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Comparison of ancestral and traditional methods for elaborating sparkling wines

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ABSTRACT

This work compares the ancestral method for elaborating sparkling wines with the most widely used traditional method. Ancestral method is a single fermentation procedure in which the fermenting grape must is bottled before the end of alcoholic fermentation whereas traditional method involves a second fermentation of a base wine inside a bottle. Macabeo grapes were used to elaborate a traditional sparkling wine and two ancestral sparkling wines, one with a low yeast population and one with a high yeast population. The findings indicate that ancestral sparkling wines have lower ethanol content and can be elaborated using lower sulphur dioxide levels. In general, ancestral sparkling wines showed similar protein concentration, higher polysaccharide content, similar or better foamability (HM) than the traditional sparkling wine., No differences were found in the foam stability (HS). In addition, the sensory analysis indicated that ancestral sparkling wines have smaller bubble size, lower CO_2 aggressivity, they seemed to have longer ageing time and were scored better than the traditional sparkling wine. These results therefore indicate that the ancestral method is of great interest for the elaboration of high-quality sparkling wines.

1. Introduction

Sparkling wines are a group of special wines characterized to produce effervescence when they are uncorked (European council, 2009; OIV, 2023a). Global production of sparkling wines is about 20 million hectoliters/year, which represents only 11% of total wine production (OIV, 2023b). Although this relatively low percentage, these wines have a large economic importance in the global wine market, which in 2022 exceeded USD 42 billion dollars (Cravero, 2023). The effervescence of sparkling wines comes from an overpressure of CO_2 that can have an exogenous origin, in the case of sparkling carbonated wines, or an endogenous origin, in the case of natural sparkling wines (European council, 2009; OIV, 2023a). Artificially carbonated wines are usually cheap low-quality products with low interest. In contrast, the CO_2 present in natural sparkling wines is obtained from alcoholic fermentation performed by yeasts in closed vessels, which leads to obtain much higher quality products (Ribéreau-Gayon et al., 2006).

There are different methodologies for obtaining natural sparkling wines depending on various aspects. Thus, natural sparkling wines can be made with one or two alcoholic fermentations, can acquire effervescence in the bottle or in an isobaric tank, and can be isobarically filtered or not before the final bottling (Jackson, 2008). However, premium sparkling wines such as Champagne, Cava or Franciacorta are produced following the traditional method (referred to as *champenoise* method in AOC Champagne), which involves a second alcoholic fermentation of a base wine inside a bottle (Kemp et al., 2015; Maujean, 1989). This second fermentation takes place inside crown sealed bottles in which *liqueur de tirage,* a mixture of still wine, sugar (around 20–24 g of sucrose/L), preadapted yeasts (1 or 2 million viable cells/mL) and a riddling agent, is added to the base wine. For the second fermentation to be successful, the yeasts need to be preadapted, and essential nutrients need to be provided (Berbegal et al., 2022; Martí-Raga et al., 2016).

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In contrast, the ancestral method is a single fermentation procedure that is historically considered the first method for producing sparkling wine (Rose, 2021). The precedent dates back to the 16th century in the Languedoc region, where in a Benedictine monastery wine was bottled and corked without completing the alcoholic fermentation (Stevenson, 2005), due to the very cold winter temperatures, alcoholic fermentation was stopped and the wine was bottled prior the full depletion of sugars. However, the warm spring temperatures reactivated the yeasts, which then finished the residual sugars and accumulated CO_2 inside the bottle. This CO_2 transformed into effervescence once the bottles were opened (Robinson and Harding, 2015).

However, the ancestral method was abandoned by most producers due to the difficulties involved in controlling this process (Jeandet et al., 2011; Panesar et al., 2017). Determining the appropriate time to bottle the fermenting must with the suitable concentration of sugars implies a very strict analytical control that was not always possible. Furthermore, stopping or at least slowing down fermentation kinetics before bottling at the adequate moment requires very low temperatures that, without the current technology, was sometimes very difficult to achieve. Finally, in the past it was also very complicated to control the yeast population inside the bottles. This lack of control in ancient times could lead to: (i) the presence of off-flavours (reduction taint) due to the excess yeast population, (ii) internal CO_2 pressures either too high or too low, (iii) extreme variation in the sparklingness, (iv) inappropriate turbidity levels and even (v) unstable microbiology products that could lead to highly volatile acidities (Dubois et al., 1998; Ribéreau-Gayon et al., 2006a,b). Evidently, all these problems led to the progressive substitution of the ancestral method for the more controlled traditional method.

The ancestral method is still used and regulated in some AOC, and AOC Blanquette de Limoux is probably the best known. Nevertheless, the sparkling wines from this AOC can also be elaborated using the traditional method, and unfortunately nowadays only a small proportion of wines are made using the ancestral method.

However, in recent years there has been a growing interest in sparkling wines made according to the ancestral method, such as *pétillant naturels* or *Pét-Nats, which* currently have great commercial success in France (Colinet, 2022; Neiman, 2018; Voisin, 2021).

In Catalonia (Spain), most of the Sparkling wines produced are elaborated by the traditional method (PDO Cava); however, in recent years there has been an increasing interest in single fermentation sparkling wines (Falgueras, 2022; Vicens, 2023). This has also led to PDO Penedès and PDO Tarragona including and regulating the process for elaborating these wines using the ancestral method.

In addition, the ancestral method has an advantage over the traditional method that can be very useful today when climate change is affecting the grape ripening process. As it is widely known, global warming is causing the grapes to reach high sugar concentrations earlier (Jones et al., 2005; Schultz, 2000) which forces harvest dates to be advanced to avoid wines with too much alcohol (Gil et al., 2013). However, harvesting earlier can sometimes mean that the grapes are not be well balanced, resulting in wines with vegetal or herbaceous characteristics (Zamora, 2014). This problem is especially worrying in the case of sparkling wines elaborated by traditional method because the base wines must be added with around 22 g of sucrose/L for the second fermentation. This means that the final sparkling wine will contain around 1.3° more alcohol, and therefore it is necessary to harvest the grapes with a potential alcohol level not exceeding 11.0% (Esteruelas et al., 2015a,b). In contrast, the ancestral method does not need sugar to be added since it only has one fermentation. It is therefore not needed to advance the harvest which makes possible working with riper grapes. This is one of the advantages of the ancestral method. Furthermore, ancestral sparkling wines do not require such high acidities because these sparkling wines are not usually aged for long time.

The ancestral method also has the advantage that it is not necessary to add SO_2 to protect the base wine during the stabilization period. Consequently, ancestral sparkling wines normally contain less SO_2 than traditional sparkling wines. Nowadays this undoubtedly represents a great advantage since the current trend in winemaking is to decrease and even eliminate sulphites owing to their negative effect on the environment (Stockley, 2005) and human health (Vally and Misso, 2012).

There is extensive scientific literature on sparkling wines made with the traditional method and many research groups have studied them (Cilindre et al., 2021; Esteruelas et al., 2015; Marchal et al., 2001; Kemp et al., 2015; Martínez-García et al., 2017; Medina-Trujillo et al., 2017; Liger-Belair & Cilindre, 2021; Wilson et al., 2022). However, there is almost no scientific literature on sparkling wines made with the ancestral method and only a few articles have appeared on the subject only very recently (Dachery et al., 2023; Makarov and Lutkov, 2021; Rossier et al., 2016). Given this lack of information and the great interest that many wineries have about the subject, the aim of this work is to compare the composition and sensory qualities of sparkling wines elaborated with the ancestral and traditional methods from the same grapes. Another objective of this work was to study the influence of the yeast population during the last step of bottle fermentation in the ancestral sparkling wine elaboration.

2. Material and methods

2.1. Chemicals

All samples and standards were handled without any exposure to light. $K_2S_2O_5$ (purity ≥97.2%), carboxymethyl cellulose (Estabicel) (purity ≥99.0%) and fumaric acid (purity ≥99.0%) were purchased from Agrovin (Alcázar de San Juan, Ciudad Real, Spain). Ethanol (purity ≥99.5%), hydrochloric acid (purity ≥37.0%), NaOH (purity ≥98.0%), sulphuric acid (purity ≥96.0%) and $CuSO_4 \bullet 5\ H_20$ (purity ≥99.0%) were purchased from Panreac (Castellar del Vallès, Barcelona, Spain). Glycerol (purity ≥99.5%), acetic acid (purity ≥99.5%), L-(+)-tartaric acid (purity ≥99.5%), L-malic acid (purity ≥97.0%), L-lactic acid (purity ≥98.0%), citric acid (purity ≥99.5%), ammonium formate (purity ≥99.9%), ammonium acetate (purity ≥99.9%), bovine serum albumin (purity ≥98.0%) and fumarase (≥300 units/mg protein) were purchased from Sigma-Aldrich (Madrid, Spain). Pectinolytic enzymes (Lallzyme) were purchased from Lallemand, Inc. (Montreal, Canada). Riddling agent (Adjuvant 92) was supplied by Station Oenotechnique de Champagne (Epernay, France). A pullulan molecular weight calibration kit Shodex P-82 was obtained from Waters (Barcelona, Spain), whereas a pullulan 1.3 kDa and four dextrans BioChemika (12, 25, 50, and 80 kDa) were obtained from Fluka (St. Louis, MO, USA). The polysaccharides used as external standards for quantification were pectins from citrus fruit (≥90%) and dextrans from Leuconostoc mesenteroides (≥99.9%) purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Enumerating the yeast population

A 10 μL aliquot of the appropriately diluted sample was dispensed into a Neubauer chamber (Leica Microsystems GMS QmbH, Leica, Germany). Total cells were counted using an optical microscope (B–510 B F, Optika, Ponteranica, Italy). The total yeast cell population was calculated considering the applied dilution factor.

2.3. Experimental design

The experiment was carried out during the 2022 harvest at the experimental winery of the Universitat Rovira i Virgili (Mas dels Frares, Constantí, Tarragona, Spain) using Macabeo grapes provided by the Regulatory Council of the PDO Tarragona. The manual harvest took place on 7 of September when the grape maturity parameters were at 18.6 ^{o}Brix, 3.32 pH and a titratable acidity of 5.2 g/L (expressed as tartaric acid). Fig. 1 illustrates the experimental design.

The grapes bunches were crushed (Delta E2, Bucher Vaslin,

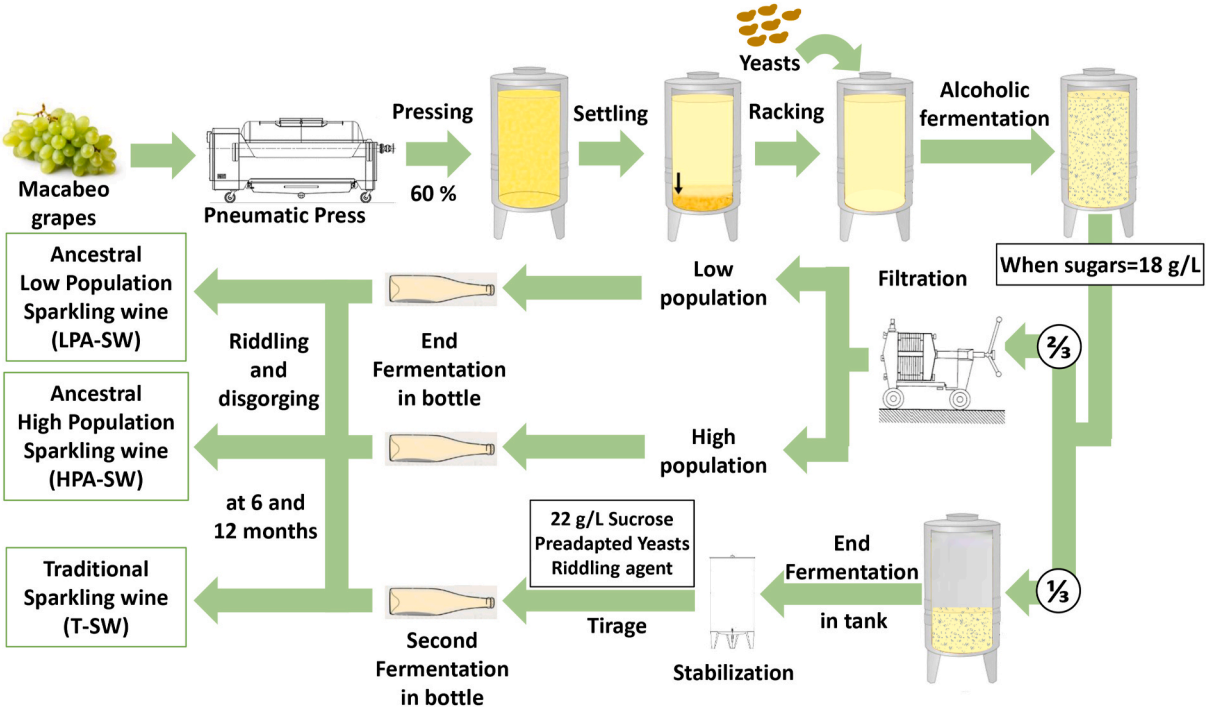

Fig. 1. Experimental design.

Chalonnes-sur-Loire, France) and pressed in a pneumatic press (M5, Marzola, Navarrete, Spain) until a yield of 0.6 L/kg was obtained. The must was immediately supplemented with 70 mg/L of $K_2S_2O_5$ and 20 mg/L of pectolytic enzyme (Lallzyme, Lallemand, Inc., Montreal, Canada) to favour settling. The must was then cold (8 °C) settled for 24 h. After settling, 200 L of clarified must were racked into a stainless-steel tank and immediately inoculated with 200 mg/L of a commercial strain of *Saccharomyces cerevisiae* (Lalvin EC1118™, Lallemand, Inc., Montreal, Canada). The temperature was maintained at 16–18 °C and the fermentation kinetics were monitored using a digital densimeter (Mettler Toledo-PortableLabTM, Cornellà de Llobregat, Barcelona, Spain). The fermenting must was acidified with 1 g/L of tartaric acid due to its low titratable acidity.

When must densities were close to 1005 kg/m^3, we started the analytical control to determine the exact time in which the residual fermentable sugars reached the appropriate value for bottling the ancestral sparkling wine (18.0 g/L). Normally, base wines are supplemented with 20–24 g/L of sucrose in the elaboration of sparkling wines by traditional method. The lower concentration of sugar in the case of ancestral sparkling wines is because the fermenting must already contain a saturating concentration of carbon dioxide, something that does not happen in the case of traditional sparkling wine, and also because a slightly lower pressure is usually sought for these wines. Once the fermenting must reached this value, around two-thirds of the volume was racked and cooled to 5 °C to slow down alcoholic fermentation and it was filtered with a 310 mm diameter plate filter (Cristalinox 310 mm, In Via, Sant Sadurní d'Anoia, Barcelona, Spain) using paper filter sheets (FIBRAFIX® AF 70, Filtrox, Santa Perpètua de Mogoda, Barcelona, Spain) to reduce the yeast population to 6.0 × 10^6 cell/mL. This fermenting must was then divided into two batches. One was kept as it was (low-population; LPA-SW) while the other was supplemented with 6 % of the non-filtered fermenting must to achieve a yeast population of 12.0 × 10^6 cell/mL (high-population; HPA-SW). The two batches were supplemented with 200 mg/L of carboxymethyl cellulose (Estabicel, Agrovin, Alcázar de San Juan, Ciudad Real, Spain) to avoid the crystallization of tartrate salts, with 0.3 g/L of fumaric acid (Laboquimia, Logroño, Spain) to inhibit malolactic fermentation (Morata et al., 2023) and with 20 mg/L of adjuvant 92 (Station Oenotechnique de Champagne, Epernay, France) to facilitate the riddling process. Then, the two fermenting musts were bottled, crown sealed and stored at 15–16 °C until disgorgement.

In parallel, the remaining one-third of the fermenting must, which had not been used to produce the two ancestral sparkling wines, was kept in the original tank until the alcoholic fermentation had finished. This base wine was then racked, sulphited (40 mg/L of $K_2S_2O_5$) and partially cold stabilized at 4 °C for one month. The base wine was then racked again and used to elaborate the sparkling wine using the traditional method (T-SW). With this purpose, the base wine was supplemented with 22 g/L of sucrose and with a population of 2.0 × 10^6 cell/mL of a commercial strain of *Saccharomyces cerevisiae* (Lalvin EC1118™, Lallemand, Inc., Montreal, Canada) previously preadapted (Berbegal et al., 2022; Martí-Raga et al., 2016). It has been described that yeast population can grow during the second fermentation of sparkling wines elaborated by traditional method until around 4.0–7.0 × 10^6 cell/mL depending of the nitrogen content and temperature (Valade and Laurent, 1999; Martínez-Rodríguez et al., 2002, Martí-Raga et al., 2016). In contrast, yeast population cannot grow in ancestral sparkling wines since yeast are already in the decline phase when the fermenting must is bottled. Therefore, the population of LPA-SW was similar to that achieved by T-SW. This base wine was also supplemented with 200 mg/L of carboxymethyl cellulose (Estabicel, Agrovin, Alcázar de San Juan, Ciudad Real, Spain), with 0.3 g/L of fumaric acid (Laboquimia, Logroño, Spain) and with 20 mg/L of adjuvant 92 (Station Oenotechnique de Champagne, Epernay, France). This base wine was then bottled, crown sealed and stored at 15–16 °C until disgorgement.

The fermentation kinetics of the two ancestral sparkling wines and the traditional wine were monitored by measuring of the accumulated pressure inside the bottle via a non-invasive methodology (L. sensor CO2, FT System, Alseno, Italy). All the sparkling wines followed the appropriate fermentation kinetics and finished after about 30 days (data not shown).

After six and twelve months of ageing at 16 °C, four bottles of each experimental group (24 bottles in total) were placed in a pupitre and the riddling process was performed manually. Once all the lees sediment

had reached the bottom (around 12 days), the bottles were disgorged by hand after freezing their neck at −28 °C using a Champagel apparatus (Maquinaria Moderna, Sant Sadurní d'Anoia, Barcelona, Spain). After adding 30 mg/L of $K_2S_2O_5$, the bottles of sparkling wine were immediately corked. Three bottles were used for physicochemical analysis and one bottle was used for sensory analysis.

2.4. Analysis of general wine parameters

The internal CO_2 pressure inside the bottles was measured using a non-invasive Laser Sensor (L. sensor CO2, FT System, Alseno, Italy). For all the other measurements, all wine samples were centrifuged at 13,000 g (Biofuge Primo centrifuge, Heraeus, Hanau, Germany) for 15 min at 4 °C to obtain clear samples and to remove carbon dioxide. The ethanol content was determined by ebulliometry (GAB Analysis Systems, Moja-Olerdola, Barcelona Spain). The concentrations of residual fermentable sugars (D-glucose and D-fructose) were measured using a commercial enzymatic kit (Enology D-GLUCOSE/D-FRUCTOSE, Biosystems, Barcelona, Spain). Titratable acidity and pH were determined following the OIV recommended methods (OIV, 2023c). The total and free sulphur dioxide content were determined using a commercial kit (GAB Analysis Systems, Moja-Olerdola, Barcelona Spain). The concentrations of glycerol, L-(+)-tartaric, L-malic, L-lactic, citric and acetic acids were measured according to Lemos Junior et al. (2019). Fumaric acid was determined following the enzymatic method proposed by Fernández-Vázquez et al. (2021).

2.5. Colour parameters

The CIE$L^*a^*b^*$ coordinates were determined following the method described by Ayala et al. (1997) using a Helios Alpha UV VIS spectrophotometer (Thermo Fisher Scientific Inc., Waltman, MA, USA). Data were processed using MSCV® software (MSCV, 2013). The total colour difference (ΔE^*ab) was calculated according to Martínez et al. (2001).

2.6. Quantification of proteins, polysaccharides and mannose by HPLC

The protein measurement was processed and analysed by HRSEC-DAD using the methodology described by Canals et al. (1998). The polysaccharide measurement was processed and analysed by HRSEC-RID using the methodology described by Ayestarán et al. (2004). Mannose was analysed by HRSEC-RID after acidic hydrolysis according to the protocol described by Quirós et al. (2012).

2.7. Measurement of foaming properties

The foam properties were measured using the Mosalux method (Station Oenotechnique de Champagne, Epernay, France) according to the procedure described by Maujean et al. (1990). Two parameters were measured: HM, the maximum foam height, and HS, the stable foam height. HM represents foamability while HS represents foam stability.

2.8. Measurement of colloidal properties

Nanoparticle tracking analysis was performed to determine the concentration and size of the colloidal bodies of the samples using NanoSight NS300 (Malvern, Worcestershire, United Kingdom) following the procedure described by Bindon et al. (2016).

2.9. Sensory analysis

All the sparkling wines at 12 months of ageing were tasted by 15 trained wine tasters, nine males and six females aged between 22 and 60. Tasting was carried out using ISO official tasting glasses (ISO, 1997). The served volume was around 50 mL and the service temperature was 6–8 °C. For each sample, the tasters were required to evaluate the intensity of eleven sensory attributes (Colour, Bubble size, Balance reduction/oxidation, Ageing, Tropical fruit, Aniseed, White fruit, CO_2 aggressivity, Structure, Acidity and Overall quality) on a scale of 1–10 (1 = 'slight intensity', 10 = 'maximum intensity'). For Colour the scale goes from very pale yellow (1) to very intense brown (10). For Bubble size the scale goes from very small bubbles (1) to very big ones (10). For Balance Reduction/Oxidation, the scale goes from the presence of evident reduction notes (1) to high oxidation notes (10). For Ageing the scale goes from very young aroma (1) to very evolved one (10). For Tropical fruit, Aniseed and White fruit the scale goes from very low intensity of these aromas (1) to very high intensity (10). For CO_2 aggressivity the scale goes from a very pleasant sparklingness (1) to very aggressive sparklingness (10). For Structure the scale goes from very light (1) to very heavy body (10). For Acidity the scale goes from very scarce (1) to very intense (10). Finally, for Overall quality the scale goes from very bad (1) to excellent (10). The value of each descriptor was expressed as the average of all the tasters. A sensory training session was held beforehand so that the tasters could agree on the criteria for each of the different sensory attributes. Samples were served randomly to avoid the tasting order having an influence.

2.10. Statistical analysis

The data shown are the arithmetic means of triplicates with the standard deviation for each parameter. Two-way ANOVA and Tukey comparison tests were carried out using the XLSTAT software (Addinsoft, Paris, France). The sensorial analysis results were analysed with the PanelCheck V1.4.2 software (Nofima Mat, Technical University of Denmark & University of Copenhagen).

3. Results and discussion

Physicochemical analyses were performed at six months of ageing (minimun time of ageing of PDO Tarragona - disgorgement after the first spring day of the next year of the harvest) and at twelve months (once the minimum nine months of ageing of PDO Cava has been exceeded). Base wine was not analysed because it does not exist in the case of ancestral sparkling wines.

3.1. General parameters

Table 1 shows the general compositional parameters of the three sparkling wines after twelve months of ageing. All the sparkling wines have an internal CO_2 pressure greater than the minimum legal 3.00 bars (European council, 2009). However, the internal CO_2 pressure was significantly higher (5.86 bars) in the traditional sparkling wine (T-SW) than in the low-population ancestral sparkling wine (LPA-SW) and high-population ancestral sparkling wine (HPA-SW), 4.84 and 4.80 bars respectively. This difference can be attributed to the different fermentable sugar content at bottling, which was 22.0 g/L of sucrose (equivalent to 23.16 g/L of D-glucose and/or D-fructose) for the traditional sparkling wine and only of 18.0 g/L of D-glucose and/or D-fructose for the ancestral sparkling wines (A-SW). This data is also reflected in the final ethanol content since it was of 12.2 % (v/v) in T-SW and 10.7 % (v/v) in the two A-SW. Glycerol shows a similar pattern; however, the differences between T-SW and the two A-SW were not significant. The lower ethanol content of A-SW can be considered an advantage nowadays because many consumers prefer wines with a lower alcohol content (Bucher et al., 2018). Another additional advantage of the lower alcohol content of the ancestral method is that the adverse effects of climate change on grape sugar accumulation can be compensated (Jones et al., 2005; Schultz, 2000).

This higher ethanol content is also probably the reason why the residual sugar concentration was slightly but significantly higher in T-SW than in A-SW because a higher alcohol content implies greater difficulties in completing the fermentation (Novo et al., 2014). Nevertheless,

Table 1
General parameters.

Parameter	Traditional				Ancestral							
					Low population				High population			
CO2 presure (bars)	5.86	±	0.15	**B**	4.84	±	0.14	**A**	4.80	±	0.17	**A**
Ethanol (% v/v)	12.2	±	0.2	**B**	10.7	±	0.1	**A**	10.7	±	0.1	**A**
Residual sugars (g/L)	0.35	±	0.02	**B**	0.24	±	0.04	**A**	0.25	±	0.01	**A**
Total SO2 (mg/L)	39	±	1	**B**	28	±	1	**A**	27	±	1	**A**
Titratable acidity (g of tartaric acid/L)	6.15	±	0.01	**C**	6.05	±	0.02	**B**	5.93	±	0.04	**A**
pH	2.99	±	0.02	**A**	2.98	±	0.02	**A**	2.95	±	0.02	**A**
L-Malic acid (g/L)	0.50	±	0.11	**A**	0.43	±	0.10	**A**	0.33	±	0.09	**A**
L-Lactic acid (g/L)	0.04	±	0.01	**A**	0.22	±	0.02	**B**	0.20	±	0.02	**B**
Citric acid (g/L)	0.19	±	0.02	**B**	0.10	±	0.02	**A**	0.08	±	0.01	**A**
Acetic acid (g/L)	0.37	±	0.01	**A**	0.39	±	0.01	**A**	0.39	±	0.02	**A**
Fumaric acid (g/L)	0.05	±	0.02	**A**	0.01	±	0.01	**A**	0.02	±	0.01	**A**

Results are expressed as mean ± standard deviation of three replicates. T-SW: Traditional sparkling wine; HPA-SW: High population ancestral sparkling wine; LPA-SW: Low population ancestral sparkling wine Different letters in a row indicate the existence of statistical difference ($p < 0.05$).

the levels of residual sugars were in all the cases very low, which indicates that all the sparkling wines had finished the fermentation optimally. In addition, the residual sugar concentration was so small in all the sparkling wines that it would not exert any sensory effect (Mao et al., 2019).

As expected, the free sulphur dioxide levels were practically non-existent (data not shown) because alcoholic fermentation mainly involves the combination of sulphur dioxide (Ribéreau-Gayon et al., 2006a,b). However, the total sulphur dioxide concentration of the T-SW was significantly higher than that of the two A-SW. The higher level of total sulphur dioxide is clearly due to the sulphur dioxide being added to protect the base wine in the traditional method, whereas in the ancestral method the fermenting must is bottled without adding this additive. Therefore, as mentioned in the introduction, this is an advantage of the ancestral method due to the current tendency in winemaking to decrease and even eliminate sulphur dioxide owing to its negative effects on the environment and human health (Stockley, 2005; Vally and Misso, 2012).

Titratable acidity of T-SW was slight but significantly higher than in both A-SW. No significant differences were found in the concentration of tartaric acid. The concentration of L-malic acid was not significantly different either, although in the case of A-SW it seems to be slightly lower. It should be noted that the concentration of L-lactic acid in the A-SW was significantly higher and the concentration of citric acid significantly lower than in the T-SW. Therefore, the lower titratable acidity of A-SW seems to be due to the development of partial malolactic fermentation. It should also be noted that A-SW partially developed malolactic fermentation although all the wines were supplemented with fumaric acid to inhibit lactic acid bacteria (Morata et al., 2023). However, the concentration of fumaric acid present in all the sparkling wines was much lower than the original added fumaric acid (0.3 g/L), which indicates that yeasts have metabolized this acid during the alcoholic fermentation. It has been reported previously that fumaric acid can be metabolized by yeasts (Jamalzadeh et al., 2012). Similar results have been reported by other authors, indicating that fumaric acid is probably transformed into L-malic acid by the action of fumarase (García-Viñola et al., 2023; Payan, 2023). In any case, it seems that the lower alcoholic level and the lower concentration of sulphur dioxide in the A-SW, as well as the disappearance of fumaric acid, favoured a partial development of lactic acid bacteria, which caused a small decrease in the titratable acidity. However, this slight decrease on the titratable acidity did not affect the pH of sparkling wines because most likely because none of the acids involved are very strong (Gancel et al., 2022). No significant differences were found in the acetic acid concentration.

3.2. Colour parameters

Table 2 shows the CIE$L^*a^*b^*$ coordinates and Table 3 the total colour difference (ΔEab^*) of the different sparkling wines at six and twelve months of ageing. All sparkling wines showed very similar CIE$L^*a^*b^*$ coordinates at six months of ageing with only a small but significant difference in the green–red colour component (a^*) of the T-SW, which

Table 3
Total colour differences (ΔEab*).

A. ΔEab* between elaboration methods		
Comparison	6 months	12 months
T vs ALP	0.32	1.04
T vs AHP	0.30	0.68
ALP vs AHP	0.05	0.91

B. ΔEab* between ageing times			
Comparison	T	ALP	AHP
6 vs 12 months	3.32	4.08	3.53

T-SW: Traditional sparkling wine; HPA-SW: High population ancestral sparkling wine; LPA-SW: Low population ancestral sparkling wine. A ΔEab* value lower than 3 units indicates that the human eye cannot distinguish the difference between two samples.

Table 2
CIEL*a*b* coordinates.

CIEL*a*b* coordinates		Traditional					Ancestral									
							Low population					High population				
L*	6	99.40	±	0.23	**A**	**β**	99.20	±	0.05	**A**	**β**	99.20	±	0.05	**A**	**β**
	12	98.50	±	0.50	**A**	**α**	97.70	±	0.80	**A**	**α**	97.90	±	0.40	**A**	**α**
a*	6	−0.70	±	0.10	**B**	**β**	−0.92	±	0.03	**A**	**β**	−0.88	±	0.02	**A**	**β**
	12	−2.70	±	0.60	**A**	**α**	−2.90	±	0.60	**A**	**α**	−3.00	±	0.30	**A**	**α**
b*	6	4.49	±	0.07	**A**	**α**	4.38	±	0.09	**A**	**α**	4.35	±	0.10	**A**	**α**
	12	6.98	±	0.04	**A**	**β**	7.62	±	0.12	**B**	**β**	6.85	±	0.02	**A**	**β**

Results are expressed as mean ± standard deviation of three replicates. T-SW: Traditional sparkling wine; HPA-SW: High population ancestral sparkling wine; LPA-SW: Low population ancestral sparkling wine. Different letters in a row indicate the existence of statistical difference ($p < 0.05$). First row (capital letters) indicates the influence the elaboration method. Second row (Greek letters) indicates the influence of ageing time.

was slightly higher than in the two A-SW. To find out whether these differences were distinguishable by the human eye, we determined the total colour difference (Δ*Eab**) between the different sparkling wines. It is generally considered that if Δ*Eab** is lower than 3 units it is not possible to distinguish between two samples (Martínez et al., 2001). The one-to-one comparison between the Δ*Eab** values of the three sparkling wines at six months of ageing generated values much lower than the 3 units threshold. Similar results were obtained when sparkling wines were compared one-to-one at twelve months of ageing. This information indicates that the three sparkling wines, regardless of the elaboration method used, have colours that are indistinguishable to the human eye. In contrast, all the sparkling wines at twelve months of ageing showed significantly lower *L** and *a** values, and especially a significantly higher blue–yellow colour component than their corresponding wines at six months of ageing. These differences indicate that the intensity of the yellow colour has increased as the ageing time also increased. Furthermore, the one-to-one comparison between the Δ*Eab** values of the three sparkling wines at twelve months of ageing with their corresponding ones at six months generated values of higher than 3 units, which indicate that the human eye can easily distinguish between them. Similar results have been previously reported (Pons-Mercadé et al., 2022; Serra-Cayuela et al., 2013), confirming a fact that winemakers know well: the longer the ageing time, the more intense the yellow colour (Kanavouras et al., 2020).

3.3. Protein fraction

Fig. 2 shows the protein fraction of the different sparkling wines at six and twelve months of ageing. The total protein concentration (Fig. 2A) was significantly similar between the three sparkling wines at six months of ageing and this trend was observed in the three molecular weight protein fractions (Fig. 2B, C and 2D). In contrast, all the sparkling wines at twelve months of ageing showed significantly higher total protein concentration than their corresponding wines at six months of ageing. This increase in total proteins can be easily explained by the yeast autolysis that takes place once alcoholic fermentation is finished (Alexandre and Guilloux-Benatier, 2006; Kemp et al., 2015; Pons-Mercadé et al., 2022). It should be noted that this increase in protein concentration was observed in all the molecular weight fractions. This apparent release of proteins due to yeast autolysis during the ageing time is very important since proteins act as surfactant agents that improve the foam characteristics of sparkling wines (Esteruelas et al., 2015; Kemp et al., 2019; Medina-Trujillo et al., 2017). It should also be noted that at twelve months of ageing, the total protein concentration of the HPA-SW was significantly lower than in T-SW, being the LPA-SW at intermediate level (no significant differences with none of the other sparkling wines). This difference is mainly due to the high molecular weight fraction (HMW), which was significantly lower in the two ancestral wines than in traditional wine.

3.4. Polysaccharide fraction

Fig. 3 shows the polysaccharide fraction of the different sparkling wines at six and twelve months of ageing. The total polysaccharide concentration (Fig. 3A) of the LPA-SW wine at six months of ageing wine was significantly lower than those of the corresponding HPA-SW and T-SW. This significant lower polysaccharide concentration of the LPA-SW was mainly due to the intermediate (IMW) and low (LMW) fractions (Fig. 3C and D), whereas no differences were found in the high (HMW) fraction. However, at twelve months the total polysaccharide concentration of the two A-SW was similar and significantly higher than that of the T-SW. It should also be noted that the total concentration of polysaccharides significantly increased with ageing time in the two A-SW whereas it did the opposite in the T-SW. This lower concentration of polysaccharides observed in the T-SW at twelve months was mainly due the LMW fraction (Fig. 3D).

The behaviour of the polysaccharides observed in the two A-SW can be easily explained. It is logical that the concentration of total polysaccharides would increase over time due to the autolysis of the yeasts. In fact, some authors have reported an increase in polysaccharide and/or mannoprotein concentration in sparkling wines during ageing (Charpentier, 2000; Pons-Mercadé et al., 2022). However, other authors did not find any increase (Martínez-Lapuente et al., 2016) or others have even reported a decrease in polysaccharides with ageing of sparkling wines (Martínez-Lapuente et al., 2013; Moreno-Arribas et al., 2000). The lower total polysaccharide fraction of the LPA-SW wine at six months of ageing could be attributable to the lower yeast population present at the beginning of the bottle fermentation. The lower the population, the

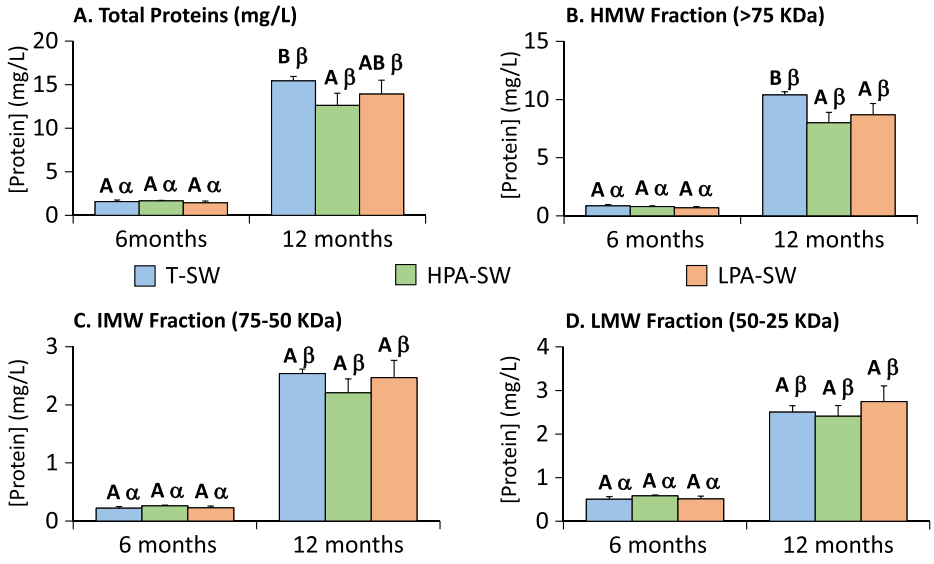

Fig. 2. Protein composition

Results are expressed as mean ± SD of three replicates. Concentration of proteins is expressed as bovine serum albumin equivalents. T-SW: Traditional sparkling wine; HPA-SW: High population ancestral sparkling wine; LPA-SW: Low population ancestral sparkling wine. HMW: High Molecular weigh Fraction; IMW: Intermediate Molecular Weight Fraction; LMW: Low Molecular Weight Fraction. Different letters indicate the existence of a statistical difference ($p < 0.05$). Capital letters indicates the influence the elaboration method. Greek letters indicates the influence of ageing time.

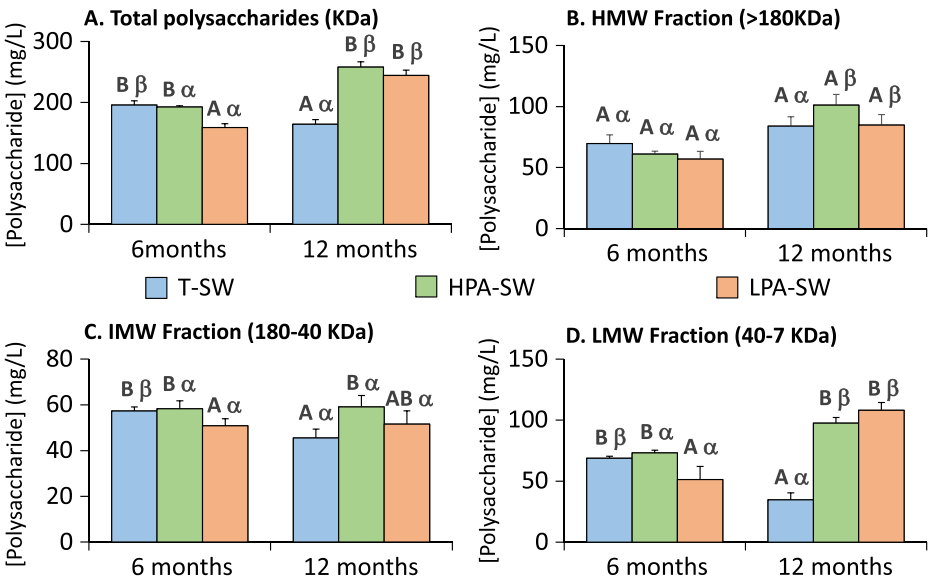

Fig. 3. Polysaccharide composition
Results are expressed as mean ± SD of three replicates. Concentration of polysaccharides is expressed as pectin and dextran equivalents. T-SW: Traditional sparkling wine; HPA-SW: High population ancestral sparkling wine; LPA-SW: Low population ancestral sparkling wine. HMW: High Molecular weigh Fraction; IMW: Intermediate Molecular Weight Fraction; LMW: Low Molecular Weight Fraction. Different letters indicate the existence of a statistical difference ($p < 0.05$). Capital letters indicates the influence the elaboration method. Greek letters indicates the influence of ageing time.

lower the polysaccharide release during the yeast autolysis. However, this difference is no longer significant after twelve months of ageing.

In the T-SW, an increase in the total polysaccharide concentration with the time of ageing would also be expected; however, the opposite actually happens. A possible explanation could be its higher ethanol content. It is well known that the solubility of polysaccharides decreases when the ethanol concentration increases (Bouchard et al., 2007). Therefore, it is possible to consider that a greater proportion of the polysaccharides released by yeast autolysis would have precipitated due to the higher alcoholic strength of this sparkling wine.

3.5. Mannose concentration after polysaccharide hydrolysis

T-SW and HPA-SW showed similar mannose concentration levels after polysaccharide hydrolysis (Fig. 4) at six and twelve months of ageing. In contrast, these levels in the LPA-SW were significantly lower than in the other two sparkling wines at both ageing times. These data confirm that the size of the yeast population exerts a significant effect on the release of mannoproteins from yeast autolysis as it was suggested in the polysaccharide data. It should be taken into account that mannoproteins are constituted by high percentages of mannose (Ribéreau-Gayon et al., 2006) and are therefore also quantified as polysaccharides (Ayestarán et al., 2004).

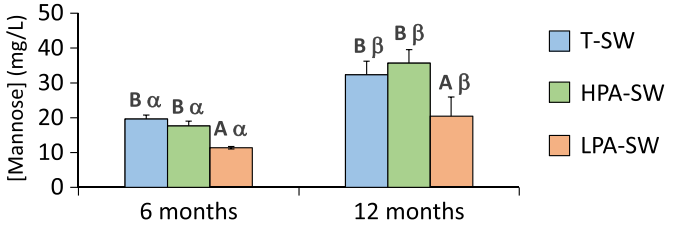

Fig. 4. Mannose concentration after polysaccharide hydrolysis
Results are expressed as mean ± SD of three replicates. T-SW: Traditional sparkling wine; HPA-SW: High population ancestral sparkling wine; LPA-SW: Low population ancestral sparkling wine. Different letters indicate the existence of a statistical difference ($p < 0.05$). Capital letters indicates the influence the elaboration method. Greek letters indicates the influence of ageing time.

The mannose concentration after polysaccharide hydrolysis significantly increased between six and twelve months in all the sparkling wines, which confirms that mannoproteins are released during ageing. Other authors have reported that the mannose/glucose ratio in the polysaccharide fraction increased with the ageing time (Alexandre and Guilloux-Benatier, 2006; Martínez-Lapuente et al., 2013).

3.6. Foaming properties

Fig. 5 shows the foaming properties of the different sparkling wines. The foamability (HM) of the two A-SW was significantly higher than that of T-SW at six months of ageing. This higher HM was maintained at twelve months of ageing in HPA-SW whereas T-SW and LPA-SW showed similar levels. The release of proteins, mannoprotein and polysaccharides due to yeast autolysis during the ageing time is very important for the quality of the foam since these compounds act as surfactant agents that improve the foam properties (Alexandre and Guilloux-Benatier, 2006; Kemp et al., 2019; Martínez-Lapuente et al., 2015; Medina-Trujillo et al., 2017). Therefore, it can seem strange that the T-SW has lower HM values than the two A-SW at six months of ageing although it has similar mannose concentration levels after polysaccharide hydrolysis than HPA-SW and higher than LPA-SW. The explanation in this case is very simple and is associated to the higher ethanol content of T-SW. It is well known that ethanol exerts a negative effect on the foamability of sparkling wines (Dussaud et al., 1994; Medina-Trujillo et al., 2017).

No significant differences were found in foam stability (HS) between the three sparkling wines after six or twelve months of aging. However, it was observed that both HM and HS, increased significantly between six and twelve months of ageing for the three sparkling wines, indicating that the foam properties improve with the ageing time for the three sparkling wines, which indicates that the foam properties improve with the ageing time. Previous studies have reported similar results for traditional sparkling wines (Cilindre et al., 2010; Pérez-Magariño et al., 2015).

3.7. Colloidal properties

To our knowledge, there is only few information about the colloidal

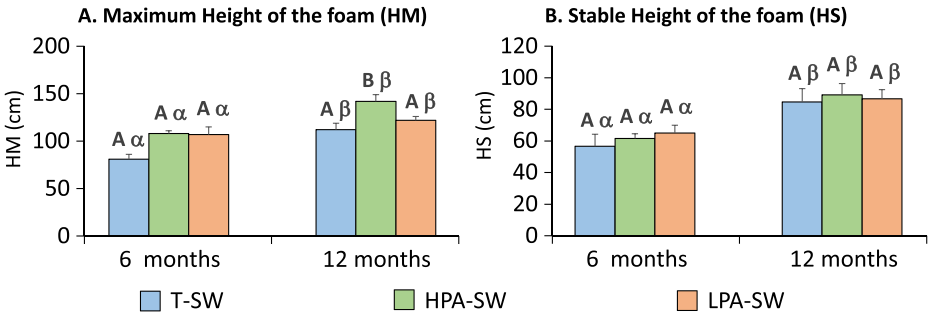

Fig. 5. Foam properties
Results are expressed as mean ± SD of three replicates. T-SW: Traditional sparkling wine; HPA-SW: High population ancestral sparkling wine; LPA-SW: Low population ancestral sparkling wine. HM: maximum height of the foam; HS: stable height of the foam. Different letters indicate the existence of a statistical difference ($p < 0.05$). Capital letters indicates the influence the elaboration method. Greek letters indicates the influence of ageing time.

composition of sparkling wines (Senée et al., 1998; Senée, Robillard & Vignes-Adler, 2001). Fig. 6 shows the colloidal properties of the different sparkling wines. The results indicate that after six months all wines had colloids approaching 250 nm in size, values that are in line with those reported, using the same method (NTA), in other wine types (Kassara et al., 2019). T-SW contained the largest colloidal particles after six and, even more, after twelve months, even though the average size of all colloids decreased during ageing, a decrease that was significant for both ancestral wines.

Interestingly, the analysis of the number of colloidal particles during ageing (Fig. 6B) reveals that T-SW contained significantly more colloids than the two ancestral wines at both six and twelve months. Altogether, data of Fig. 6 seem to indicate that the T-SW had more and bigger colloids than the two ancestral wines. Given that colloids are made of wine macromolecules, mostly protein and polysaccharides in white wines, one would expect T-SW to be both richer in these macromolecules and, thanks to their recognised foam-promoting effects, have better foamability parameters. However, this was not the case. In fact, T-SW contained similar protein content (see Fig. 2A), the same or less total polysaccharides (see Fig. 3A), and the same or worse HM and HS parameters (see Fig. 5) than the two ancestral wines. A potential explanation for the apparent discrepancies between the here presented findings and literature knowledge about the foam-promoting factors could lie on the differences in turbidity of the wines. Since ancestral sparkling wines have not been finned as T-SW was, they must contain a higher level of insoluble particles. This greater presence of non-soluble particles could be responsible for greater absorption of colloid-forming molecules (proteins and polysaccharides), with a consequent lower number of colloidal particles present in these wines (Fig. 6B). Despite being smaller and in present in lower number, the colloidal particles present in the ancestral wines were sufficient to produced comparable or better HM and HS than T-SW. Another explanation on the differences found could lie on the differences on the ethanol content since other parameters like pH or ageing temperature were the same for all the wines (Senée et al., 2001; Dufrechou et al., 2012).

3.8. Sensory analysis

Fig. 7 shows a spider web chart to illustrate the sensory analysis

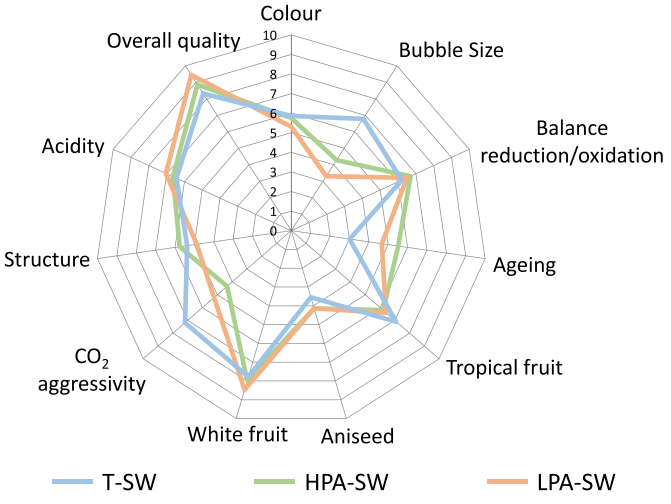

Fig. 7. Sensory analysis of sparkling wines at 12 months of ageing
Results are expressed as mean of 15 tasters. T-SW: Traditional sparkling wine; HPA-SW: High population ancestral sparkling wine; LPA-SW: Low population ancestral sparkling wine. The presence of asterisk indicates the existence of significant differences ($p < 0.05$).

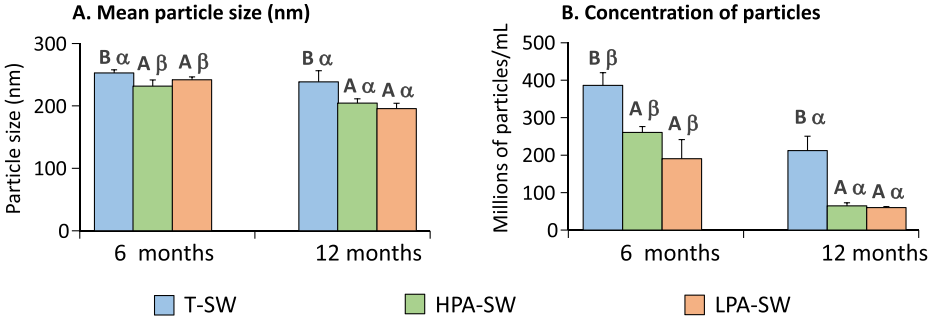

Fig. 6. Colloidal properties
Results are expressed as mean ± SD of three replicates. T-SW: Traditional sparkling wine; HPA-SW: High population ancestral sparkling wine; LPA-SW: Low population ancestral sparkling wine. Different letters indicate the existence of a statistical difference ($p < 0.05$). Capital letters indicates the influence the elaboration method. Greek letters indicates the influence of ageing time.

results for the sparkling wines at twelve months of ageing. No large differences were detected in colour, balance reduction/oxidation, tropical fruit, white fruit, structure or acidity between the different sparkling wines. In contrast, the panel found that the bubble size and the CO_2 aggressivity of the T-SW were higher than in the two A-SW. A possible explanation for the lower bubble size and CO2 aggressivity of ancestral sparkling wines would be their lower internal pressure. The panel also considered that the T-SW less evolved than the two A-SW. This perception could be associated to the higher initial yeast population of both ancestral sparkling wines that would make the effects of autolysis more visible. The differences between both A-SW were small, but the bubble size of LPA-SW was slightly smaller and the CO_2 aggressivity slightly higher than in HPA-SW. Finally, according to the overall quality, the panel ranked the sparkling wines from best to worst in the following order: LPA-SW, HPA-SW and T-SP.

4. Conclusions

Regardless of the drawbacks and advantages of each one of these elaboration methods, our results show that the ancestral sparkling wines have lower ethanol content and can be elaborated using lower sulphur dioxide levels than traditional sparkling wines. In addition, the two ancestral sparkling wines showed in general similar protein concentrations and higher polysaccharide concentrations than the traditional sparkling wine. The mannoprotein concentration of HPA-SW, measured as the percentage of mannose after polysaccharide hydrolysis, was similar than that of T-SW. In contrast, this value was significantly lower in the LPA-SW, which indicates that the size of the yeast population exerts an effect on the release of mannoproteins from yeast autolysis. In general, A-SW showed similar or better foamability (HM) than T-SW, whereas no differences were found in foam stability (HS). Finally, a trained panel found that the A-SW had a smaller bubble size, lower CO_2 aggressivity, seemed to have a longer ageing time and were better scored than T-SW. This study therefore confirms the interest of ancestral method for elaborating high-quality sparkling wine. The panel also considered that the overall quality of LPA-SW was higher than that of HPA-SW, which confirms that it is necessary to reduce the yeast population before bottling. Further studies are needed, especially with longer ageing times, to increase our knowledge on ancestral sparkling wines and how their elaboration procedure can be improved.

Notes

The authors declare no competing financial interest.

CRediT authorship contribution statement

Arnau Just-Borràs: Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Ekaterina Moroz:** Formal analysis, Software. **Pol Giménez:** Formal analysis, Methodology. **Jordi Gombau:** Formal analysis, Methodology. **Elisa Ribé:** Conceptualization, Resources. **Angels Collado:** Conceptualization, Resources. **Pedro Cabanillas:** Visualization, Supervision. **Matteo Marangon:** Data curation, Supervision, Writing – original draft. **Francesca Fort:** Conceptualization, Supervision. **Joan M. Canals:** Conceptualization, Supervision, Software, Project administration, Funding acquisition. **Fernando Zamora:** Conceptualization, Supervision, Project administration, Funding acquisition, Validation, Data curation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare no competing financial interest.

Data availability

Data will be made available on request.

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