

Oxygen consumption rate of lees during sparkling wine (Cava) aging; Influence of the aging time

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ABSTRACT

Sparkling wines elaborated with a traditional method need to age in the bottle in contact with wine lees because yeast autolysis enriches the wines in colloids and improves their effervescence, foam and aromatic complexity. It is generally considered that lees protect the wine against oxidation because they consume small amounts of oxygen that can permeate the crown cap. However, to our knowledge there is no specific study on this subject using lees from real sparkling wine. Therefore, the oxygen consumption rate (OCR) of the lees of sparkling wines from the first to the ninth year of aging time was measured using a noninvasive fluorescence measurement method. The results indicate that lees really consume oxygen and that their OCR tended to decrease with the wine aging time. These data suggest that the lees' capacity to protect against oxidation decreases over time, which could affect the ability of sparkling wines to age properly.

Keywords: Sparkling wine, Traditional method, Cava, Lees, Oxygen consumption, Aging time.

28

29 **1. Introduction**

30

31 Sparkling wines made using the traditional method follow an elaboration process that involves two
32 fermentations (Maujean, 1989). The first fermentation transforms the grape juice into base wine following
33 a conventional winemaking process. In contrast, the second fermentation, also named “prise de mousse”
34 occurs inside a bottle after the wine is enriched with a solution called “liqueur de tirage”, which provides
35 the necessary sugar for the second fermentation (around 22 g of sucrose/L) (Kemp, Alexandre, Robillard, &
36 Marchal, 2015). This “liqueur de tirage” also contains some co-adjuvants, such as bentonite and/or
37 alginates, to favor the riddling process (Vanrell, Canals, Esteruelas, Fort, Canals, & Zamora, 2007), and
38 yeasts, which have previously been acclimatized to ensure that the second fermentation comes to a
39 successful end. Once the second fermentation has finished, which can last between three and six weeks,
40 the sparkling wines remain in contact with the yeasts, called lees, for a long time so that the autolysis
41 process takes place (Alexandre, & Guilloux-Benatier, 2006).

42

43 During this ageing period, the yeast cells release many parietal and cytoplasmic compounds that have a
44 large influence on the sparkling wine’s quality (Feuillat, & Charpentier, 1982; Pozo-Bayón, Martínez-
45 Rodríguez, Pueyo, & Moreno-Arribas, 2009). Among the various substances released during the autolysis
46 process, the following substances can be highlighted due to their effects on the sensory attributes of
47 sparkling wine: proteins (Luguera, Moreno-Arribas, Pueyo, Bartolome, & Polo, 1998), mannoproteins
48 (Martínez-Lapuente, Guadalupe, Ayestarán, & Pérez-Magariño, 2015), peptides (Moreno-Arribas, Pueyo, &
49 Polo, 1996), amino acids (Martinez-Rodriguez, Carrascosa, Martin-Alvarez, Moreno-Arribas, & Polo, 2002),
50 polysaccharides (Martinez-Lapuente, Guadalupe, Ayestaran, Ortega-Heras, & Perez-Magariño, 2013), lipids
51 (Pueyo, Martínez-Rodríguez, Polo, Santa-María, Bartolomé, 2000) and nucleotides (Charpentier, Aussenac,
52 Charpentier, Prome, Duteurtre, & Feuillat, 2005).

53

54 Proteins, mannoproteins, peptides and polysaccharides play an important role improving the quality of the
55 effervescence and increasing the foam stability (Martínez-Rodríguez, Carrascosa, Barcenilla, Pozo-Bayón,
56 Polo, 2001; Kemp, Conde, Jegoud, Howell, Vasserot, & Marchal, 2019). It has also been reported that
57 polysaccharides and mannoproteins play a positive role in improving mouthfeel (Gawel, Smith, Cicerale, &
58 Keast, 2018), and that some proteins and peptides released by yeasts can contribute to wine sweetness
59 (Marchal, Marullo, Moine, & Dubourdiou, 2011). In addition, some amino acids, peptides and nucleotides
60 can contribute to the umami taste (Vilela, Ines, & Cosme, 2016) and have been described as flavor
61 enhancers. Finally, it has also been reported that amino acids and lipids are aroma precursors (Styger, Prior,

62 & Bauer, 2011) and consequently their release from yeast cells can also contribute to the aromatic
63 complexity of sparkling wines.

64

65 However, natural autolysis is a slow process that takes a long time to achieve a real organoleptic effect.
66 This means that great sparkling wines require a long aging time (several years) in order to acquire all the
67 richness and complexity that autolysis provides. In sparkling wines, yeast autolysis does not begin until 2–4
68 months after the completion of secondary fermentation (Todd, Fleet, & Henschke, 2000). Autolysis starts
69 with a passive excretion of amino acids and other small molecules. This process, called exorption, lasts
70 between 3 and 6 months (Morfaux, & Dupuy, 1966). Later, true autolysis begins with degradation of
71 membranes (cell and vacuolar), causing the progressive hydrolytic degradation of all cell structures
72 (Alexandre, & Guilloux-Benatier, 2006), whose fragments can be released into the wine. Once autolysis has
73 begun, cell wall polysaccharides and mannoproteins, proteins, lipids and nucleotides increase very slowly,
74 completely transforming the composition of the sparkling wine and therefore its sensory attributes (Kemp
75 et al., 2015).

76

77 For these reasons, most of the sparkling wines made with the traditional method have established
78 minimum aging times that guarantee that autolysis has had an effect on the composition and quality of the
79 product. In the particular case of AOC Cava a minimum aging time of 9 months has been established.
80 However, the finest sparkling wines from this AOC are usually aged for a much longer time. In fact, AOC
81 Cava distinguishes between two other types of sparkling wine with a longer aging time: “Reserva” and
82 “Gran Reserva” with a minimum aging time of 15 and 30 months respectively. Some prestigious wineries
83 even produce Cavas with much a longer aging time, despite the fact that there is no specific category to
84 distinguish them.

85

86 Nevertheless, a long aging time can sometimes have some drawbacks, the clearest of which is a very rapid
87 evolution (Riu-Aumatell, Bosch-Fuste, López-Tamames, & Buxaderas, 2006) that causes the premature
88 appearance of oxidized characters (Pons, Nikolantonaki, Lavigne, Shinoda, Dubourdieu, & Darriet, 2015).
89 Sparkling wines are better protected against oxidation than still wines due to their higher internal pressure
90 in carbon dioxide, which makes it difficult for oxygen to enter through the crown cap. However, gas
91 exchange takes place through the crown cap so that carbon dioxide can exit (Gerard Liger-Belair, &
92 Villaume, 2011) and oxygen from the air can enter inside the bottle even under these conditions (Valade,
93 Bunner, Tribaut-Sohier, Tusseau, & Moncomble, 2011). There is some variability in the literature about the
94 oxygen permeability of the different crown caps used for the second fermentation of sparkling wine.
95 Valade, Tribaut-Sohier, Bunner, Laurent, Moncomble, & Tusseau (2007) reported an oxygen permeability
96 ranging from 0.35 to 2.5 mg of O₂/L/year depending on the cap liner, and other authors have reported

97 similar values (Kemp et al., 2014). Evidently the higher the oxygen transfer rate the faster the wine
98 oxidation. Different levels of oxidation have been reported in still wines (Godden et al., 2001; Mas, Puig,
99 Lladó, & Zamora, 2002) and sparkling wines (Mas, Puig, Lladó, & Zamora, 2001) depending on the
100 permeability of the stopper used.

101

102 Another reason why sparkling wines are more protected against oxidation than still wines is the presence
103 yeast lees inside the bottle. It has been reported that yeast lees exert antioxidant activity (Gallardo-Chacón,
104 Vichi, Urpí, López-Tamames, & Buxaderas, 2010) and consume oxygen (Salmon, Fornairon-Bonnefond,
105 Mazauric, Moutounet, 2000; Fornairon-Bonnefond, Camarasa, Moutounet, & Salmon, 2002; Fornairon-
106 Bonnefond, & Salmon, 2003). Consequently, the presence of lees slows down the oxidative evolution of the
107 wine by consuming the oxygen that permeates the crown cap. The mechanism by which the lees consume
108 oxygen is not clear, but some authors associate it with the oxidation of membrane lipids, sterol fractions in
109 particular (Fornairon-Bonnefond, 2000; Fornairon-Bonnefond, & Salmon, 2003); however, it may also be
110 due to the fact that lees can release glutathione (Kritzinger, Bauer, & du Toit, 2013). Regardless of the
111 mechanism by which the lees consume oxygen, it is clear that this oxygen consumption is probably the
112 main reason why sparkling wines can usually age for a far longer time than still white wines.

113

114 There is no doubt that lees can consume the small amounts of oxygen that can permeate the stopper
115 protecting the wine against oxidation. However, it would be interesting to determine whether this ability to
116 consume oxygen is very long lasting and therefore can protect the sparkling while it is in contact with the
117 lees or rather it decreases or even disappears after a certain aging time. In fact, it has been reported that
118 the antioxidant capacity (FRAP/DPPH assays) as well as the thiol groups content of sparkling wine lees
119 decrease progressively over the aging time (Gallardo-Chacón et al., 2010). It has also been reported that
120 the ability of lees to consume oxygen in a model wine solution decreases over time (Salmon et al., 2000;
121 Fornairon-Bonnefond et al., 2002; Fornairon-Bonnefond et al., 2003). This is, in our opinion, a key question
122 since it determines the maximum aging time that can be applied to a sparkling wine without it
123 deterioration.

124

125 The aim of this work was to study the oxygen consumption rate (OCR) of the sparkling wine lees of AOC
126 Cava with an aging time from 1 to 9 years. To our knowledge there is no specific study on the capacity of
127 the lees present in a bottle of sparkling wine to consume oxygen or on how this capacity evolves over a
128 long aging time.

129

130

131 **2. Materials and methods**

132

133 *2.1. Chemicals*

134

135 Absolute ethanol, L-(+)-tartaric acid and sodium hydroxide pellets were purchased from Panreac
136 (Barcelona, Spain). Copper (II) sulfate pentahydrate and iron (III) chloride hexahydrate were purchased
137 from Sigma–Aldrich (Madrid, Spain).

138

139 *2.2. Base wine elaboration*

140

141 The experiment was carried out during nine consecutive vintages (2008-2016) in Juve & Camps winery (AOC
142 Cava, Sant Sadurní d'Anoia, Barcelona, Spain). The base wines were made in the most similar way possible,
143 although some differences between each vintage are inevitable. Monovarietal base wines in all the vintages
144 were elaborated with three autochthonous cultivars: Xarel.lo (VIVC Prime name: Xarello; VIVC Variety
145 number: 13270), Macabeo (VIVC Prime name: Viura; VIVC Variety number: 13127) and Parellada (VIVC
146 Prime name: Parellada; Variety number VIVC: 8938). Xarel.lo and Macabeo grapes came from the Juvé &
147 Camps vineyards in Sant Sadurní d'Anoia (Barcelona, Spain; 41°26'47.42" N and 1°49'0.63" E) at 165 meters
148 above sea level and Parellada grapes from the Juvé & Camps vineyards in Mediona (Barcelona, Spain;
149 41°29'48.6" N and 1°39'56.9"E) at 506 meters above sea level.

150

151 For each vintage the grapes were harvested at the appropriate maturity level for obtaining base wines for
152 sparkling wine production. More specifically, the total soluble solid content (°Brix) varied between 17.3 and
153 19.5 for Xarel.lo, between 16.2 and 18.3 for Macabeo and between 16.2 and 18.3 for Parellada. The
154 titratable acidity (g of tartaric acid/L) varied between 5.5 and 8.0 for Xarel.lo, between 4.6 and 7.2 for
155 Macabeo and between 5.1 and 7.8 for Parellada. The grapes were immediately pressed in a pneumatic
156 press to obtain a yield of 0.6 L/kg of grape juice. The grape juices were immediately sulfited with 100 mg/L
157 of K₂S₂O₅ and pectinolytic enzymes (20 mg/L) were added to facilitate settling. After 24 h, clean grape juices
158 were racked into the fermentation stainless steel tanks and were immediately inoculated with 200 mg/L of
159 selected yeasts (*Saccharomyces cerevisiae* - IOC 18-2007; Institut Œnologique de Champagne, Epernay,
160 France). When necessary, titratable acidity was corrected with tartaric acid up to 6.0 g of tartaric acid/L. All
161 alcoholic fermentations were performed at 16-18 °C. Once alcoholic fermentation was finished, base wines
162 were racked and kept at 18-20 °C until spontaneous malolactic fermentation was completed. Base wines
163 were then racked again, sulfited (40 mg/L of K₂S₂O₅) and cold stabilized.

164

165

166 2.3. *Sparkling wine elaboration*

167

168 Once the monovarietal base wines were considered to be stable, they were used to obtain a classic blend
169 of the AOC Cava in all the vintages. Each vintage, a blend of 50 % of Xarel.lo, 30 % of Macabeo and 20 % of
170 Parellada, was used to produce sparkling wines (Cava) with the traditional method. Briefly, all the base
171 wines were supplemented with 22 g/L of sucrose, 40 mg/L of a mixture of bentonite and alginates as
172 riddling agent (Adjuvant MO; Station OEnotechnique du Champagne, Epernay, France) and 2×10^6 cells/mL
173 of a pre-adapted yeast culture (*Saccharomyces cerevisiae* - IOC 18-2007; Institut Œnologique de
174 Champagne, Epernay, France). The wines were then bottled, crown corked and stocked at 12-15 °C until
175 disgorgement. This procedure was employed during the nine consecutive vintages with the aim of
176 reproducing as much as possible the same elaboration conditions in all the vintages studied.

177

178 2.4. *Experimental design*

179

180 The experimental design used for determining the lees' oxygen consumption rate was an adaptation of
181 those previously described by [Navarro et al. \(2016\)](#) and [Pascual et al. \(2017\)](#) for determining the oxygen
182 consumption rate of oak chips and oenological tannins respectively.

183

184 Three months after the "tirage" of youngest sparkling wine (vintage 2016), when the second fermentation
185 was completely finished, three bottles were placed in a "pupitre" and the "remuage" process was
186 performed manually. The sparkling wines from the other vintages were also disgorged three months after
187 having completed 1 to 8 years of aging respectively. Consequently, we have worked with lees from the first
188 to the ninth years of aging. Once all the lees sediment was in the neck of the bottle, disgorging was
189 performed without freezing to facilitate the recuperation of the lees. The lees sediment of each bottle was
190 recovered in a plastic bucket and immediately transferred to a centrifuge tube. The tubes were centrifuged
191 (5 minutes at 10,000 rpm). Then the pellet was washed with 10 mL of saline solution (NaCl 0.9% v/v) and
192 centrifuged again. This operation was repeated twice. The lees were then ready for measuring their oxygen
193 consumption rate.

194

195 We used a model wine solution composed of ethanol (12% v/v) and tartaric acid (4 g/L) adjusted to pH = 3.5
196 with sodium hydroxide. This solution was enriched with 3 mg of Iron/L, in the form of iron (III) chloride
197 hexahydrate, and 0.3 mg of copper/L, in the form of copper (II) sulfate pentahydrate. These metals were
198 added because they play a central role in the mechanism of wine oxygen consumption ([Danilewicz, 2003](#)).
199 We worked with this model wine solution and not with real wine because the naturally occurring phenolic
200 compounds would compete with the lees in oxygen consumption, making it impossible to determine their

201 real oxygen consumption rate of the lees. However, it should be noted that the lees extracted from the
202 sparkling wines probably had absorbed some phenolic compounds from the wine ([Gallardo-Chacón et al.,](#)
203 [2010](#)) that could play a role in its ability to consume oxygen.

204

205 This model wine solution was saturated in oxygen (around 8.0 mg/L) by bubbling with air for 10 min
206 immediately before the beginning of the experiment.

207

208 The sparkling wine lees the first to the ninth year of aging time of each bottle were placed individually in
209 clear glass flasks (66 mL) into which a pill had previously been inserted (PreSens Precision Sensing GmbH,
210 order code: SP-PSt3-NAU-D5-CAF; batch number: 1203-01_PSt3-0828-01, Regensburg, Germany) for
211 measuring dissolved oxygen noninvasively by luminescence (Nomasense TM O2 Trace Oxygen Analyzer by
212 Nomacor S.A., Thimister Clermont, Belgium). We used flasks with a very small volume to concentrate the
213 lees and reduce the time of the experiment. The bottles were completely filled with the model wine
214 solution and closed immediately after with a shutter and a crown cap to minimize the volume of
215 headspace. The bottles were then gently shaken to resuspend the lees. The flasks were kept at 20 ± 2 °C
216 during the entire time. Oxygen was measured ([Diéval, Vidal, & Aagaard, 2011](#)) immediately after sealing the
217 bottles and every day during the first month and then measurements were taken at increasing intervals
218 until the end of the experiment (365 days). [Fig. 1](#) shows a schematic representation of the experimental
219 design. Two types of control were prepared. Control-A bottles were filled with the oxygen-saturated model
220 wine solution without adding lees, and Control-B bottles were filled with the same model wine solution but
221 after bubbling for 10 minutes with nitrogen to eliminate oxygen. Control A was used to verify that the
222 model wine solution did not consume oxygen and Control B was used to verify the tightness of the closure
223 system. Oxygen consumption was calculated by subtracting the concentration at each time from the initial
224 oxygen concentration for each sample. All assays were performed in triplicate.

225

226 *2.5. Statistics*

227

228 All data are expressed as the arithmetic average \pm standard deviation of three replicates. One-factor
229 analysis of variance (ANOVA) was carried out using the SPSS 15.0 software (SPSS Inc., Chicago, IL).

230

231 **3. Results and discussion**

232

233 [Fig. 2](#) shows the oxygen consumption kinetics of the lees from sparkling wines from the first to the ninth
234 year of aging time. The oxygen consumption of the Control-A model wine solution (without adding lees)
235 and the oxygen intake in Control-B (solutions without oxygen) were very low and can be considered

236 negligible (data not shown). In contrast, the oxygen consumption of all the samples containing lees
237 increased over time, demonstrating that the lees really have the ability to consume oxygen. All samples
238 showed similar behavior with higher oxygen consumption during the first 30 days, which later smoothed
239 out, reaching an asymptotic profile in some cases. This initially higher oxygen consumption may be related
240 to a rapid initial exorption of substances from inside the lees because the external medium is initially free
241 of these compounds. Once equilibrium is reached, the rate of oxygen consumption decreases as it would
242 only depend on the actual rate of lees autolysis. Moreover, the lees were observed to have a different
243 oxygen consumption capacity depending on the aging time of the sparkling wine from which they were
244 extracted. Looking at the graph it can be clearly seen that the lees of the first, second and third year show
245 higher initial slopes than the older lees. It can be seen that the lees of the first three years, especially those
246 of the second year, consume much more oxygen than the lees of later years. It therefore seems clear that
247 the ability of the lees of sparkling wines to consume oxygen increases between the first and second year
248 and after tends to decrease throughout the aging period.

249 [Fornairon-Bonnefond, & Salmon, \(2003\)](#) reported that the oxygen consumption of lees in a model solution
250 was between 1 and 4 $\mu\text{mol of O}_2/\text{h}\cdot 10^{10}$ cells from the second month to the third year of wine aging. This
251 means a total oxygen consumption of between 125 and 500 mg of oxygen/L.year, considering that the total
252 population of yeast inside a sparkling wine bottle is around 6×10^6 cells/mL ([Martinez-Rodriguez et al.,](#)
253 [2002](#)). Evidently these values are too high and certainly much greater than our results. However, these
254 studies were not performed with lees from sparkling wines and they used a less precise and invasive
255 method for measuring oxygen (Clark electrode). In any case, the decrease in the ability of lees to consume
256 oxygen over time has been previously reported ([Salmon et al., 2000](#); [Fornairon-Bonnefond et al., 2002](#);
257 [Fornairon-Bonnefond et al., 2003](#)). Moreover, other authors have measured the antioxidant capacity of lees
258 of sparkling wines and their results agree with our data as they reported a progressive decrease over time
259 during aging ([Gallardo-Chacón et al., 2010](#)).

260
261 However, the quantitative comparison of the oxygen consumption kinetics of the different lees is not
262 evident from looking at Figure 2. To better quantify the real ability of lees to consume oxygen, we applied
263 the kinetic model proposed by [Pascual et al. \(2017\)](#) to determine more precisely how the oxygen
264 consumption capacity of the lees of sparkling wines decreases according to their aging time. This model
265 consists in displaying the inverse of consumed oxygen versus the inverse of time.

266
267 [Fig. 3](#) shows the results obtained for the sparkling wine lees from the first to the ninth year of aging. It can
268 be seen that satisfactory linear regression coefficients were obtained in all cases. This confirms that this
269 mathematical model works quite well. According to this mathematical model, the following equation can

270 be established: $1/[O_2] = A/t + B$. This equation describes the relationship between the consumed oxygen
271 versus time and is shown in [Fig. 4A](#), which also shows how the consumed oxygen can be cleared up, how
272 the first derivative is obtained, and finally how the oxygen consumption rate at time zero (OCR_{to}) can be
273 determined, which corresponds to the inverse of the slope of the initial equation. This OCR_{to} indicates the
274 initial oxygen consumption rate by the lees.

275

276 This modelling can also be used to compare the experimental data ([Fig. 4B](#)) with the theoretical data ([Fig.](#)
277 [4C](#)) obtained after applying this model. As it can be seen in these figures, the mathematical model
278 reproduces quite well the experimental kinetics of oxygen consumption by the lees from the sparkling
279 wines of different aging time. These results demonstrate the validity of the modelling.

280

281 [Fig. 5A](#) shows the OCR_{to} of the sparkling wine lees from the first to the ninth year of aging time calculated
282 using the model described above. These OCR_{to} values were corrected to be referred to the volume of the
283 original sparkling wine bottles (750 mL), considering the volume of the flasks (66 mL) in which the oxygen
284 measurements were performed and are expressed in mg/L. According to these data, OCR_{to} was highest in
285 the first year lees, then decreased significantly in the second and third year lees, and finally decreased
286 significantly again in the older lees. These results confirm more accurately that the capacity of the lees to
287 consume oxygen decreases with the aging time. Nevertheless, the initial oxygen consumption at time zero
288 does not match the annual capacity of oxygen consumption exactly, as can be seen in [Fig. 5B](#). Considering
289 the total oxygen consumption in 365 days, the second year lees are capable of consuming slightly more
290 than double the oxygen than the lees of the first or third years. Subsequently, the annual oxygen
291 consumption decreases drastically in the older lees. The surprising behavior of the second year lees could
292 be related to the described progress of the autolysis process which, according to some authors, starts
293 slightly after 4 months and is more intense during the second year ([Todd et al., 2000](#); [Fornairon-Bonnefond](#)
294 [et al., 2002](#); [Alexandre, & Guilloux-Benatier, 2006](#); [Kemp et al., 2015](#)). Some authors have related the
295 oxygen consumption to the lipids from the cell membranes ([Salmon et al., 2000](#); [Fornairon-Bonnefond, &](#)
296 [Salmon, 2003](#)), especially to certain sterol fractions ([Fornairon-Bonnefond, 2000](#)). Since autolysis is more
297 intense during the second year, it is logical that there is more availability of these lipids and there may
298 be other substances capable of consuming oxygen. In any case, it is clear that the lees' oxygen
299 consumption capability decreases drastically after the first three years of aging since older lees (fourth
300 to ninth years) have much lower OCR values than young lees (first to third years).

301

302 Therefore, it is logical to consider that the balance between the entrance of oxygen through the crown cap
303 and the consumption of oxygen by the lees must determine the evolution of the sparkling wine. As long as
304 the lees' oxygen consumption capacity is greater than the oxygen intake, the sparkling wine will be

305 protected against oxidation. However, what would happen if the lees stop consuming enough oxygen?
306 When this occurs, oxygen will be most likely consumed by other wine components, especially by phenolic
307 compounds, which probably will generate browning and the appearance of hydrogen peroxide that will
308 oxidize other wine compounds in the absence of free sulfur dioxide. This process, that is the main
309 responsible of the loose of quality caused by oxidation, can only be stopped by the presence of free sulfur
310 dioxide, glutathione, or when lees are present and consume enough oxygen. Evidently, sparkling wine
311 oxidation will be greater or lesser depending on the composition of the wine, which is largely dependent on
312 the vintage and the production process. To better illustrate this balance Fig. 6 shows the accumulated
313 oxygen consumption by the lees, considering the addition of their annual oxygen consumption over the
314 aging time. This curve fits reasonably well with a logarithmic equation ($[O_2] = 0.7078 \times \ln(t) + 0.351$; $r^2 =$
315 0.9895). Fig. 6 also shows what the oxygen intake across the crown cap would be considering the minimal
316 value of oxygen permeability reported by Valade et al. (2007). The comparison of the two curves is just a
317 theoretical approximation, but even so it provides very interesting information.

318

319 According to this approach the oxygen intake across a crown cap of low permeability remains below the
320 accumulated oxygen consumed by the lees during the first three years of aging time and exceeds it at
321 around three and a half years. More precisely the exact interception point is at 3 years and 7 months. This
322 means that after this aging time, the oxygen consumed by the lees would not be high enough to
323 compensate the oxygen intake across the crown cap. This would leave the sparkling wine without a
324 sufficient defense against the oxidation that oxygen intake could cause. It should be taken into account that
325 the considered permeability was very low and that any increase in this permeability would entail an earlier
326 point of intersection in time. As an example, with a 20% higher permeability the intersection would take
327 place just after two years. As mentioned above, this is only a theoretical approach based on our results;
328 however, it illustrates what may occur during sparkling wine aging.

329

330 Finally, this study has been performed only with one yeast strain. It could be hypothesized that some yeast
331 strains could consume more oxygen and faster than other ones. In that case the use of yeast strains with
332 higher ability to consume oxygen could be a strategy to extend the aging time of sparkling wines without
333 the appearance of oxidation notes. However, further studies are needed to verify this hypothesis.

334

335 4. Conclusions

336

337 It can be concluded that lees of sparkling wines elaborated using the traditional method have a real
338 capacity to consume oxygen and consequently to protect sparkling wines against oxidation. However, the
339 lees' capacity to consume oxygen decreases drastically after three years of aging, which could seriously
340 affect the ability of sparkling wines to age properly. These data also explain what AOC Cava winemakers

341 know empirically. Only some high quality sparkling wines made using the traditional method can age more
342 than three years without being affected by oxidation.

343

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349

350 **Declaration of Competing Interest**

351 The authors declare that they have no known competing financial interests or personal relationships that
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353

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357

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Figure 1. Experimental design for measuring oxygen consumption

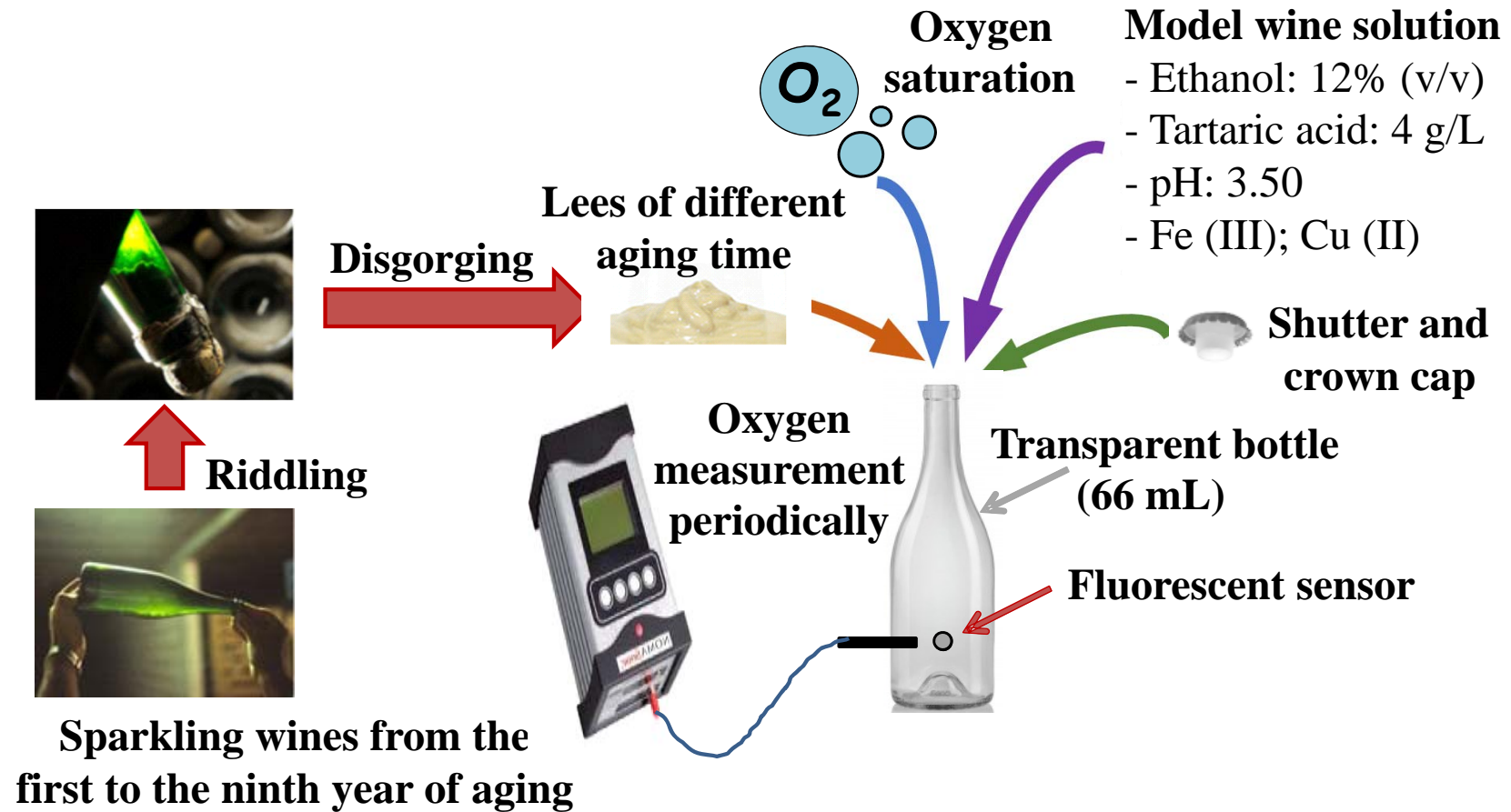
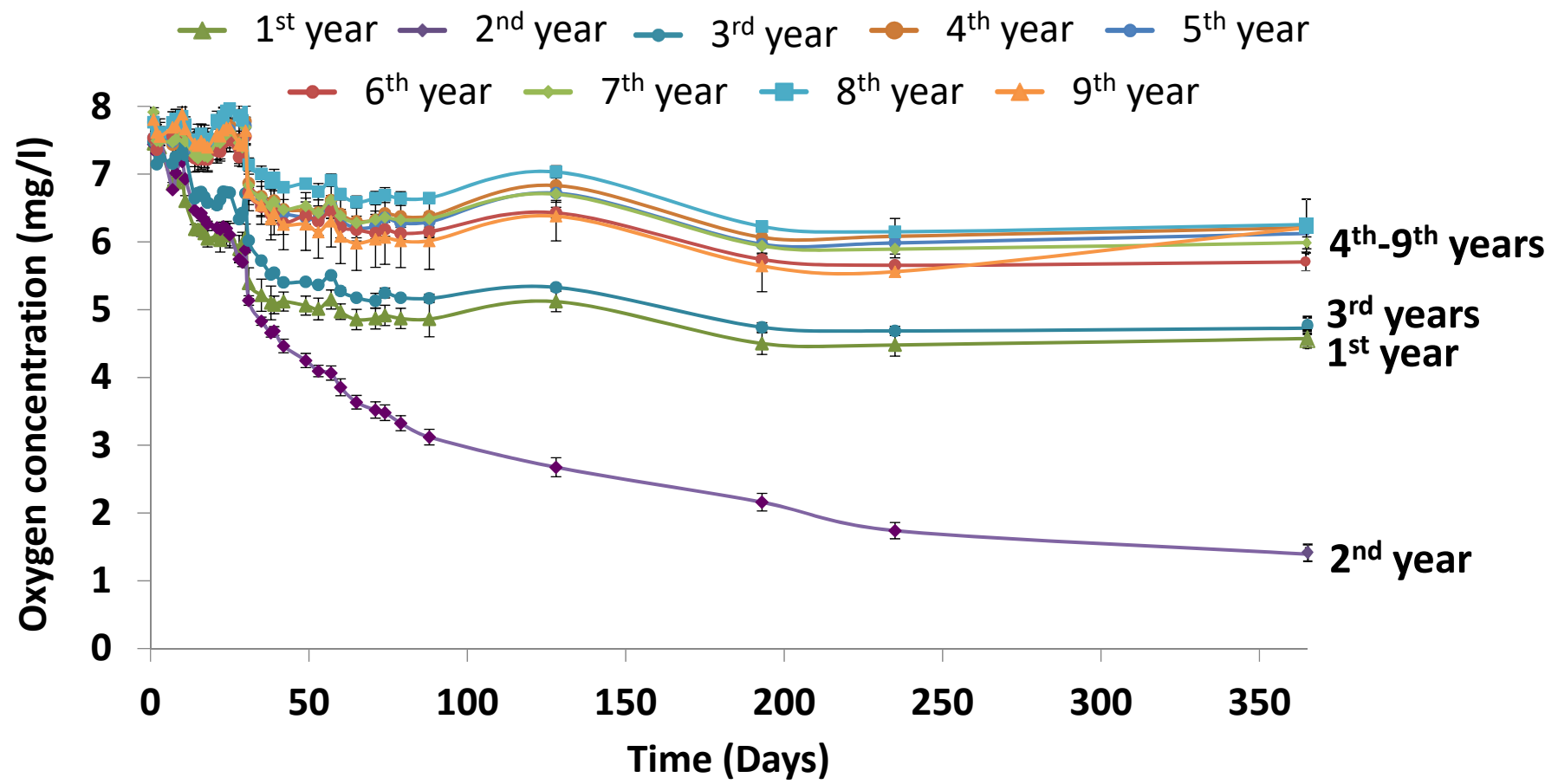


Figure 2. Oxygen concentration evolution inside bottles with lees extracted from sparkling wines of different aging time.



All data are expressed as the average of 3 replicates \pm standard deviation

Figure 3. Application of the oxygen consumption model to the lees of different aging times

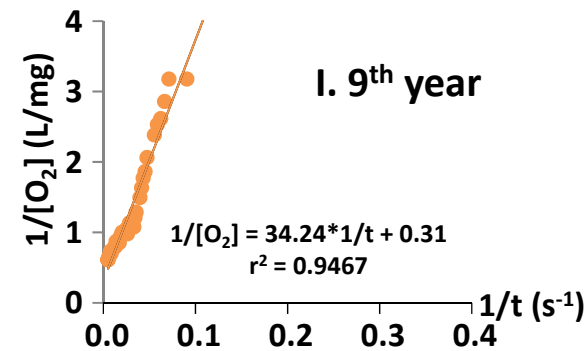
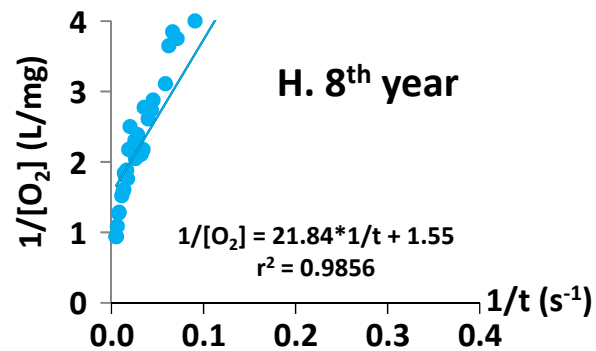
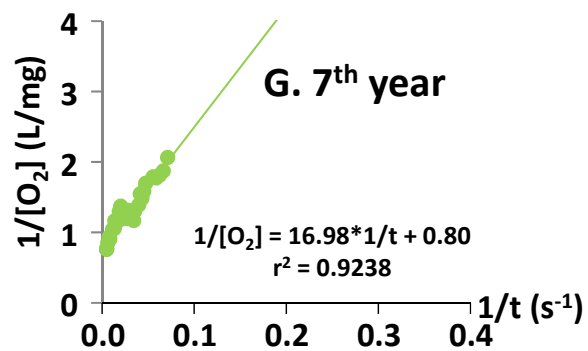
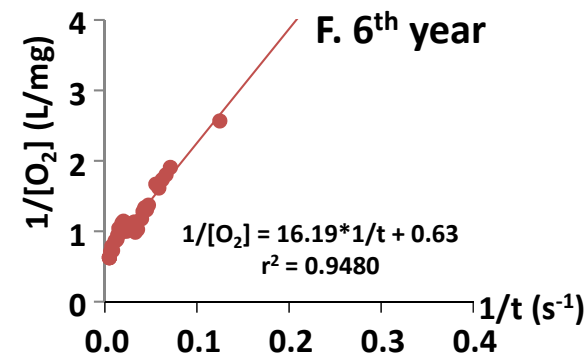
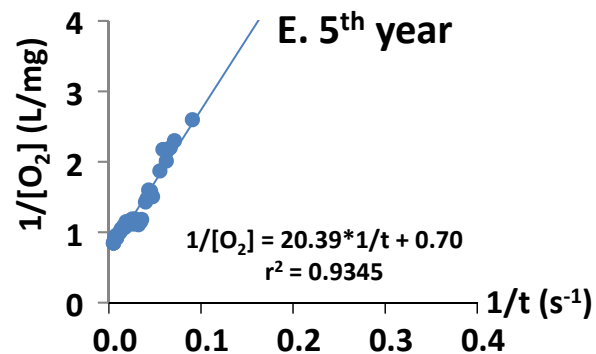
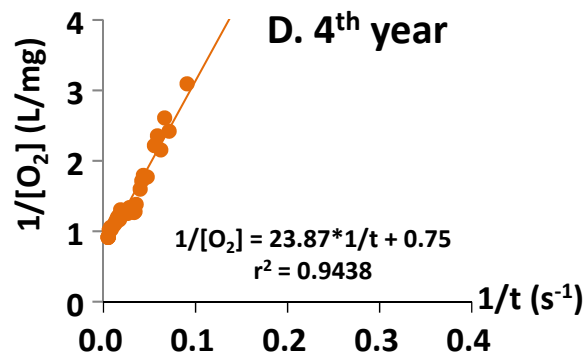
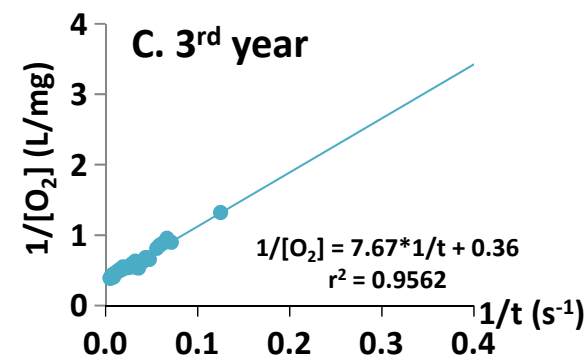
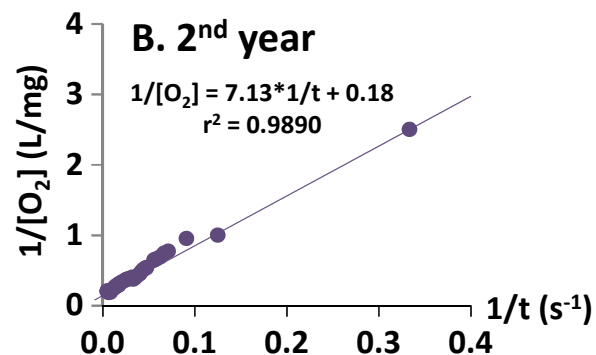
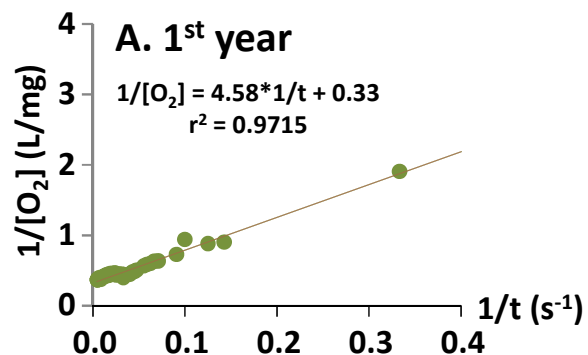
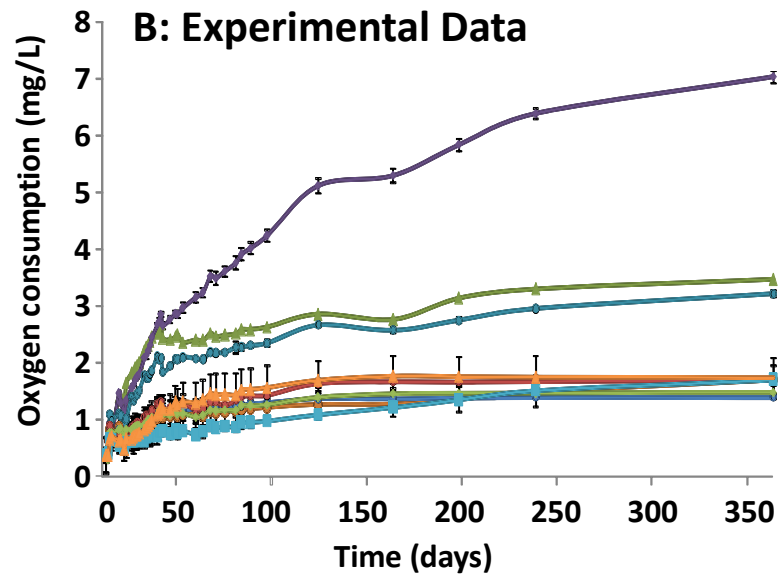


Figure 4. Modelling of the oxygen consumed by lees

A. Calculation of the Oxygen Consumption Rate at time zero (OCR_{t_0})

$$\frac{1}{[O_2]} = \frac{A}{t} + B \xrightarrow{1. \text{ Clear up } [O_2]} [O_2] = \frac{t}{A + Bt} \xrightarrow{2. \text{ First derivative}} \frac{d[O_2]}{dt} = \frac{A}{(A + Bt)^2} \xrightarrow{3. \text{ For } t=0} \frac{d[O_2]}{dt} = \frac{A}{A^2 + 2ABt + B^2t^2} = \frac{1}{A} = OCR_{t_0}$$



—▲— 1st year —■— 2nd year —●— 3rd year —◆— 4th year —□— 5th year —◇— 6th year —▽— 7th year —○— 8th year —△— 9th year

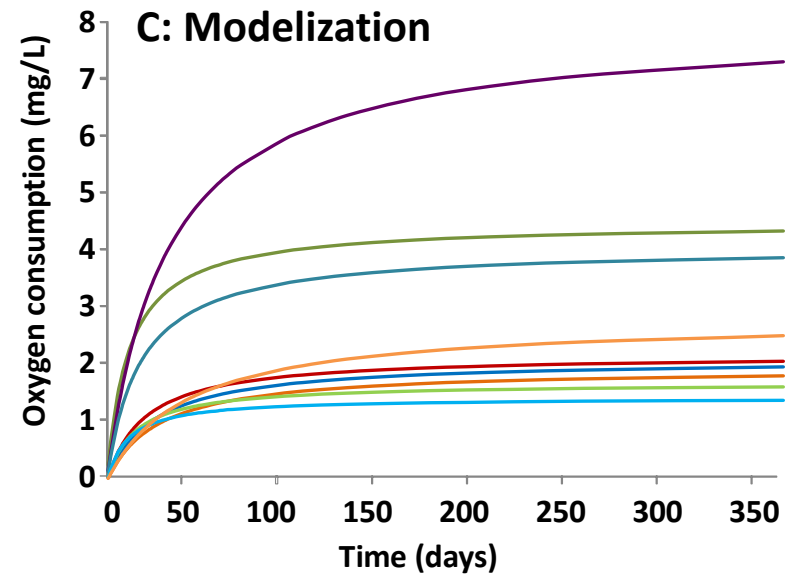


Figure 5: Oxygen Consumption Rate (OCR) of the different aged lees from 1 to 9 years old

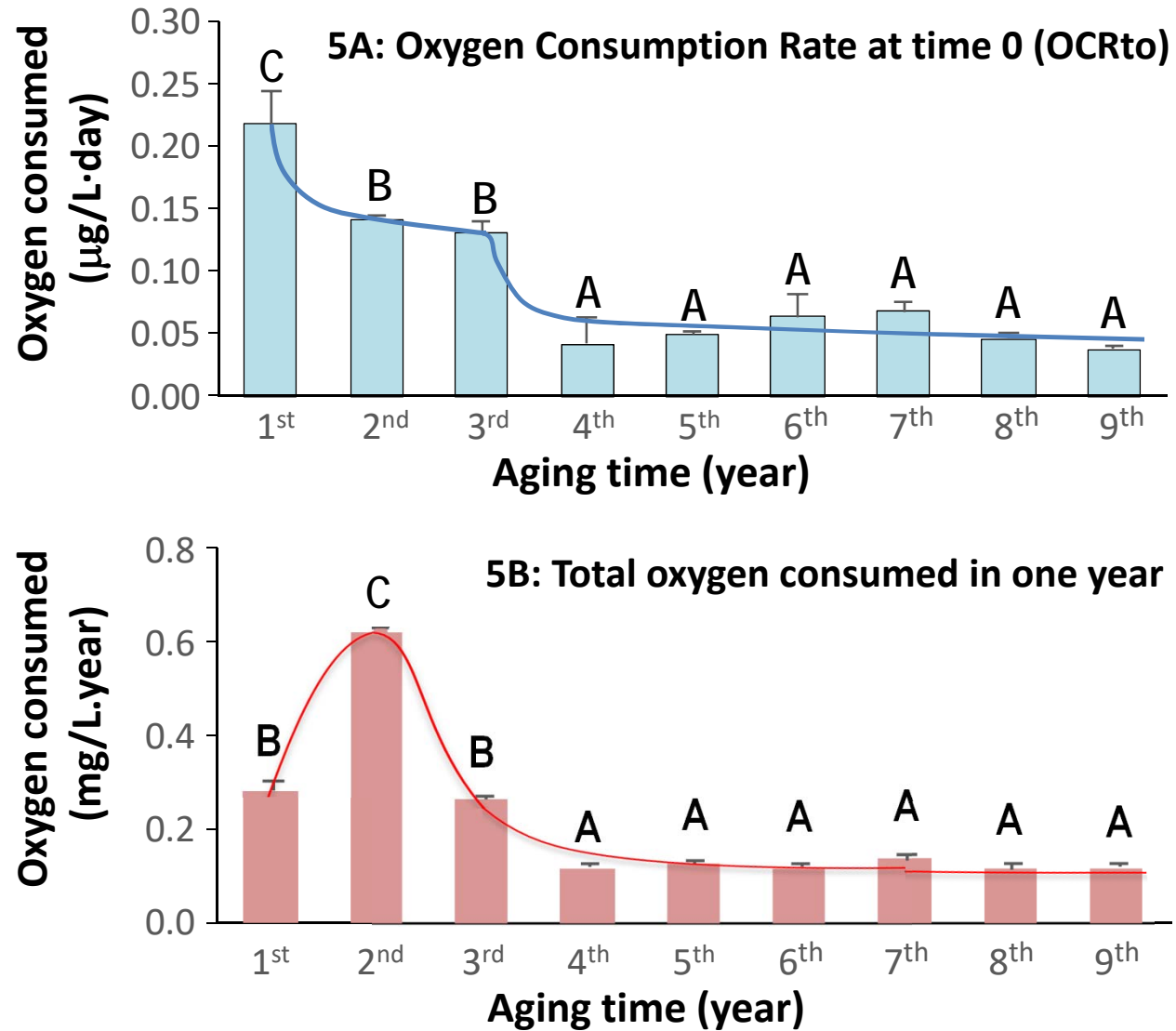


Figure 6. Accumulation of oxygen consumed by the lees in comparison with the oxygen intake of the crown cap

