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New Insights about the Influence of Yeasts Autolysis on Sparkling Wines Composition and Quality

Pere Pons-Mercadé, Pol Giménez, Glòria Vilomara, Marta Conde, Antoni Cantos, Nicolas Rozès, Sergi Ferrer, Joan Miquel Canals and Fernando Zamora

Abstract

Sparkling wines elaborated using the traditional method undergo a second fermentation in the bottle. This process involves an aging time in contact with the lees, which enriches the wine in various substances, especially proteins, mannoproteins and polysaccharides, thanks to the autolysis of the yeasts. As a result of this yeast autolysis, sparkling wines benefit from better integration of carbon dioxide and a clear sensory improvement, especially in the case of long aging. This chapter synthesizes the main results that our research group has obtained about the influence of yeasts autolysis on sparkling wines composition and quality during last years, making special emphasis on the capacity of the lees to release proteins and polysaccharides as well as on their capacity to consume oxygen and thus protect the sparkling wines from oxidation.

Keywords: sparkling wines, yeast autolysis, proteins, polysaccharides, oxygen consumption

1. Introduction

In obedience to the European Regulation CE 1493/99 [1], sparkling wines differ from still wines in the level of internal pressure of carbon dioxide that must be higher than three bars. Sparkling wines are classed in the function of the CO₂ origin in two main categories: gasified wines when the carbon dioxide is from an exogenous source and natural sparkling wines when it comes from endogenous fermentation.

Gasified wines are produced simply by injecting carbon dioxide until reaching the desired internal pressure. Normally, these gasified wines have no geographical references, are very cheap and have much lower sensory quality than natural sparkling wines. Given their small interest in their sensory point of view they will not be considered in this chapter. In contrast, natural sparkling wines are obtained using a natural fermentation keeping all or a great proportion of the carbon dioxide inside the vessel in which it has been fermented.

There are different elaboration methods of natural sparkling wines depending on the type of vessel (bottle or tank), time of lees contact, the procedure of eliminating the lees, or if they have had one or two alcoholic fermentations. Moreover,

some of these natural sparkling wines are protected by *Appellations d'origine contrôlées* (AOC) such as *Champagne*, *Cava*, *Francia Corta*, *Prosecco*, *Asti*, *Crémant de Bourgogne*, etc.... In that case, each AOC determines the elaboration method, authorized varieties and aging time.

Sparkling wines considered as top quality, such as *Champagne*, *Francia Corta* and *Cava*, are mainly produced by the traditional method, also called for Champagne AOC “*méthode champenoise*”. The main characteristic of the traditional method is that after a first fermentation to obtain the base wine, a second fermentation, also called “*prise de mousse*”, is performed inside a closed bottle [2, 3]. This second fermentation inside the bottle, and especially the aging time in contact with the lees, completely transform the sparkling wine composition and represents therefore the main differential factor regardless of other sparkling wines produced using other methods [4–6]. During the time of contact of the wine with the lees, several processes occur (**Figure 1**) that explain why the sparkling wines produced by the traditional method generally have higher quality and complexity and are much better considered by the consumers.

Briefly, once the second fermentation is completed, yeast autolysis begins [7]. Autolysis consists of the degradation process of yeast cell structures [8]. Autolysis involves the participation of hydrolytic enzymes, which, by degrading cell structures, cause the release of many substances such as amino acids, peptides, lipids, proteins, nucleotides, mannoproteins and polysaccharides [9–15]. The release of peptides, proteins, mannoproteins and polysaccharides favors the integration of carbon dioxide, which improves the perception of effervescence in the palate and increases the foam stability [6, 16]. Mannoproteins and polysaccharides also play a positive sensory role by improving mouthfeel [17], whereas some peptides and proteins can contribute to wine sweetness [18]. Some amino acids, peptides and nucleotides are also reported to participate in the umami taste [19] and to be flavor enhancers. Finally, amino acids and lipids have been described as aroma precursors [20] that contribute to the aromatic complexity of sparkling wines.

It has been also reported that yeast lees exert antioxidant activity [21] and recently it has been demonstrated the ability of the lees to consume oxygen [22]. The mechanism by which the lees consume oxygen is not clear but it could be related to the oxidation of membrane lipids [23] or with their content in glutathione [24]. Regardless of the mechanism by which lees consume oxygen, it is clear that their presence slows down the oxidative evolution of the wine by consuming the oxygen that permeates the crown cap. This oxygen consumption by lees is probably the main reason why sparkling wines can usually age for a longer time than still white wines.

In synthesis, yeast autolysis completely modifies the composition of the sparkling wine and therefore also its sensory quality. For all these reasons, the most important AOC (*Appellation d'Origine Contrôlée*) for sparkling wines has established minimum

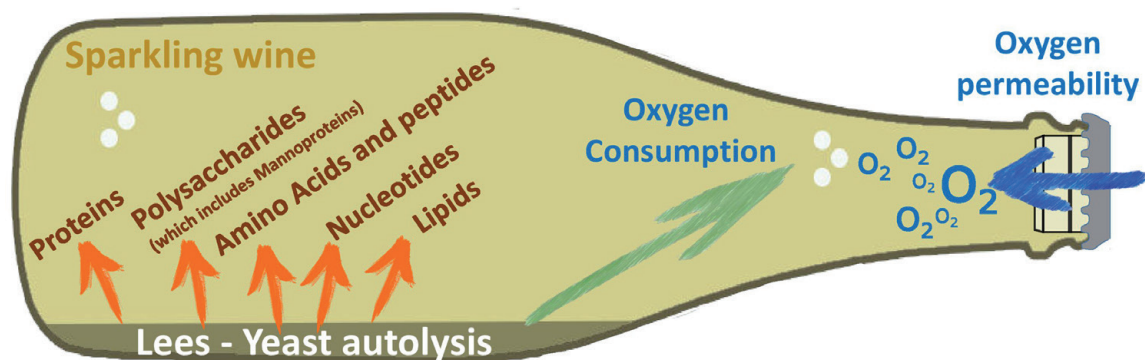


Figure 1.
Influence of the lees on sparkling wine composition.

ageing times to ensure that autolysis exerts an effect on their composition and quality. For the AOC Cava, the minimum ageing time is 9 months, though its premium sparkling wines are usually aged for longer. The AOC Cava contains two other categories of sparkling wines with extended ageing times. These are the Reserva and Gran Reserva, whose minimum ageing times are 15 and 30 months, respectively. Certain prestigious wineries produce Cavas with an even longer ageing time.

It appears, therefore, that autolysis favors the quality of sparkling wines, at least during the first few years. However, other phenomena take place in parallel—such as aromatic and color oxidation or an excessive lees flavor—which can damage the sensory qualities of these wines [22]. Therefore, we can ask ourselves until what time of aging the quality of the product is favored.

The chapter aims is to synthesize the main results that our research group has obtained on the influence of yeast autolysis on the composition and quality of sparkling wines. This study was carried out studying nine consecutive vintages and was developed in the PhD thesis of Pere Pons entitled “Yeasts autolysis on the manufacture of sparkling wines; influence of aging time on the release of polysaccharides and proteins and the consumption of oxygen by the lees” [25] that was part of the projects GLOBALVITI (global solution to improve wine production against climate change based on robotics, IT technology and biotechnological strategies and vineyard management) and CAVAWINNER (Study and Technological Improvement of the Traditional Processes for the Production of Cava) funded by the Spanish Centre for the Development of Industrial Technology (CDTI - CIEN program). To our knowledge, this is the longest time ever studied about sparkling wines from the AOC Cava.

2. Materials and methods

Figure 2 illustrates the experimental design. Briefly, this study was carried out using sparkling wines from nine consecutive vintages (2008–2016) from the Juve & Camps winery (AOC Cava, Sant Sadurní d’Anoia, Barcelona, Spain). All these

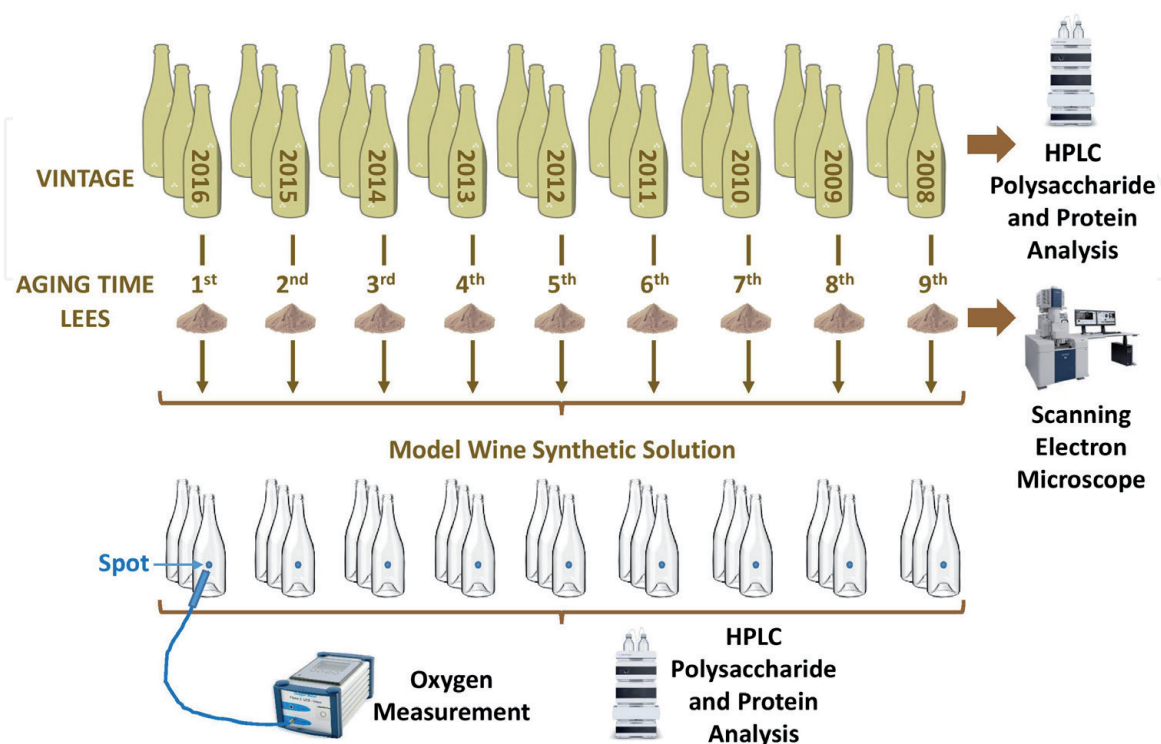


Figure 2.
Experimental design.

sparkling wines were produced with grapes from the same vineyards and were elaborated as similarly as possible. The youngest sparkling wine (2016) was disgorged 3 months after “tirage” and the sparkling wines from the other vintages were also disgorged 3 months after having completed 1–8 years of aging, respectively. In all the cases the lees were recovered, washed, resuspended in a model wine solution and bottled for subsequent analysis. These bottles were inserted with a pill for measuring dissolved oxygen by luminescence (Nomasense™ O2 Trace Oxygen Analyzer). Aliquots of the lees from the nine consecutive vintages were also used for ultrastructural observation using scanning electron microscopy [15]. Consequently, this study was performed with sparkling wines and lees from the first to the ninth years of aging.

The sparkling wines were used for color [26], polysaccharides [27] and proteins [28] analysis, for measuring the foaming properties [29] and for tasting. In parallel, the oxygen concentration was measured periodically in the bottles in which the lees were transferred [22]. Exactly 1 year later the solution was centrifuged and used for polysaccharides and protein analysis [27, 28].

3. Results and discussion

Table 1 shows the CIELab coordinates of the sparkling wines. As expected, the blue-yellow CIELab component (b^*) clearly increased as the aging time increased. These data confirm a fact that is well known by winemakers: the intensity of the yellow color progressively increases over time. **Table 1** also shows the foaming properties of these sparkling wines. Both the maximal height of the foam (foamability [HM]) and the stable height of the foam (foam stability [HS]) showed a similar tendency, they increased between the first and second year of aging and decreased progressively afterward.

Figure 3 shows the polysaccharide concentration of the sparkling wines of the nine consecutive vintages. In general, no clear trend was detected either in the total concentration of polysaccharides or in any of its different fractions of different molecular weight. This lack of tendency seems to contradict what should be expected from yeast autolysis, as it should theoretically increase its concentration over time. Nevertheless, other authors also found no clear trend in the evolution of the polysaccharide fraction during the aging of sparkling wines on lees [13, 30, 31].

A possible explanation for this lack of trend maybe that polysaccharides are simultaneously released and removed from the media. Yeast autolysis may be a source of polysaccharides and mannoproteins [32]. However, polysaccharides can also disappear by precipitation [13], absorption by the riddling agents [33] and enzymatic degradation [30]. In addition, the variability among vintages may overlap making it very difficult to detect any tendency.

Figure 4 shows the protein concentration of the sparkling wines of the various vintages. Similar to what happened with polysaccharides, no clear trend was observed throughout aging time, neither in the concentration of total protein nor in any of its fractions of different molecular weight. Once again, these results may appear to contradict what is expected from yeast autolysis. However, other authors have also reported a similar erratic behavior [34–36].

Similar to what happened with polysaccharides, this lack of tendency may be related to a balance between the proteins released from yeast autolysis and those that disappear due to bentonite absorption and enzymatic degradation [9, 34, 35, 37]. Furthermore, the variability in the protein concentrations of each vintage can make it difficult to conclude.

Since no tendency was observed for either polysaccharides or proteins, it was decided to study the release of these macromolecules from the lees using a different

Aging time (years)	Lightness (L*)				Green-red component (a*)				Blue-yellow component (b*)				Foamability-Hs (mm)			Foam stability-Hs (mm)				
1st	98.0	±	0.2	C	-0.88	±	0.06	CD	6.88	±	0.12	A	87	±	1	C	71	±	3	C
2nd	98.1	±	0.1	C	-1.02	±	0.04	BCD	7.69	±	0.08	B	170	±	1	E	93	±	3	E
3rd	97.9	±	0.1	C	-1.11	±	0.02	AB	8.78	±	0.07	C	110	±	5	D	81	±	1	D
4th	97.7	±	0.2	BC	-1.11	±	0.07	AB	8.66	±	0.21	C	80	±	4	BC	72	±	2	C
5th	97.4	±	0.0	B	-1.09	±	0.08	AB	9.87	±	0.13	E	76	±	4	BC	68	±	1	BC
6th	97.6	±	0.1	BC	-1.25	±	0.08	A	9.59	±	0.07	DE	61	±	1	A	53	±	3	A
7th	96.8	±	0.5	A	-1.05	±	0.03	ABC	9.39	±	0.02	DE	73	±	1	ABC	58	±	2	A
8th	97.6	±	0.1	BC	-1.00	±	0.10	BCD	10.77	±	0.19	F	71	±	4	AB	54	±	1	A
9th	97.4	±	0.2	B	-0.92	±	0.10	BCD	10.99	±	0.34	F	74	±	1	ABC	59	±	1	AB

Results are expressed and as mean ± standard deviation of three replicates. Different letters indicate the existence of statistical difference ($p < 0.05$). Adapted from Pons-Mercadé et al. [15].

Table 1.
CIELAB coordinates and foaming properties of the sparkling wines of different aging time.

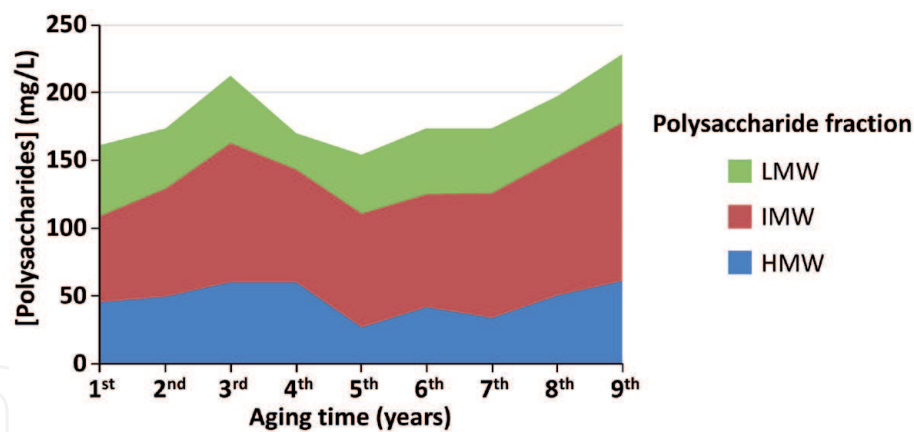


Figure 3. Changes in the polysaccharide content of sparkling wines over ageing time. LMW, low molecular weight fraction (40–75 kDa); IMW, intermediate molecular weight fraction (180–40 kDa); HMW, high molecular weight fraction (>180 kDa). Adapted from Pons-Mercadé et al. [15].

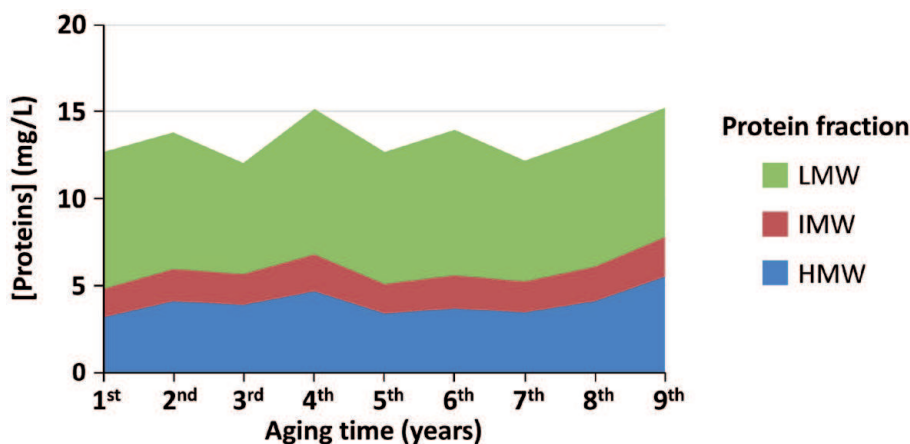


Figure 4. Changes in the protein content of sparkling wines over ageing time. LMW, low molecular weight fraction (50–25 kDa); IMW, intermediate molecular weight fraction (75–50 kDa); HMW, high molecular weight fraction (>75 kDa). Adapted from Pons-Mercadé et al. [15].

approach consisting of analyzing the wine model solutions that had been in contact with the lees for a year. **Figure 5** shows the obtained results. The polysaccharides released in this model wine solution by the lees (**Figure 5A**) increased between the first (roughly 4 mg/L) and the second (roughly 6 mg/L) year of aging while decreasing progressively in the later vintages, reaching a minimum value in the ninth year of ageing (roughly 0.90 mg/L). The mannose concentration obtained from the hydrolysis of this polysaccharide (**Figure 5B**) showed very similar, which would confirm that they were mainly mannoproteins.

The total protein concentration released from the lees of different aging times in a model wine (**Figure 5C**) showed a similar pattern to that of the polysaccharides reaching a maximal value in the third year (roughly 0.32 mg/L) and a minimum value in the ninth year of aging (roughly 0.17 mg/L).

To reproduce the cumulative release effect of polysaccharides and proteins over the lees aging time, the concentrations of both macromolecules released from the first to the ninth year were added (**Figure 5D and E**). This simple approach shows a clear increase in the accumulated concentrations of both macromolecules over the ageing time. The total accumulation of polysaccharides at the end of the 9 years was 26.6 mg/L, while that of proteins was 2.4 mg/L. These values should be taken with caution since they reflect just one approach. Nevertheless, this data indicates that

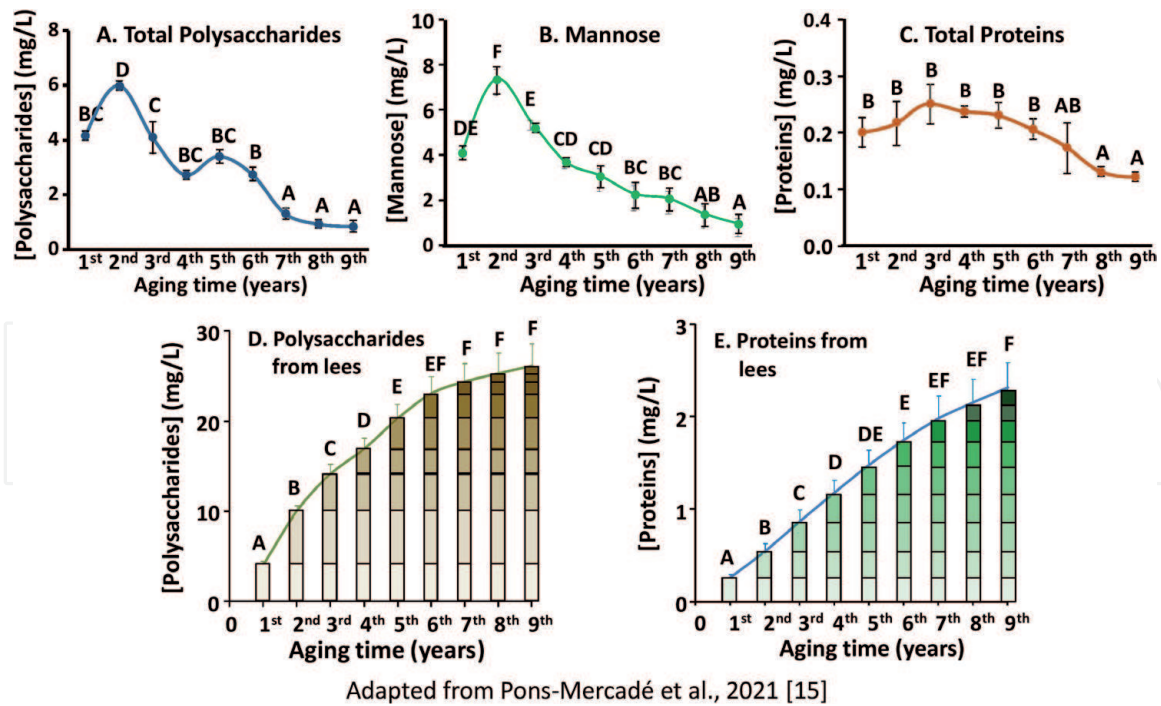


Figure 5. Changes in the polysaccharide and protein content of sparkling wines over ageing time. A. Total polysaccharides; B. Mannose; C. Total protein; D. Polysaccharides released by lees; E. Proteins released by lees. Adapted from Pons-Mercadé et al. [15].

the release of polysaccharides and proteins from the lees during the ageing process was much lower than the usual concentrations present in these sparkling wines.

Another approach was carried out to illustrate the distribution of polysaccharides and proteins according to their origin (from the autolysis of lees or base wine) throughout the aging time. **Figure 6** shows the percentage of polysaccharides (A) and proteins (B) from lees autolysis or base wines for total concentration in the sparkling wines. This figure clearly shows that the percentage of polysaccharides and proteins from lees autolysis was extremely low in the young sparkling wines. That means that during the first year of ageing, sparkling wine had only 2% of proteins and 3% of polysaccharides from the lees. These percentages increased as

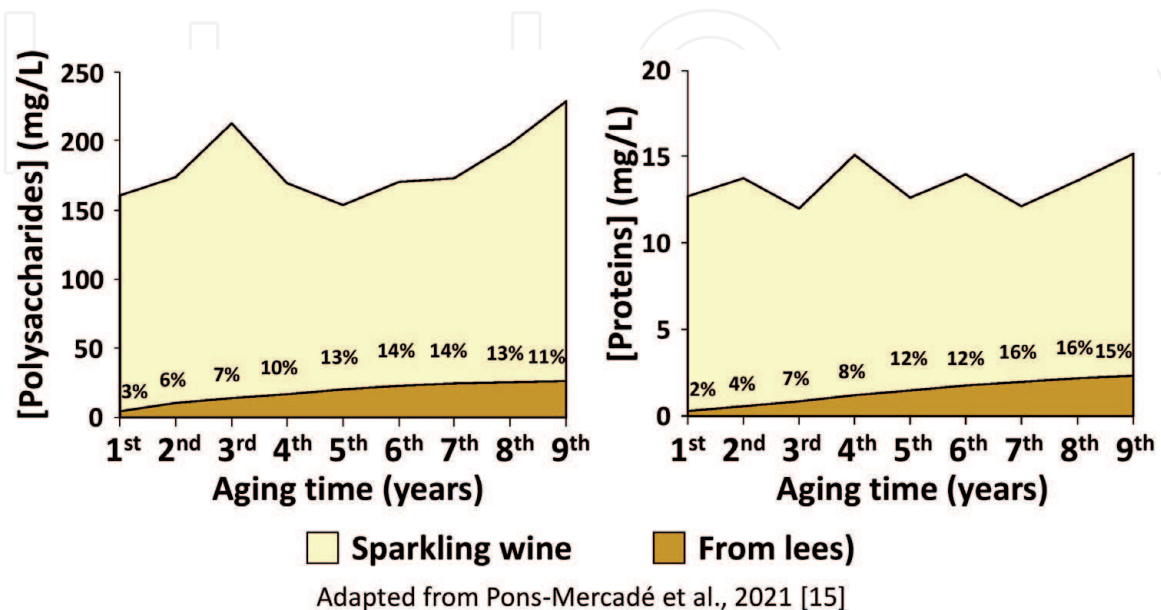


Figure 6. Distribution of proteins and polysaccharides of sparkling wine according to their origin. Adapted from Pons-Mercadé et al. [15].

the ageing time increased and reached maximal values in the seventh year of ageing (14% for polysaccharides and 16% for proteins).

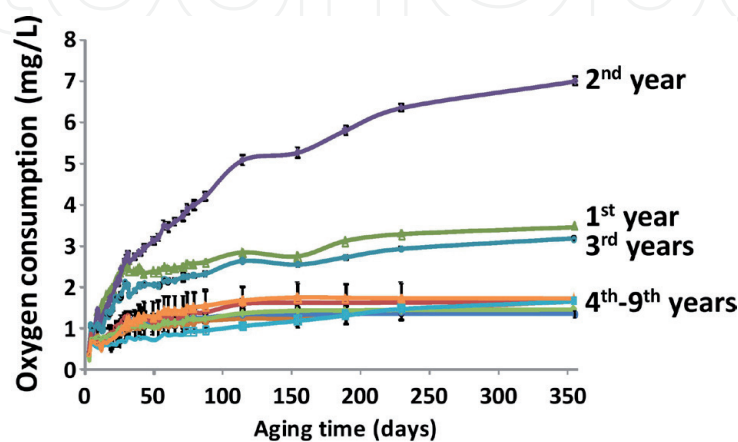
It is necessary to point out that only 88% of sparkling wines from AOC Cava are aged between 9 and 15 months and 78% of champagnes are aged between 12 and 36 months (data from the regulatory councils). Consequently, the majority of sparkling wines produced by the traditional method have percentages of polysaccharides and proteins from lees autolysis below 7%, and this value should even be lower in the youngest sparkling wines, especially those not produced by the traditional method.

As it was explained above, the bottles, in which the lees extracted from the sparkling wines were resuspended in a model wine solution, had been inserted with a pill for measuring dissolved oxygen by luminescence. The content of these bottles was saturated in oxygen and the oxygen concentration was monitored periodically for a year. **Figure 7** shows the oxygen consumption kinetics of the lees from sparkling wines from the first to the ninth year of aging time [22]. The oxygen consumption of the Control-A model wine solution (without adding lees) and the oxygen intake in Control-B (solutions without lees and oxygen) were very low and can be considered negligible (data not shown). In contrast, the oxygen consumption of all the samples containing lees increased over time, demonstrating that the lees can consume oxygen.

Moreover, this graph clearly shows that the lees of the first 3 years, especially those of the second year, consume much more oxygen than the lees of later years. It, therefore, seems clear that the ability of the lees of sparkling wines to consume oxygen increases between the first and second year and after tends to decrease throughout the aging period. The kinetic model proposed by Pascual et al. [38] was applied to these data to determine more precisely the total oxygen consumption capacity of the lees of these sparkling wines. **Figure 8** shows that the lees from the second year are capable of consuming nearly the double oxygen than those of the first or third year. Subsequently, the annual oxygen consumption decreases drastically in the older lees.

The higher oxygen consumption of the lees of the second year could be related to the described progress of the autolysis process which, according to some authors, starts slightly after 4 months and is more intense during the second year [7, 32, 39, 40]. It should also be noted that the maximal oxygen consumption-ability of the lees of the second-year match with the maximal polysaccharide and protein release and with the maximal levels of the foaming parameters [15]. All of these data seem to indicate that autolysis is at its peak during the second year of aging.

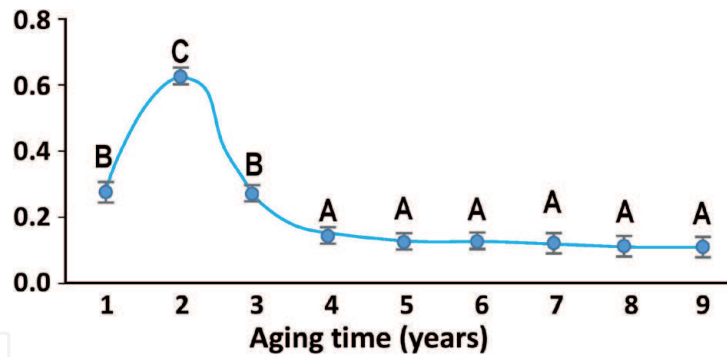
In any case, it seems that the oxygen consumption by the lees decreases drastically after 3 years of aging whereas the entrance of oxygen inside the sparkling wine



Adapted from Pons-Mercadé et al., 2021 [22]

Figure 7.

Oxygen consumption by lees extracted from sparkling wines of different aging times. Adapted from Pons-Mercadé et al. [22].



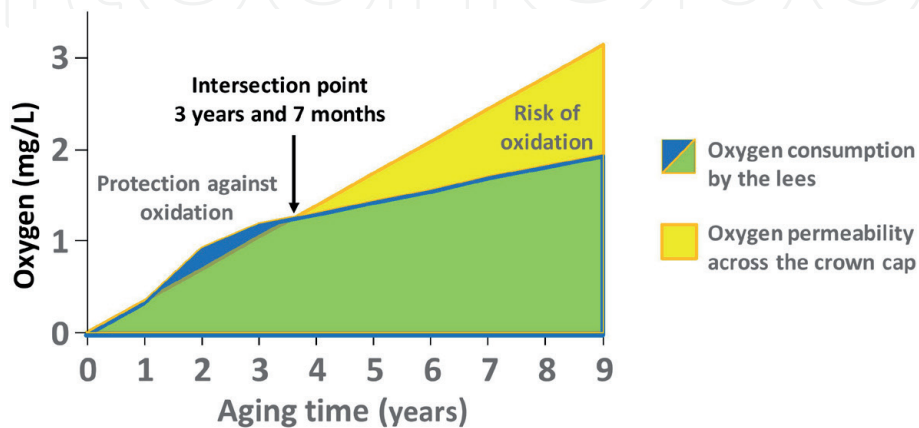
Adapted from Pons-Mercadé et al., 2021 [22]

Figure 8. Total oxygen consumed in 1 year by the lees extracted from sparkling wines of different aging times. Adapted from Pons-Mercadé et al. [22].

through the crown cap seems to be constant [41]. As long as the lees' oxygen consumption ability is greater than the oxygen permeation, the sparkling wine will be protected against oxidation. However, we can wonder what would happen when the lees stop consuming enough oxygen? When this happens, oxygen will be consumed by other wine components, especially by phenolic compounds, which will cause browning and the appearance of hydrogen peroxide that will oxidize other wine compounds in the absence of free sulfur dioxide, especially aroma compounds. Oxidation will be greater or lesser depending on the composition of the sparkling wine, which is largely dependent on the vintage and the production process.

Figure 9 try to illustrate this complex balance showing the accumulated oxygen consumption by the lees in comparison with the oxygen intake across the crown cap considering the minimal value of oxygen permeability reported by Valade et al. [41]. The comparison of the two curves is just a theoretical approximation, but even so, it provides very interesting information.

According to this approach, the oxygen permeability across the crown cap remains below the accumulated oxygen consumed by the lees during the first 3 years of aging time and exceeds it at roughly three and a half years. More exactly the interception point is at 3 years and 7 months. This data indicates that after this aging time, the oxygen consumed by the lees would not be high enough to compensate for the oxygen entrance which would probably lead to wine oxidation. It should be taken into account that this calculation was done considering the minimal value of permeability reported for crown caps and that any increase in this permeability



Adapted from Pons-Mercadé et al., 2021 [22]

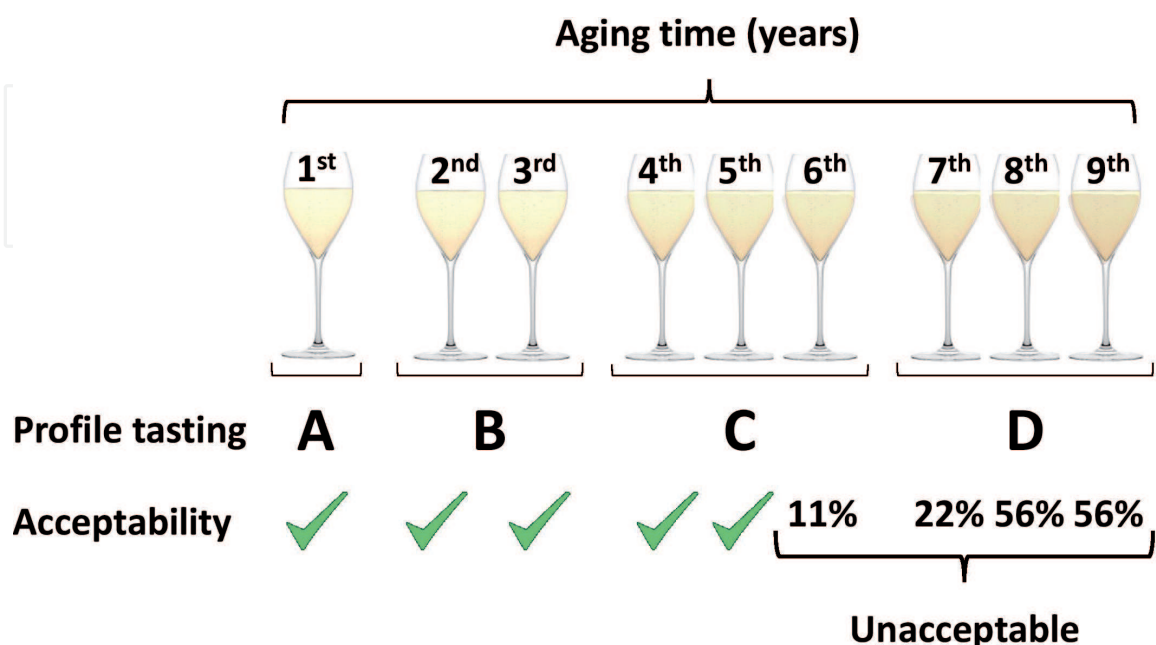
Figure 9. Accumulation of oxygen consumed by the lees in comparison with the oxygen permeability of the crown cap. Adapted from Pons-Mercadé et al. [22].

would therefore entail an earlier point of intersection in time. For instance, with a 20% higher permeability the intersection would take place just after 2 years of aging. As aforementioned, this is only a theoretical approach based on our results but it is very useful to illustrate what happens during sparkling wine aging.

All these sparkling wines were tasted by a trained panel and the main results are synthesized in **Figure 10**. The panel was asked to blindly classify sparkling wines based on their age. The panel successfully appreciated the chronological order of these sparkling wines since established four statistically significant groups depending on their sensory perception of their aging time: Group A, which the panel considered the youngest (first year of aging); Group B (second and third year of aging); Group C (fourth to sixth years of aging) and Group D (seventh to ninth years of aging).

All panelists considered the five youngest vintages of sparkling wines as “acceptable” for consumption under their qualitative sensory criterion. However, some of them considered that after this aging time the sparkling wines were “unacceptable”. These data indicate that after 5 years the sparkling wines began to be affected by excessive ageing. It should be pointed out that these sensory data match well with the previous considerations about the balance between the oxygen consumption by the lees and the oxygen permeability across the crown cap. According to these results, the oxygen consumed by the lees started to be not enough to compensate for oxygen intake through the crown cap after 3 years and 7 months of ageing. After this time, the sparkling wine does not have enough defense against oxidation. Under these conditions, its sensory quality may begin to deteriorate, though the effects of this oxidation will also depend on its chemical composition and storage conditions. In the present study, sensory deterioration seems to begin after the 5th year of aging.

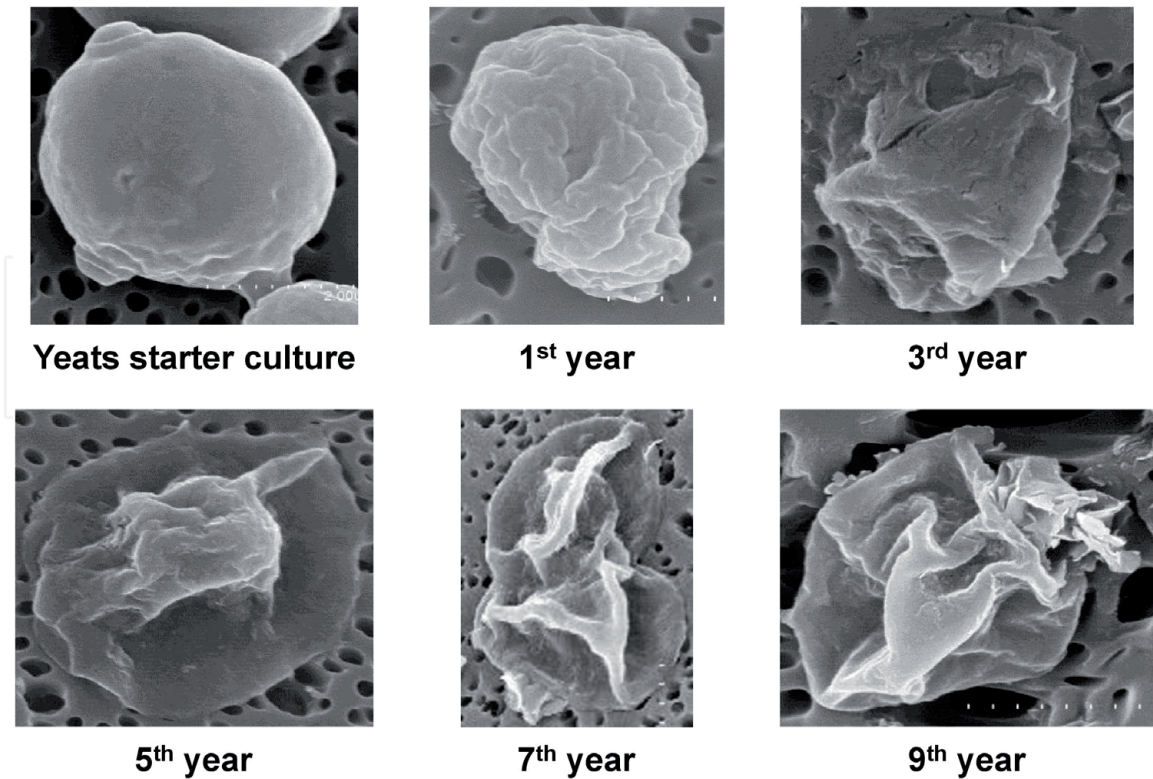
Finally, some photographs of the yeasts were taken using a scanning electron microscope (SEM) [15] to visualize the yeast autolysis process in the sparkling wines aged by up to 9 years (**Figure 11**). These pictures show how the structures of the yeast cells are progressively degraded, folded and deflated. In the first image, which shows the yeast of the starter culture used for the second fermentation of the last vintage (2016), the yeast cell seems very healthy since it is elongated, ovoid and turgid without any wrinkle or folds. Several bud scars can even be identified. The



Adapted from Pons-Mercadé et al., 2021 [15]

Figure 10. Sensory analysis of the sparkling wines of the nine consecutive vintages. Adapted from Pons-Mercadé et al. [15].

YEAST AUTOLYSIS



Adapted from Pons-Mercadé et al., 2021 [15]

Figure 11.

Monitoring yeast autolysis overtime using scanning electron microscopy. Adapted from Pons-Mercadé et al. [15].

second image shows what happens after the second fermentation (3 months later): the yeast cell has lost some turgor and is beginning to display wrinkles and folds. Two-years later, in the third year of ageing, the yeast cell is even more degraded and wrinkled and begins to deflate. At the fifth year of ageing, the yeast cell is completely flattened at the edges and retains only a little turgor in the middle, which is full of wrinkles and folds. In the seventh year of ageing, the yeast cell is even more degraded and deflated and the center of the cell has crumbled, wrinkled and flattened. Finally, in the ninth year, the yeast cell has completely collapsed and some of its structures are broken.

4. Conclusions

It can be concluded that the lees of sparkling wines elaborated using the traditional method have a real capacity to release proteins and polysaccharides. However, the proportion of polysaccharides and proteins from lees autolysis is very low in the young sparkling wines, roughly only 2–3% in the first year of ageing and around 7% in the third. This suggests that the real impact of polysaccharides and proteins from lees autolysis in the sparkling wines disgorged before the end of the first year should be very low. Wine producers should bear this conclusion in mind since most sparkling wines elaborated by the traditional method are aged for less than 1 year and those made by other methods are aged even less. Consequently, only sparkling wines aged for longer would therefore benefit from a greater presence of polysaccharides and proteins from yeast autolysis.

It can also be stated that lees consume oxygen and therefore they protect sparkling wine against oxidation. However, the lees' capacity to consume oxygen decreases

drastically after 3 years of aging, reaching values lower than those of the theoretical oxygen permeability of the crown cap after about 3 years and a half of aging. Producers of sparkling wines should also bear in mind because after this time the ability of the lees to protect against could not be enough. Some panelists considered that the quality of the sparkling wines was negatively affected after 5 years of aging due to excess oxidation. These data explain what AOC Cava winemakers know empirically. Only some high-quality sparkling wines made using the traditional method can age more than 3 years without being affected by oxidation, and in this case, sparkling wines reach an extraordinary level of complexity that only long aging can provide.

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Conflict of interest

The authors declare no conflict of interest.

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