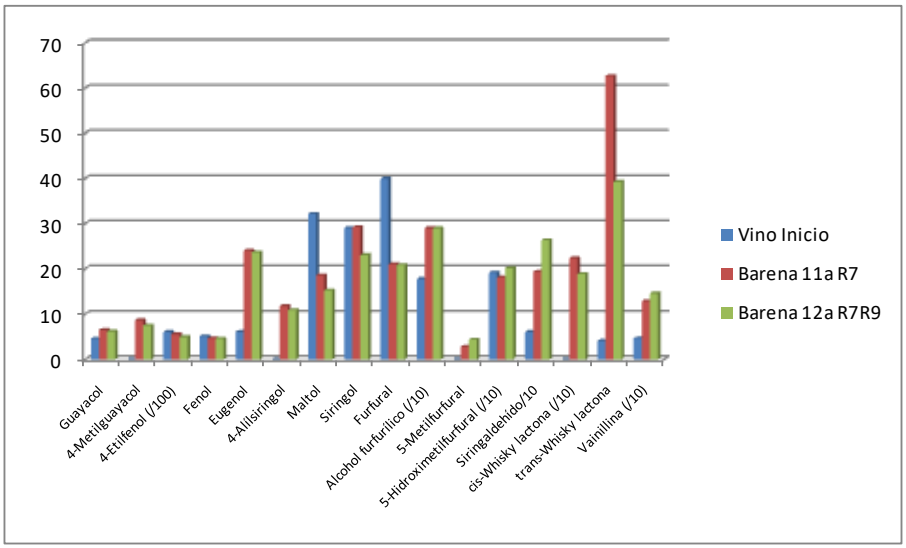

***Figure 5**: Graph showing wood aromas in the same wine comparing the control with the wine in regenerated barrels and with 1 and 2 times, (concentrations in µg / L).*

5-. Test on various wines of the cabernet sauvignon variety (Bordeaux):

This test comes from France and is done using the cabernet sauvignon variety. Barrels from the same cellar are all compared with the same wine. The first using traditional cellar washing, the second using the same system with the addition steam, the third in Barena® barrels and the fourth in new barrels. What can be seen is that the new barrel is the one that stands out the most in the transfer of wood aromas. This as can be expected. However, the second one is the Barena® barrel. The most notable results are observed at the level of cis-methyloctolactone, vanillin, hydroxylactone and siringol. *(Analysis carried out by Laboratorios Excell France).* Graph showing wood aromas in the same wine comparing the control with the wine in regenerated barrels and with 1 and 2 times, (concentrations in µg / L.

***Table 1**: results of the analysis of wine aromas in different barrels*

Tipo de lavado µg/L de vino	Barrica L. Clásico	Barrica Vapor + SO2	Barrica Barena	Barrica Nueva
Furfural	89	88	88	129
Metil-5-furfural	4	5	5	86
Alcohol furfurílico	23	29	17	24
Guaiacol	8	10	10	14
Trans-metiloctolactona	1	2	0	2
Cis-Metiloctolactona	137	154	158	321
Metil-4-guaiacol	1	1	1	7
Fenol	5	5	5	6
Etil-4-guaiacol	7	7	7	3
0-cresol	1	1	1	1
m-p-cresol	2	2	2	2
Eugenol	28	24	27	41
Etil-4-fenol	46	40	41	9
Isoeugenol	1	1	1	11
Siringol	31	50	53	63
5-hidroximetilfurfural	42	41	52	84
Alil-4-siringol	8	9	13	45
Vainillina	55	72	96	231
Siringaldehido	104	141	175	731

Figure 6 from the same test shows the same results as in the previous case, but grouping the chemical compounds into olfactory families, where it can be seen that the new barrel stands out from the aromatic standpoint. Nevertheless, the Barena® regenerated barrel is in second place, where levels of lactones and phenolic aldehydes are the ones that present the largest number of differences compared to the systems with classic cellar maintenance. It can be surmised that the differences found analytically here are more qualitative than quantitative.

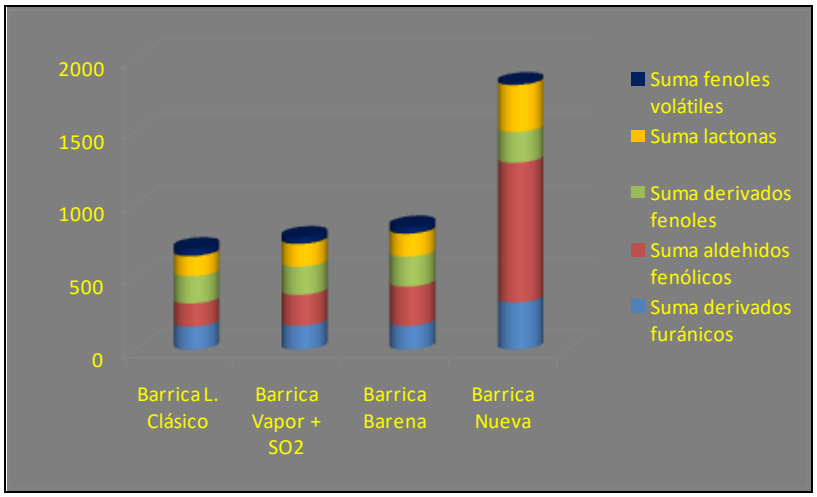

***Figure 6**: graph showing aromas grouped by families. (concentrations in µg / L).*

Figure 7 shows the level of tannins taken from different types of barrels in wine from the same test. The most notable is that the highest release of ellagic tannins is observed in the level of the Barena® regenerated barrel. This was, predominantly, thanks to the small-scale renewal of the inner layer of the wood that is produced from the stripping system by blasting with quartz particles. In second place, there is the new barrel and later, at a significant distance, the barrels maintained with the classic system of the winery.

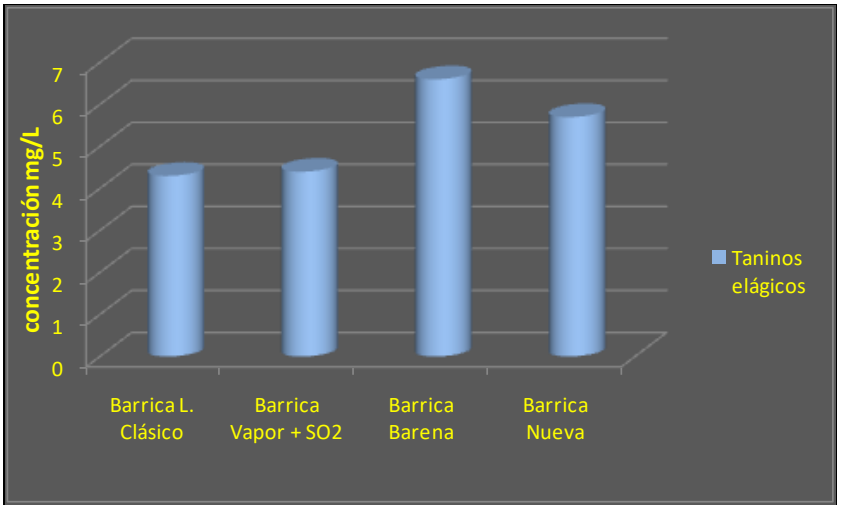

***Figure 7:** Graph showing the concentration of ellagic tannins in the different barrels.*

6-. Test on a wine from the cabernet sauvignon variety; effect on tannins (Bordeaux):

Figure 8 compares the same aged wine kept in barrels with classic cellar washing in their third year, against the same wine aged in a Barena® regenerated barrel, which are also three years old. The most remarkable thing about the level of results can be seen in the level of differences. It is the regenerated Barena® barrel that releases more methyloctolactone and syringaldehyde compared to the barrel used as a control. *(Analysis carried out by Laboratorios Excell France).*

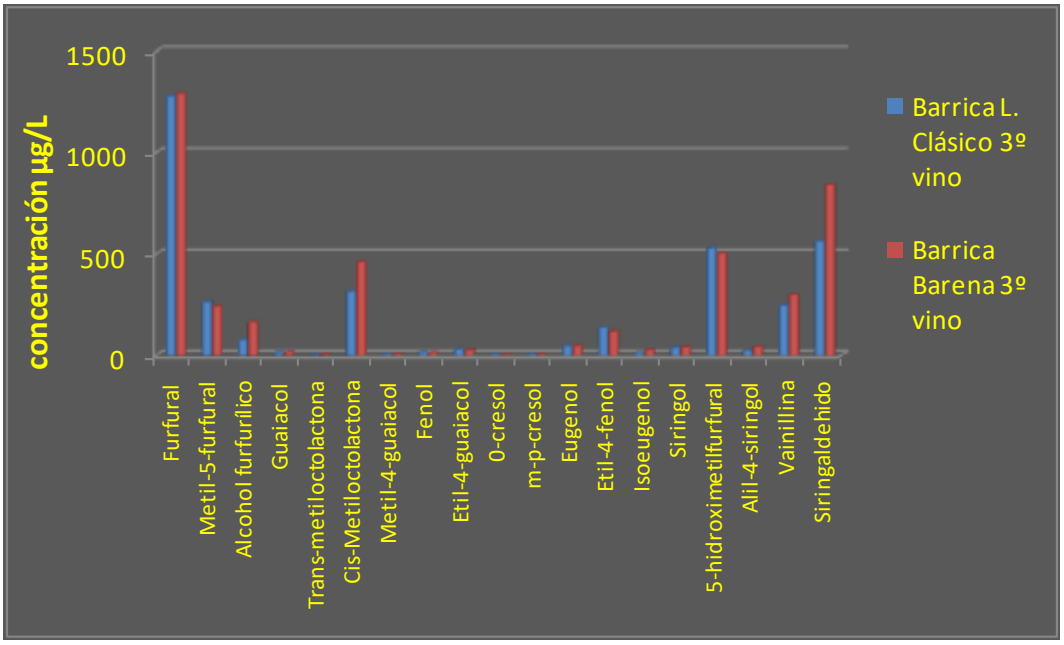

***Figure 8**: Graph showing wood aromas in two different barrels, one of them with Barena regeneration.*

7-. Aromatic quality according to the origin of the oak: regenerated oak versus derivatives:

For this study, the criteria published in the International Food Research magazine were followed: "Criteria to discriminate between wines aged in oak barrels and macerated with oak fragments". Purificación Hernández-Orte, Ernesto Franco, Carlos González Huerta, Juana Martínez García, Mariano Cabellos, Julián Suberviola, Ignacio Orriols and Juan Cacho in 2014. This publication collected the results of aroma analysis from more than 200 samples, managing to differentiate between those aged in barrels and those which were in contact with oak chips.

In our case, 23 samples of Spanish wines were analysed following this same methodology. Of the 17 wines, 7 of them were aged in barrels for more than 6 months, two of them in new barrels (Rja Crz11, Rja Crz 10 Com 1, Rja Crz 10 Com 2, Rja Am Nv 13, Rja Fr Nv 13, Rja Am 3a 12 and Rja Fr 3a 12), 5 were aged in regenerated barrels (Bar. 10m Crz12, Bar. 9m RF Crz05, Bar. 9m RA Crz07, Bar. 9m RF Crz08 and Bar. 9m RA Crz05), 6 of them were in contact with chips (TG, SCA, DC 310, DC 190, S11 T + and S11 Tm) and 5 wines had mixed aging in both oak barrels and using oak chips or fermented with oak chips (DC 210, T11 T +, T11 Tm, Chip + Barr and Chip in Ferm.)

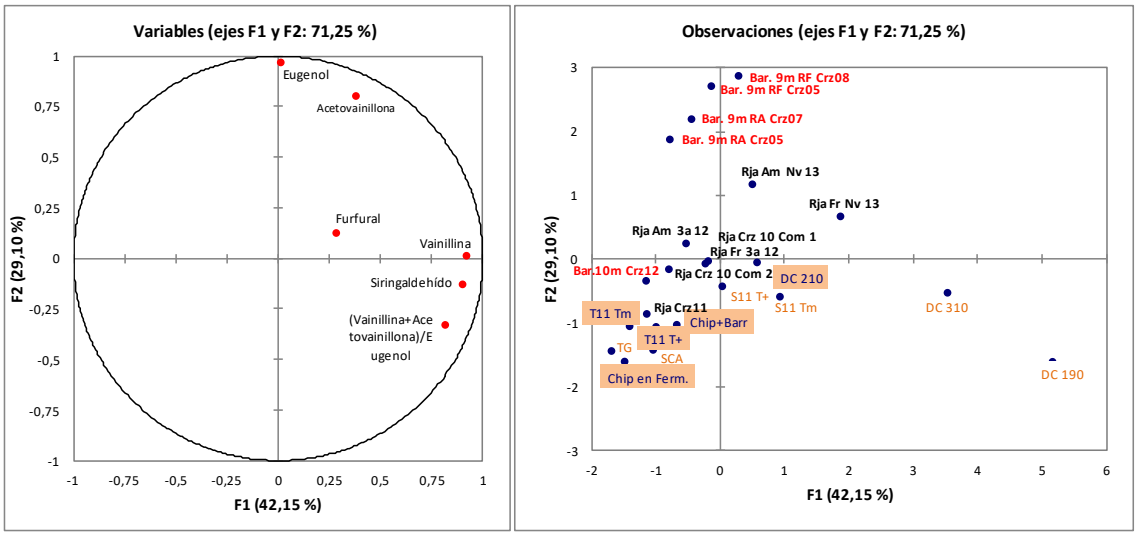

***Figure 9**: Analysis of the main components of oak aromas against 23 wine samples (aged in barrels: Rja Crz11, Rja Crz 10 Com 1, Rja Crz 10 Com 2, Rja Am Nv 13, Rja Fr Nv 13, Rja Am 3a 12 and Rja Fr 3a 12, aged in regenerated barrels: (Bar.10m Crz12, Bar. 9m RF Crz05, Bar. 9m RA Crz07, Bar. 9m RF Crz08 and Bar. 9m RA Crz05, in contact with chips: (TG , SCA, DC 310, DC 190, S11 T + and S11 Tm and mixed aging in barrels and chips: DC 210, T11 T +, T11 Tm, Chip + Barr and Chip in Ferm.*

As you can see in Figure 9, there is a very efficient regrouping of samples. In the lower part, the samples with oak chips (light brown color) are placed next to samples, which although they have been in contact with the barrel, were also supplemented with chips (blue color and brown box). In the very upper part of this area of the graph are the samples aged in barrels, the samples with new barrels being located in the highest part of this area of the graph. The samples aged in regenerated barrels are associated with each other and are located in the highest part of the second axis, with the exception of one of them, correlated with eugenol and acetovainillone. The ACP factor structure manages to explain 71.25% of the variance and has managed to efficiently differentiate between the samples according to their type of aging. This corroborates the results published in the work cited above.

8-. Tests on Crianza and Tempranillo Reserve wines in the D.O.Ca. Rioja:

Parallel trials are presented below, one with 2012 Tempranillo wine aged for 9 months in standard cellar barrels, versus regenerated barrels. In this test, the aim is to study the effect on new unaged wine intended for the Crianza type. In the second test, the same type of monitoring is carried out, but using a 2011 wine destined for the Reserve type, but in this case, the same wine is aged for 24 months in standard cellar barrels and one of them for 18 months in standard barrels. 6 final months in regenerated barrels, looking for the final refinement without the woody aromas being noticed too much.

The results of the first Crianza-type wine can be seen in figure 10, where increases of 16.5% are seen in eugenol, 39% in furfural, 43% in 5-methylfurfural, 32% in syringaldehyde, 9.7% in cis -whiskylactone, 26.9% in vanillin and 4.7% in acetovainillone for wines aged in regenerated barrels.

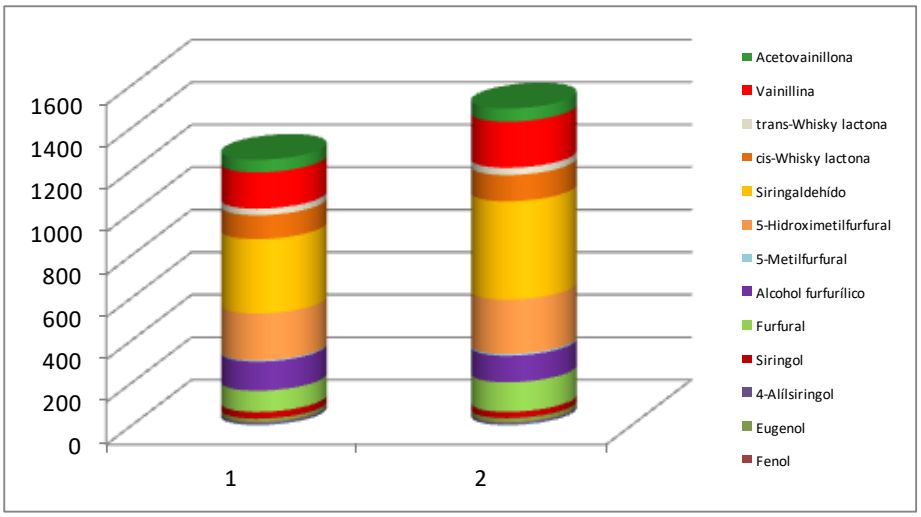

***Figure 10**: Analysis of the aromatic components of wood for the same 2012 wine aged for 9 months in standard cellar barrels compared with in regenerated barrels.*

In regard to the second Reserve type wine (figure 11), the increases observed in wine of 18 months in standard barrels, and 6 months in regenerated barrels, compared to the same sample of 24 months in standard barrels, were 24.2% for guaiacol, 14% for eugenol, 51.8% for furfuryl alcohol, 60.4% for 5-methylfurfural, 17% for cis-whiskeylactone, 11.7% for vanillin and 12.5% for acetovainillone.

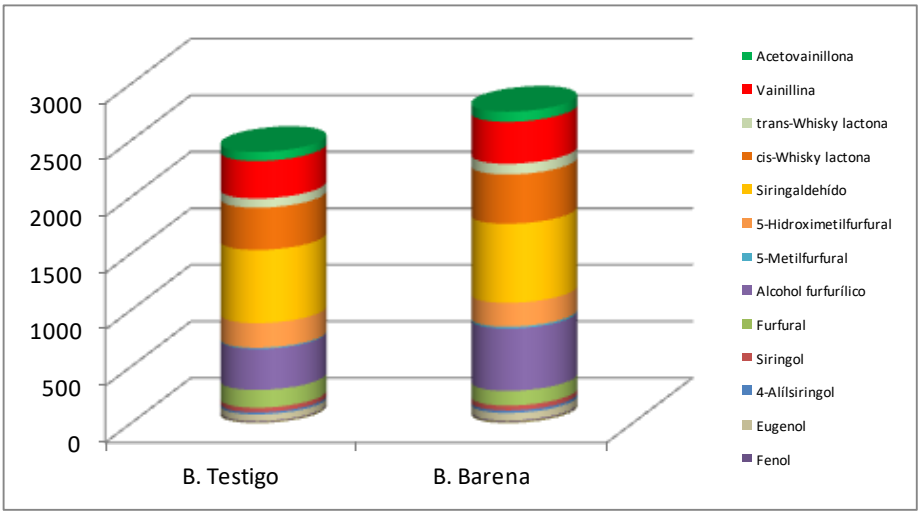

***Figure 11**:* Analysis of the aromatic components of the wood for the same 2011 Reserva wine, but in this case, the same wine has been aged for 24 months in standard cellar barrels, and one of them for 18 months in standard barrels plus 6 months more in regenerated barrels.

These results are fascinating, when it is taken into account that the average prices per litre of wine from new French oak barrels (€ 0.75 / litre) and American oak (€ 0.38 / litre) with 4 years of active use, the prices of regenerated barrels (€ 0.06 / litre) and the average prices of chips (€ 0.03-0.02 / litre), aging in regenerated barrels clearly gives added value to the wine with very profitable investments at low cost.

THE MICROBIOLOGICAL EFFECT OF THE BARENA® TREATMENT ON BARRELS.

A total of 434 barrels analysed before and after the Barena® treatment have been compared. These correspond with different wineries, different cooperages and with barrel ages between 1998 and 2010 (figure 13).

To carry out a homogeneous and representative sampling, a new method has been implemented. Using a metal brush in sterile conditions, the bottom of the barrel is brushed for one minute. Subsequently, 2 litres of sterile water are added and the sample is collected. Comparing this method with those usually used for sampling barrels (Filling the barrel with sterile water followed by an incubation period), allows us to avoid false negatives after treatment, as well as to discern whether the contamination of the barrel is either superficial or deep.

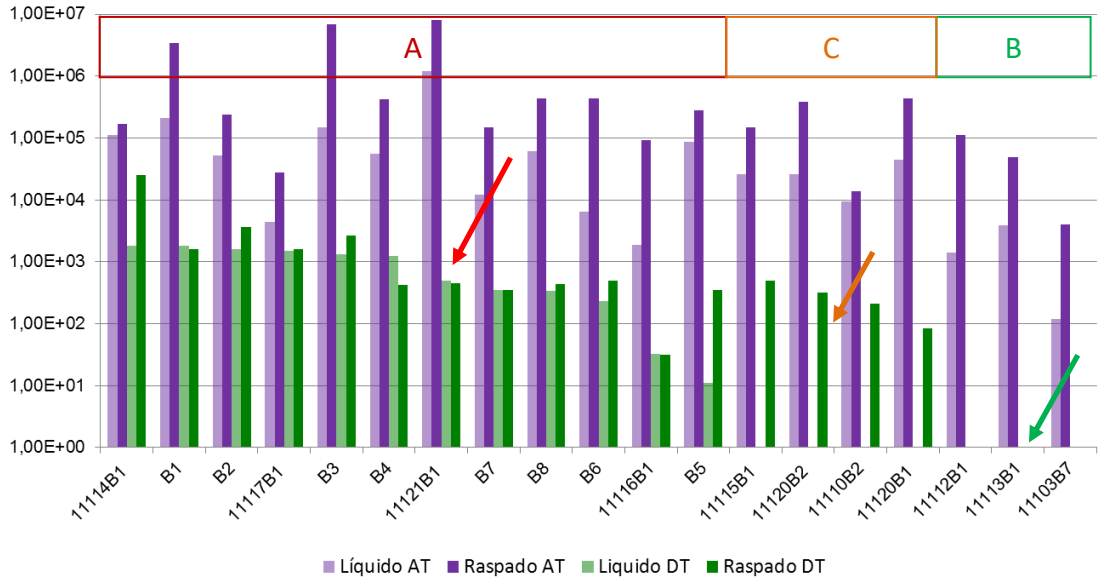

Figure 12: Group A barrels with deep brettanomyces contamination, positive counts after treatment (BT) with both methods. **Group B** barrels with surface contamination, negative BT counts with both methods. **Group C**, risk of false negative, positive BT count with the metal brush scraping method and negative with the conventional (liquid) method. (BT = Before Treatment)

Of the total number of barrels analyzed, 67% correspond to American oak barrels, 23% to French oak and the rest to Hungarian, Russian and mixed oak barrels.

The analitical technique used is Real-time PCR, for operational reasons due to the barrel treatment line work rate, as this technique allows a result in hours in comparison to the traditional one that takes 8 days. The expression of the results is measured in Genomic Units (UG / ml)

Before their treatment, only 12.31% had an initial Brettanomyces contamination of less than 103 UG / ml (3 Logarithmic units), the rest of them exceeded this figure, and 53.54% had an

initial contamination of more than 104 UG / ml (4 units Log), even reaching, in some cases, to over a million UG / ml (6 units Log).

Once treated by the Barena® method, initial contamination tends to decrease by between 2.5 and 3 logarithmic units (see figure 13). This means that if the initial contamination was 103 UG / ml (3 Log units) it is reduced to less than 10 UG / ml, and if the starting contamination is greater than 106 UG / ml (6 Log units), it is then reduced to 103 (3 Log).

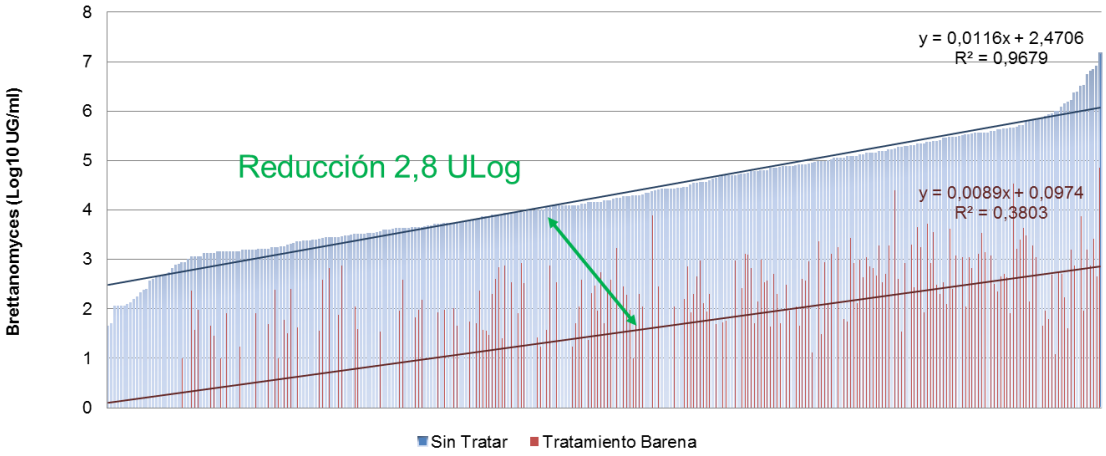

Figure 13: Concentration of Brettanomyces, displayed in logarithmic units, in 434 barrels analysed before and after the Barena treatment. The trend line and the correlation coefficient have been added for each group.Concentración de *Brettanomyces*, expresado en unidades logarítmicas, en 434 barricas analizadas antes y después del tratamiento Barena. Se añade la línea de tendencia y su coeficiente de correlación para cada grupo.

Currently, using the PCR technique, the DNA of the species sought is detected, which implies that the result is obtained from live, dead and viable colonies, but not cultivable ones. In order to verify the disinfection status, controls were carried out using a traditional culture. Brettanomyces did not grow in any of the cases, so although DNA from the species remains, it corresponds with dead cells. Additionally, microscopic observations were made (figure 14) and controls for the presence of microbial flora indicators (mesophilic aerobes at 30 ºC), state of disinfection indicators in the barrel after treatment, in 99.9% of cases the microbial count was null. (figure 15)

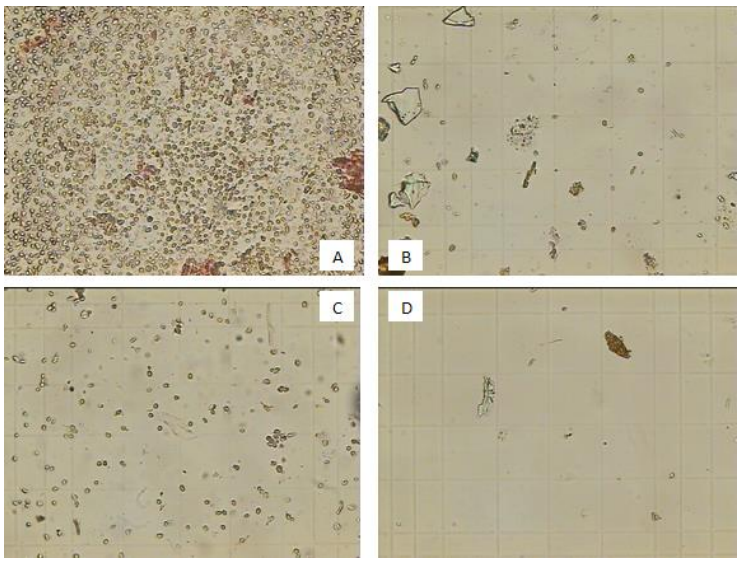

Figure 14: Total cell count in Neubauer chamber. Optical microscopy image, x400 magnification. A- Untreated barrel, initial contamination greater than 107 CFU / ml. B- Barrel that has been previously treated. C- Untreated barrel, initial contamination at around 105 CFU / ml. D- Barrel that has been previously treated.

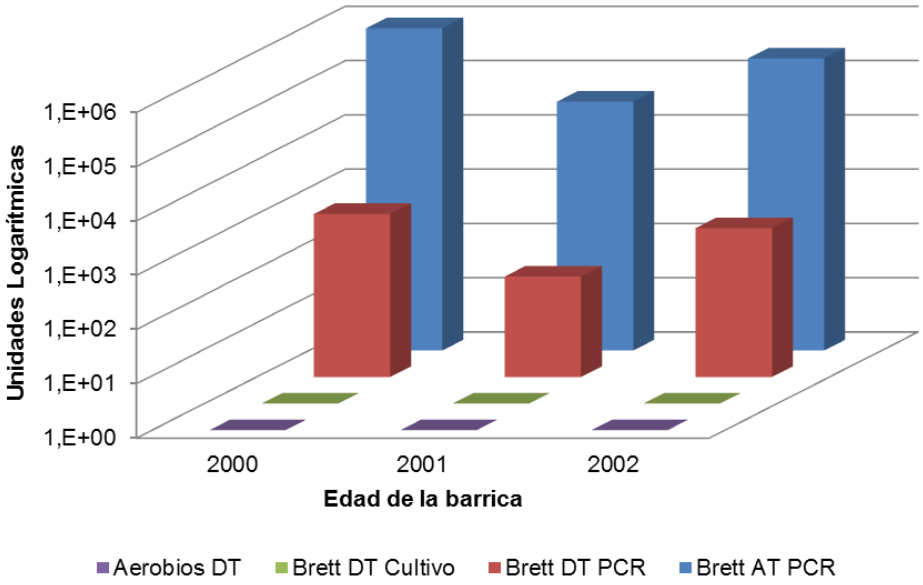

Figure 15: Verification of the disinfection status of 34 barrels after treatment (AT) compared to the same barrel before treatment (BT). (PCR method and culture mediums).

SENSORY EFFECTS OF WINES PRODUCED IN BARENA® BARRELS

In this trial, a blind tasting was performed on two different wines from the same cellar of the Tempranillo variety preserved in barrels treated with the Barena® method, and for barrels equivalent in age and type from the cellar. The tasting descriptions are as follows:

- Wine Tasting A; Barena® barrels:

-. Visual Phase: Cherry red with violet hints, with a high colour intensity in the heart of the glass.
-. Aromatic phase: Very intense and rounded. The fruit aromas appear in an unagitated glass, with expressions of blackberry, red fruit and licorice notes,while remaining very varietal. The oak, which seems new, is also highly noticeable, with very pleasant cinnamon, vanilla, coconut and sweet pastries scents. Within the great complexity of the wine, we find very subtle dairy aromas, with toffee, strawberry caramel and cream. Tobacco, incense and balsamic aromas are also present.
-. Tasting phase: This wine is extremely flavoursome, intense, well balanced on the palate, and very fruity in its evolution. The acidity brings a lot of freshness. The tannins are sweet, delivering a pleasant sweet sensation in the mouth. The retronasal is very clean and elegant, with a long finish.
>>> This is the most preferred wine of the two. It is very balanced on the fruit-wood level and the wood is the cleanest and finest of all. The evolution in the glass over time is very positive and it improves as the wine breathes. This is a crianza with a lot of class and distinction. It could last even longer in the barrel.

- Wine Tasting A; Standard barrels:

-. Visual phase: Cherry red with violet hints, with a high colour intensity in the heart of the glass.
-. Aromatic phase: It is less complex than the previous one. It also has less fruit, resulting in more spice and with more marked lactic tones.
-. Tasting phase: In the mouth, it presents a profile similar to the previous one, but is flatter, with less body and the tannins are earthier. It is also differentiated by the finish, which is more bitter. The retronasal is very marked by smoked and toasted aromas, reminiscent of charred wood, caramel and coal.
>>> This is a very pleasant wine, but smoke and toasted flavours mask the fruit, reducing its presence. As for the wood tannins, they are not as noble and appear somewhat bitter..

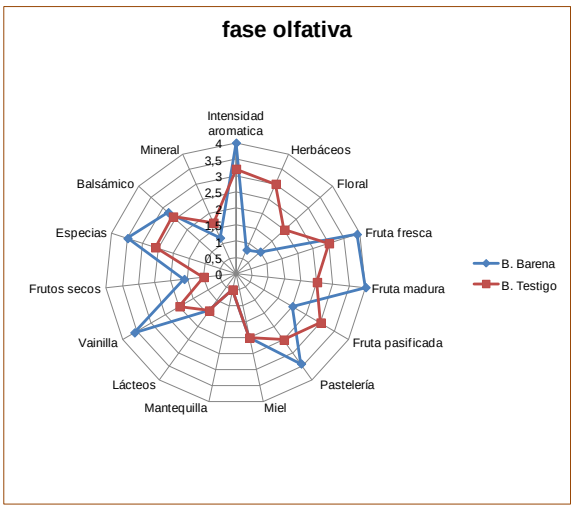

Figure 14: Spider web graph of the olfactory phase for wine A

- Wine Tasting B; Barena® barrels:

-. Visual phase: Cherry red with violet hints, with a high colour intensity in the heart of the glass, appearing more violet and somewhat more opaque.
-. Aromatic phase: On the nose, wood aromas are at the forefront, which is present in the form of raw, lightly toasted, new wood. The fruit is somewhat more in the background, but is easily noticable. It also has aromas of nuts, hazelnuts, pistachios, sweet nougat and pine nuts. Caramel aromas surround these scents, reminiscent of caramelised almonds. It also has aromas of dry grass, in addition to presenting balsamic, menthol and licorice ones
-. Tasting phase: In the mouth it has a lot of volume and tannic structure. It is very compact and concentrated. It has a lot of wood tannin, which is somewhat dry, but interesting in terms of concentration.
>>> This is the first in preference in the second series. The wood is raw and not as complex as in the first wine, with less toasted aromas. The high tannicity makes it a ready-made wine to remove from the barrel. This characteristic makes it interesting for blending and to reinforce softer wines. It evolves very well in the glass, opening and increasing in complexity.

- Cata Vino B; Barricas estándar:

-. Visual phase: Cherry red with violet hints, with a high colour intensity in the heart of the glass.
-. Aromatic phase: This wine is more closed and flatter. At first it is not very expressive, with more vegetal aromas of asparagus and dry straw, presenting clean, new leather, as well as smoked and toasted aromas. It seems to be the oldest of all, being the most tertiary and evolved.
-. Tasting phase: In the mouth this is the weakest tasting wine, with the most marked and naked notes of acidity. It does not have as much fullness of body when compared to the first one in series B. The tannins are astringent, and somewhat metallic and dry. This one is the worst regarding mouth feel, due to the phenolic component.
>>> This is the least preferred wine from the second series, and the worst of all four. It could be identified as a wine stored in old barrels and this fact makes the fruit scarcely present, tainting the wine. The wood does not live up to expectations.

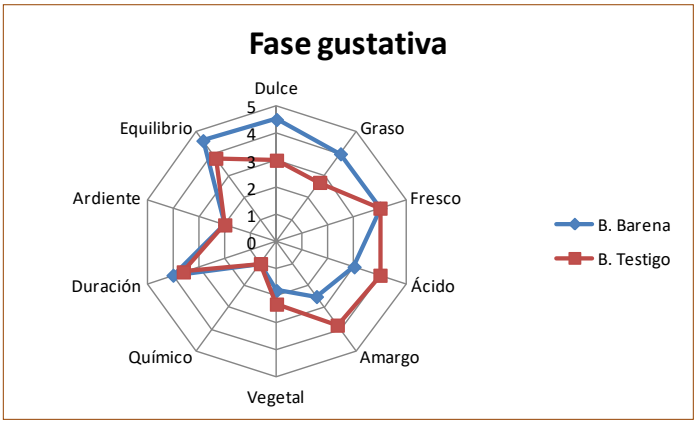

Figure 15: Spider web graph of the gustatory phase for wine B

GENERAL CONCUSIONS:

- The Barena® method regenerates the used barrels completely, eliminating the tartrate film, respecting the toasting layer and allowing the wine to be enriched and enlivened with its own wood aromas.

- Wines aged in regenerated barrels are able to increase their aromatic complexity, in terms of both typical oak aromas and in those of toasted wood.

- The kinetics in the transfer of wood aromas is proportional to the period of time the wine is stored in regenerated barrels.

- The treatment disinfects the interior of the oak barrels in 99.9% of cases observed in the extensive sampling that has been carried out. The microbiological improvement of the wood in the barrels, and subsequently, of the wine contained in them, allows for "softer" oenological techniques in respect to protection against contaminating microorganisms.

- As for the sensory aspect, wines aged in barrels treated using the Barena® method are wines which display sharper fruit aromas, more evident notes of wood and a rounder and more balanced mouth feel compared to the same wine in used barrels.

- The regeneration gives used barrels a new lease of life, increasing their time in the cellar as active containers so as to improve the quality of the wine.

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