ANCESTRAL SPARKLING WINES; COMPARISON WITH TRADITIONAL SPARKLING WINES AND PROCEDURES TO IMPROVE THEIR QUALITY

Arnau Just Borràs

UNIVERSITAT ROVIRA i VIRGILI

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Ancestral sparkling wines; Comparison with traditional sparkling wines and procedures to improve their quality

Doctoral Thesis

Directed by: Dr. Fernando Zamora Marín and Dr. Joan Miquel Canals Bosch

Department of Biochemistry and Biotechnology

Research group on OENOLOGICAL TECHNOLOGY (TECNENOL)

TARRAGONA, 2024

UNIVERSITAT ROVIRA i VIRGILI

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Fernando Zamora Marín i Joan Miquel Canals Bosch professors titulars del Departament de Bioquímica i Biotecnologia de la Facultat d'Enologia de la Universitat Rovira I Virgili.

FAN CONSTAR que aquest treball titulat "**Ancestral sparkling wines; Comparison with traditional sparkling wines and procedures to improve their quality"** que presenta **Arnau Just Borràs** per l'obtenció del títol de Doctor, ha estat realitzat sota la nostra direcció al Departament de Bioquímica I Biotecnologia de la Universitat Rovira I Virgili.

Fernando Zamora Marín and Joan Miquel Canals Bosch, full professors of the Department of Biochemistry and Biotechnology at the Faculty of Oenology of the Rovira i Virgili University.

HEREBY CERTIFY that this work entitled "**Ancestral sparkling wines; Comparison with traditional sparkling wines and procedures to improve their quality**" presented by **Arnau Just Borràs** for the obtaining of the Doctoral degree, has been carried out under our supervision at the Department of Biochemistry and Biotechnology of the Rovira i Virgili University.

Tarragona 31st April 2024

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Oh, que cansat estic de la meva
covarda, vella, tan salvatge terra,
i com m'agradaria d'allunyar-me'n,
nord enllà,
on diuen que la gent és neta
i noble, culta, rica, lliure,
desvetllada i feliç!
Aleshores, a la congregació, els germans dirien
desaprovant: "Com l'ocell que deixa el niu,
així l'home que se'n va del seu indret",
mentre jo, ja ben lluny, em riuria
de la llei i de l'antiga saviesa
d'aquest meu àrid poble.
Però no he de seguir mai el meu somni
i em quedaré aquí fins a la mort.
Car sóc també molt covard i salvatge
i estimo a més amb un
desesperat dolor
aquesta meva pobra,
bruta, trista, dissortada pàtria.

-Salvador Espriu- Assaig de càntic en el temple

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I'm forever blowing bubbles,
Pretty bubbles in the air,
They fly so high, nearly reach the sky,
Then like my dreams they fade and die.
Fortune's always hiding,
I've looked everywhere,
I'm forever blowing bubbles,
Pretty bubbles in the air.
- Jaan Kenbrovin

A tots els que, com les bombolles,
han marxat massa aviat.

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"*Tots volem ser com ells, són herois del nostre temps-Pilseners*"

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"Vivat academia, vivant professores! vivat membrum quodlibet, vivant membra quaelibet, semper sint in flore!-himne URV"

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"No estava gens clar si un dia arribaria fins a Montego Bay. El que ningú es pensava és que acabaria anant a Roma via Budapest - Skatalà."

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Resume

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The ancestral method is a technique to produce sparkling wines. Its origin seems to be associated with benedictine monks that discovered this procedure at the XVI century in Languedoc French region. It consists of bottling the fermenting must before the complete consumption of sugars. This way, the wine ends its fermentation inside the bottle provoking a CO_2 accumulation. This overpressure inside the bottle results in sparklingness once the bottle is opened. This method was almost abandoned for winemakers and substituted by traditional method (or champenoise) because it is an easier and more controllable process.

Nowadays, more and more sparkling wine producers have recovered this old way to produce sparkling wines. The reason of this reborn is the research to produce diverse wines to satisfy new consumer demands and to attract potential new consumers. It must be highlighted that there is not a well-defined procedure to follow correctly ancestral method, which causes that every producer has interpreted this method on its way.

This lack of a precise procedure originates a great heterogeneity in the existing commercial products. This heterogeneity is not necessarily a bad characteristic since originates a wider product gamma. However, sometimes it could originate faulty wines due to bad oenological praxis at the winery. For that reason, it is needed to suggest certain guidelines to winemakers to improve the quality offered in these products.

It should also be highlighted that currently global warming is drastically influencing the grape maturity process which is affecting the wine composition and quality. The main problems that are facing sparkling wine producers are the increase in the sugar concentration and the loss of acidity caused by the accelerated ripening of the grapes. It is obvious that none of these consequences are good for the quality of sparkling wines since it requires the advance of the harvest which could originate not well-balanced grapes.

In this context, ancestral sparkling wines could have some advantages in front of traditional method. Furthermore, ancestral method has some advantages such as the possibility to work with riper grapes since no sugar is added for a second fermentation, or the opportunity to work with lower levels of sulphur dioxide since it does not require a stabilization step before bottling. Nevertheless, the ancestral method has some critical points that when are not well controlled can result into faulty wines.

An assessment of commercial ancestral sparkling wines was set to determine the main chemical (acidic composition, polysaccharides, proteins, and volatile compounds concentration), physical (foaming properties and colour) and sensorial characteristics that define these products. Results showed a greater heterogeneity in ancestral than in traditional sparkling wines. It was also found that some faults such as high turbidity, scarce sparklingness and odd aromas were sometimes present on the ancestral commercial wines.

An improvement of the methodology was studied to avoid these common errors. For this purpose, the same grape juice was vinified in three different ways: i) ancestral method with low yeast population in bottling; ii) ancestral method with high yeast population in bottling; iii) traditional method. Foam characterisation, colloidal bodies determination, macromolecular composition (polysaccharides, proteins, and mannose) and sensorial analysis were determined. The results showed that most of the usual faults detected in some ancestral sparkling wines can be prevented by controlling yeast population during bottling, adding riddling agents and selecting the adequate bottling time.

It was also investigated the use of cationic exchange as a helpful tool for wineries to fight against higher pH and low acidities of grape musts that climate change is bringing with it. Cationic exchange resins showed up as a good technique to reduce

pH and increase the grape must acidity with no other physicochemical or sensorial impacts on the wine.

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Introduction

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1. The grape vine

Vitis vinifera is a flowering plant from the Genus *Vitis* that it is included in the *Vitaceae* family. The vine plant was domesticated 6000-8000 years ago in the Transcaucasia region. It is a deciduous plant that produces accessory fruits full of berries or grapes from which the wine is obtained (De Lorenzis, Mercati, et al., 2019; McGovern, Jalabadze, et al., 2017).

Grape culture occupies 7.3 million hectares around the world, and around 258 million hectolitres of wine are produced per year (2022 data). The wine market has progressively increased since the 2000s, and its worldwide value is estimated to be about 37.6 billion EUR. The wine market can be divided according to the product type, and thus bottled still wine represents 53% of traded volumes whereas sparkling wines represent only 11%. Other categories of wine, such as bag-in-box, bulk, vermouth, and distillates, represent lower percentages of traded volumes (OIV, 2023b).

Most of the vine crops and wine production are in Europe, more specifically in the Mediterranean basin, and Italy, France, and Spain are the three countries with the highest wine production in the world. Therefore, the viticulture and oenology sector is very valuable for the economy of some European regions (Champagne, Douro Valley, Bordeaux, Valpolicella, Rioja, and Priorat, among others) and all over the world (OIV, 2023b; Wine & Spirit Education Trust, 2016).

Due to the importance of the wine market and its economic value, climate change is becoming a great concern in the sector because it could lead to massive economic losses (Bernetti, Menghini, et al., 2012).

Certain adaptative oenology techniques have been proposed with the aim of mitigating the effects of global warming, which is already affecting the winemaking sector. The most important ones will be introduced later in this literature review.

1.1. The grape berry

From when the flower of the vine is fertilized until the grapes are picked, the different parts of the grape bunch (**Figure 1**) will develop and undergo several changes.

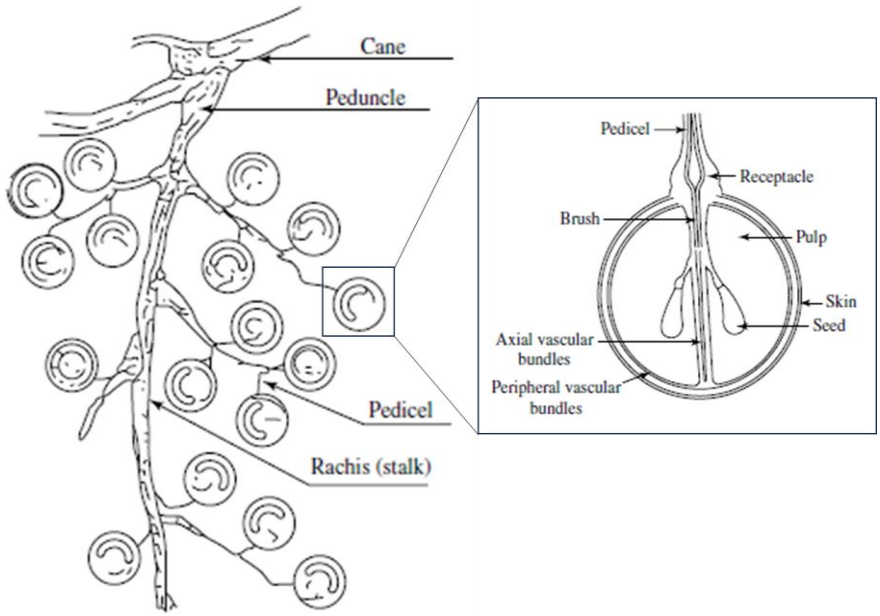

Figure 1: Parts of a grape bunch, from Ribéreau-Gayon, Dubourdieu, et al. (2006).

-Stalk: this is the woody part that connects the berries to the bunch. It is only 3-7% of the total weight of the bunch. It contains many phenolic compounds, so its presence during winemaking can cause greater astringency and bitterness. The part that touches the berry is known as the pedicel and it determines what the bunch looks like depending on its length and thickness (Reyneir, 2012).

-Seeds: these make up between 0 and 6% of the berry weight. Seeds play a very important role in red wine production because they can release between 20 and 50% of the polyphenols in the final wine. Before *véraison* is completed, seeds are at their largest size. After *véraison* the seeds "ripen", which means their tannin concentration decreases but their polymerization degree increases. Seeds bring nitrogen to the grape juice, which is important for the metabolism of the yeast (Ribéreau-Gayon, Dubourdieu, et al., 2006).

-Skins: grape skins are a set of different heterogenous layers that can vary in thickness and organization between varieties. The skin makes up from 8 to 20% of the fresh weight of the berry. Citric acid is a predominant substrate in the skin because other acids like L-malic acid are degraded during the ripening or because, like L-(+)-tartaric acid, they can be esterified with phenolic acids. Other secondary products like phenolic compounds or aromatic precursors accumulate in the skin during the maturation process (Lecas & Brillouet, 1994). The different parts of the skin are explained below.

- The Cuticle is the most external skin layer (**Figure 2**) composed by one layer of cells covered with hydrophobic waxes like pruin and hydroxylated fatty acids that protect the grape against adverse climatic factors and the attack of fungal diseases (Martínez-Lapuente, Guadalupe, et al., 2019).

- The Epidermis and hypodermis (**Figure 2**) are the internal parts of the skin where most of the colour, aroma, and flavour constituents are found. The epidermis is formed by tangentially elongated cells that can vary in thickness in the different grape varieties. The hypodermis is formed by cells that are bigger the closer they are to the seeds. The hypodermis external cells have thick walls in contrast to the more internal ones that have thin membranes (Hellman, 2003).

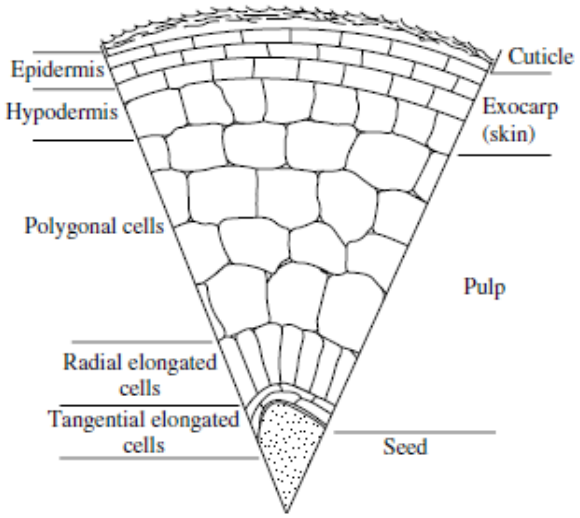

Figure 2: Grape parts, from Ribéreau-Gayon, Dubourdieu, et al. (2006).

- The pulp is the largest part of the berry. It is constituted mainly by thin-membrane cells that contain the grape must in which many substances are dissolved. The most important substances dissolved in the must are D-glucose and D-fructose, at a ratio of almost 50/50 when grapes are mature. The total D-glucose + D-fructose concentration can go from 150 to 240 g/L (or even more) in mature grapes. Other sugars are also present but at very low concentrations. In addition, cations are important in the grape pulp, specifically potassium which it is the most present and plays an important role in sugar accumulation and enzyme activation, turgor control and cell expansion through osmotic potential regulation, the regulation of cytosolic pH and the control of long phloem transport (Coetzee, Walker, et al., 2017).

1.2. Grape maturation

Maturation is a transformation process in which the grapes develop until reaching the characteristics necessary for winemaking. However, the natural objective of the

vine is not to satisfy the wine producer, but rather to generate mature seeds that can germinate. Therefore, some substances are accumulated in the grape to protect and feed the germ in the first stages of germination. This divergence between the aims of nature and the winegrower has led to the different kinds of maturity being described (van Leeuwen, Barbe, et al., 2022).

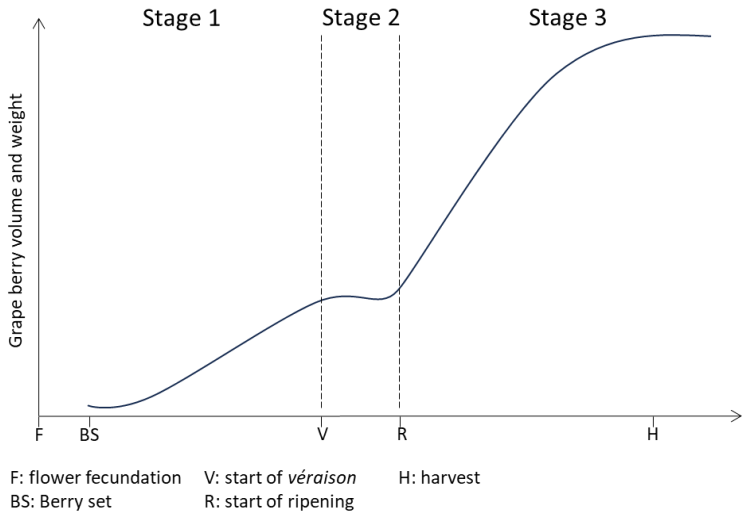

Figure 3: Growth stages of the grape berry.

The grape cycle starts when flowers are fertilized. After two weeks of ovule fertilization the grape starts to develop by cellular division. The pericarp parts (exocarp, mesocarp and endocarp) and the seed start to differentiate. The development of the grapes and all berries has always been described according to three stages (**Figure 3**) (Jackson & Lombard, 1993).

1. Rapid herbaceous growth: this lasts around two months (45-65 days) in which grapes gain volume and weight. During this period cellular division and grape growth are under the control of auxins, cytokinins and gibberellins, which act as cellular growth and division promotion hormones (Conde, Silva, et al., 2007). During this phase grape berries have a high respiratory rate and carbon fixation occurs through the accumulation of L-

(-)malic acid as an energy source in the fleshy cells. In addition, large concentrations of L-(+) tartaric acid accumulate in the peripheral cells during this period, which remain more or less constant until harvest (Possner & Kliewer, 1985).

During herbaceous growth the grapes are not able to accumulate a lot of sugars because they use them for their homeostasis and seed growth (Possner & Kliewer, 1985).

2. *Véraison:* This phase can last around 8 to 15 days. The berries do not gain a large amount of weight or volume in this period. It is a short phase in which the skins lose their chlorophyll and acquire colour by accumulating anthocyanins and/or flavonols (Lecas & Brillouet, 1994). It can be considered the beginning of the ripening process and involves a huge amount of physicochemical and biochemical reactions inside the berry and the plant, which are conditioned by climate conditions.

   Two main "hormonal" changes in the phytohormones synthetized by the plant occur during this period: growth phytohormones decrease and abscisic acid, ethylene, and the stress phytohormone increase (Conde et al., 2007).

   The L-malic acid accumulated now will be used as an energy source because the glycolysis pathway is stopped, and as a substrate for gluconeogenesis, which will lead to sugar accumulation (Possner & Kliewer, 1985; Ribéreau-Gayon, Dubourdieu, et al., 2006).

   If ammonia is the nitrogen compound most present in the grapes during the herbaceous growth (more than 80% of total nitrogen), at this point it will be progressively substituted by amino acids. L-arginine and L-proline are generally the predominant ones (Blouin & Cruège, 2003).

After *véraison* it is considered that seeds would be able to generate new vines if they were planted. This point is usually known as **physiological maturity**.

3. Maturation: During this period of 35 to 55 days the grape will double in size because cellular growth resumes. The respiration rate decreases, and enzymatic reactions will start a lot of physicochemical changes. The ammonium and L-malic acid concentrations will decrease substantially.

   On the other hand, the sugars, abscisic acid, and potassium cation concentration will increase. Potassium plays an important role in the translocation of synthesized sugars inside cell vacuoles. Other cations (like calcium) will accumulate in the skin cells or (like magnesium) in the pulp cells (Possner & Kliewer, 1985). The decrease in acidic concentration is inversely related to the accumulation of sugars and this correlation will determine the best time to harvest the grapes when sugars reach the perfect concentration, and the acidic composition of the berries is still appropriate. This is known as the **Technological maturity.**

   It is also important to note that aromatic and phenolic compounds accumulate during this phase due to the activation of secondary anabolic pathways. This accumulation is very important because the concentration of phenolic substances will determine which product the oenologist is able to work with. When phenolic substances reach their peak of accumulation, oenologists talk about **phenolic and aromatic maturity** (van Leeuwen et al., 2022).

2. Climate change and vitiviniculture

Maturation dynamics are affected by three main physical variables: temperature, light or radiation, and available water (Jones, 2022; Spayd, Tarara, et al., 2002). These three factors on a world scale reach the combined optimum point in certain climatic zones known as the viticulture or vine belt. The vine belt contains 95% of the territories where vine is cultivated. It corresponds to a latitude zone between 50°N-40°N in the northern hemisphere and 30°S-40°S in the southern hemisphere (E. Müller, 2019; Tischelmayer, 2021).

Obviously, these factors will depend on some other permanent factors, such as grape varieties, rootstocks, kind of soil, vine conduction system, plantation density and climate or microclimate conditions.

i) Temperature affects the entire vine cycle and is considered the most influential factor affecting vine productivity (Jones, 2022). The optimum temperatures for vegetative growth are in a range between 20 and 35°C (Gladstones, 2011; Greer & Weedon, 2012). Authors consider that temperatures over 35-40°C can harm the vine and affect its growth and photosynthetic activity (Köse, 2014; Luo, Ma, et al., 2011). During maturation, temperatures around 20°C with low temperature oscillation between the day and night are optimal for sugar accumulation in grape berries. If the day temperatures are too high, sugars will be concentred in other parts of the plant; however, if temperatures are too low (under 10°C) the vine will not be able to produce grapes (Ribéreau-Gayon, Dubourdieu, et al., 2006).

The “growing degree days” (GDD) is the measure of the days that are above 10°C from 1 April to 31 October. The GDD unit is used to divide the viticulture zones in function of what is called the Winkler Index (Winkler, Cook, et al., 1962). This index considers that the grape vine does not grow if temperatures are below 10°C. If temperatures are over this limit heat, the average day temperature is summed to

the others to obtain a total result. The resultant number determines how optimal a zone is for vine growing. Different growing degree days will lead to different maturation patterns that the authors divided into five categories from least to most GDD, the coldest zones being under 850ºC and the hottest being over 2700ºC. Recently, new and more precise methodologies that include other climatic variables have been proposed (Parker, García de Cortázar-Atauri, et al., 2020).

It is important to note that high temperatures accelerate some metabolic pathways related to maturation, such as sugar accumulation (Parker et al., 2020), and grape development, such as L-malic acid accumulation and degradation. While optimal growing temperatures will lead to optimal accumulation of L-malic acid before *véraison*, temperatures over 30ºC lead to premature L-malic acid degradation, and therefore, premature loss of acidity (Lakso & Kliewer, 1978). Temperatures that are too high or low also lead to a decrease in the accumulation of phenolic compounds because they force the competence between primary metabolism and secondary metabolism and also because high temperatures can provoke oxidation (Cataldo, Eichmeier, et al., 2023; Gutiérrez-Gamboa, Zhang, et al., 2021).

Finally, temperatures that are too high will also lead to low amino acids synthesis in the grape and the degradation of some aromatic precursors before the harvest (Gutiérrez-Gamboa, Carrasco-Quiroz, et al., 2018; Gutiérrez-Gamboa et al., 2021). In contrary, cooler temperatures during the maturation process will enhance the sequential accumulation of aromatic precursors without them being degraded.

ii) Light. In optimal winegrowing zones light does not limit the photosynthetic activity. It is known to influence floral induction and once the grapes have developed, light has a large effect on sugar accumulation and the decrease of acidity. Light also activates phenolic synthesis. Therefore, in zones where the grapes do not receive enough light there may be a deficiency in colour (Dokoozlian

& Kliewer, 1996). Finally, light has also been found to degrade some "herbaceous" aromas like methoxypyrazine (Plank, Hellman, et al., 2019).

iii) Water supply is determinant for every living organism. Water supply is essential for almost every metabolic reaction and grape vines are no exception. Usually, vines are moderately restricted in water supply to obtain the better-quality wines. The annual pluviometry in premium winegrowing zones is usually around 700-800mm; therefore, if this pluviometry is not reached, supportive watering can be applied (Ribéreau-Gayon, Dubourdieu, et al., 2006). Water is most necessary before *véraison*. If there is not enough water at this point, the grapes will be smaller than usual, which will lead to smaller yields, a smaller production and probably economic losses.

On the contrary, if rainfall is excessive, it can lead to the development of fungi diseases and the grapes bursting (Reynolds, 2020). Excessive rainfall or watering after *véraison* can lead to a decrease in the concentration of phenolic and aromatic compounds and a loss in acidity due to the increase in potassium imports (Ribéreau-Gayon, Dubourdieu, et al., 2006).

Historically these factors could vary within a smaller or wider range between vintages; however, nowadays it has been proven that climate change and global warming will lead to new conditions that winegrowers will have to adapt to. Industrialization led to an increase in the levels of carbon dioxide, methane, and nitrous oxide emissions during the 19th, 20th and the beginning of the 21st centuries (IPCC, 2023). This accumulation of carbon dioxide in the atmosphere leads to a greenhouse effect all over the world, which provokes global warming. Currently, although some policies are being applied to reduce carbon dioxide emissions to decrease the worst greenhouse effects, the world is already suffering the effects of the emissions generated several years ago.

Global institutions like the UN have already recognized that Earth's global temperature is increasing by 0.2°C per decade through its Intergovernmental Panel on Climate Change (IPCC, 2018). This global warming process will also lead, among other important impacts, to rainfall becoming concentrated in short but intense periods that will probably lead to flooding periods combined with long-lasting periods of drought.

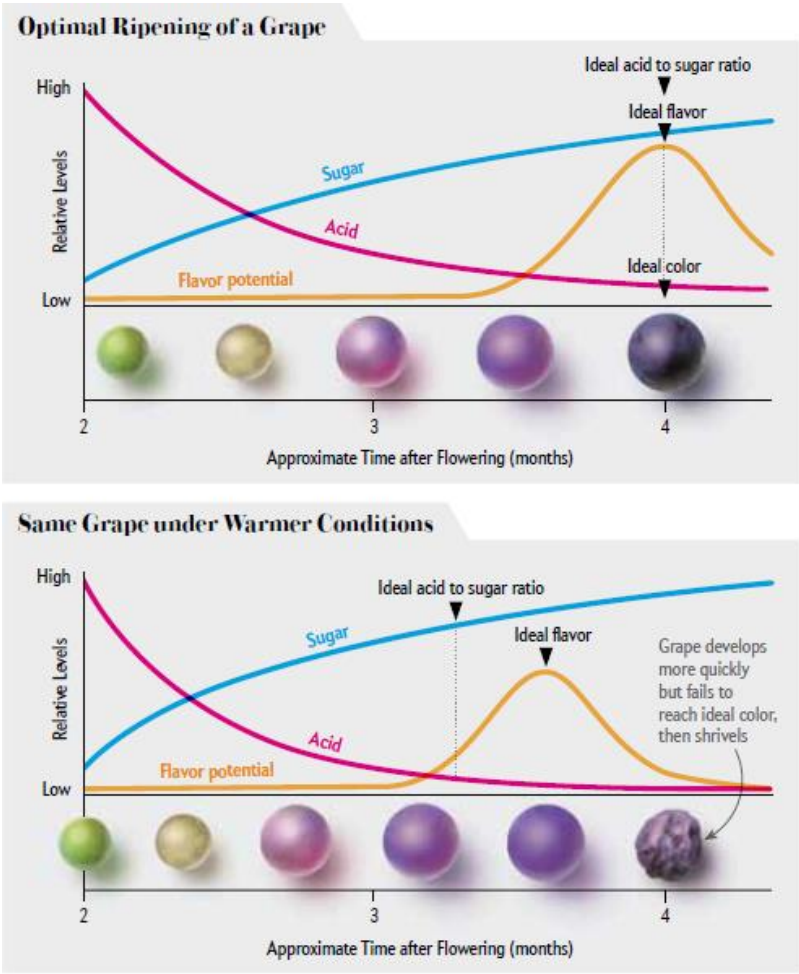

Figure 4: Changes in the grape maturation process due to hotter than usual weather (Nicholas, 2015).

This change in the rules of the game will have a large impact on the viticulture belt. The increase in temperature and the change in distribution of precipitation will mean that the optimal belt for vine growing will move to higher latitudes and altitudes that were previously considered too cold. In contrast, zones that where at the maximum range of heat for viticulture will probably be excluded from this belt (Mosedale, Abernethy, et al., 2016).

Currently, warm to hot winegrowing areas are having heatwaves during the maturation process, which leads to an increase in the ripening rate (OIV, 2023b). This phenomenon has caused the technological maturity to advance and consequently the harvest date has also advanced in the last 10-30 years. The earlier harvest time is related to preserving acidity and avoiding having a sugar concentration that is too high, which would lead to the wine having an alcoholic content that is too high (Gonzalez, Guindal, et al., 2021). Low acidities lead to high pH that can be related to a loss of freshness and health safety of the product (**Figure 4**) (Mira de Orduña, 2010).

Other factors related to climate change include the appearance of new diseases like *Xylella fastidiosa* and the more common attacks of other diseases like *black root*. Warm winters and the change in the pluviometry will lead grapevine diseases to change according to an unknown pattern (Bois, Zito, et al., 2017; Godefroid, Cruaud, et al., 2019).

Mitigating the effects of climate change on wines

Since the effects of climate change have started to be noticeable in wine, several methodologies that aim to mitigate these effects have been proposed. A handful of viticultural and oenological tools have been proposed to try to solve the different problems that climate change is provoking from the first steps of vine growing to the last stages of wine production.

2.1. Viticultural techniques

Viticultural techniques generally work on delaying the start of the vegetative growth or delaying ripening. Most of these techniques would be able to work synergistically if they were combined (Gutiérrez-Gamboa et al., 2021; Van Leeuwen, Destrac-Irvine, et al., 2019).

a) The use of old local varieties better adapted to dry climates like the Mediterranean seems to be a good tool for working against the effects of climate change on viticulture areas that are not so used to droughts (Antolín, Salinas, et al., 2022; Tzortzakis, Chrysargyris, et al., 2020). Other methodologies like using rootstocks adapted to the new conditions (Verslype, Nascimento, et al., 2023), selected late-ripening or drought-resistant clones or developing new varieties via cross breeding have shown optimal results (Riquelme, Martinez-Cutillas, et al., 2018).

b) Some crop cultural measures, such as late winter pruning (Moran, Bastian, et al., 2018), double pruning (Martínez-Moreno, Sanz, et al., 2019), green pruning & shoot trimming and removing leaves to delay the sugar accumulation and the maturation process (Kliewer & Dokoozlian, 2005), have been applied in some winegrowing regions for centuries and now have been discussed as tools that could help the wine industry to fight against climate change. Other cultural measures, like the conduction system, should be revised in areas that could experience a decrease in pluviometry. Areas, such as Mediterranean areas, should be studied further due to their efficiency in semi-arid environments (Santesteban, Miranda, et al., 2017).

c) Mulching is a kind of cover that can be applied in the space between vines. It can be done with living plants, straw, or other non-alive vegetal material, or even stones or plastic (non-organic mulching) (Ross, 2010). The main objective of the cover is to reduce thermal and hydric stress during heatwaves and droughts and

can improve soil healthcare due to the uncompacting properties that plant roots have and because mechanical labour is not necessary. Mulching with organic matter also provides higher carbon fixation in the soils. It also helps control the vigour of the vines because it establishes a beneficial competence between the vine and the other crops in heavy rain seasons (Fraga & Santos, 2018; Holzapfel, Smith, et al., 2016).

There are all kinds of cover crops or mulching with live vegetal material. While some winegrowers advocate for the use of native species, others plant certain types of plants to improve soil performance. Some kinds of legumes have been found to improve the nitrogen composition in soils (Abad, Hermoso De Mendoza, et al., 2021).

While the possible hydric competence of cover crops and the grapevine is a risk that a lot of winegrowers consider before using it, mulching using organic material like straws has shown very good water retention and decreases soil temperature. The use of straw mulches is also a slow technique for adding organic matter to the soil, which always depends on the climate conditions as they can accelerate the disintegration of the straw (Li, Chai, et al., 2022; Myburgh, 2013).

Plastic and other inorganic materials used for munching tend to play a role in cool climate conditions because they are used to generate heat in the soil or reflect light onto the leaves to accelerate budding. Plastic or aluminium also provide higher water retention than organic matter in dry climates but there is the inconveniences of deterioration and cost (Ross, 2010).

d) The use of anti-transpirant cooling chemicals, or physical mechanisms like shading nets. Chemical products like calcium carbonate or kaolin work as sunscreen agents for grapes, and they also reduce transpiration and water loss through stomata (Dinis, Malheiro, et al., 2018). Kaolin has also been found to improve the phenolic

composition in grapes because it protects these molecules from oxidation during ripening under heat stress (Dinis, Bernardo, et al., 2016). Shading nets work as a mechanism for reducing the temperature over the photosynthetic area of the vine, leading to a decrease in thermal stress during heatwaves; however, they have inconveniences related to price, deterioration, and visual impact.

2.2. Oenological techniques for reducing ethanol content

Oenology tools are also being used in wineries to try to mitigate the problems that grape juices and wines can have due to the adverse weather conditions during the maturation process. Most of the oenological tools were developed before climate change began to resolve certain *terroir* inconveniences, such as too low or high acidities or a lack of colour or too much herbaceous astringency.

The oenology strategies applied during the vinification process are in accordance with the wine that the winery is producing. Oenologists can mainly work with two solutions or a combination of these two (Zamora, 2014).

1. Harvest when alcoholic and pH levels are optimal and accept that the concentration of aromatic precursors and phenolic compounds will not yet be the adequate (Barnuud, Zerihun, et al., 2014; Nicholas, 2015).

2. Wait until aromatic and phenolic maturity and accept that the alcoholic content and pH will probably be high (Mira de Orduña, 2010).

The second solution seems to be the most satisfying because it is easier to balance misadjusted pH and ethanol content than the phenolic and aromatic profile. Alcohol levels can be lowered before, during or after fermentations.

2.2.1. Before fermentation

Lowering alcoholic content by adding water is prohibited in Europe; however, it is allowed in other winegrowing countries such as Chile, Argentina, Australia, and New Zealand (Christmann, Vanderlinde, et al., 2021). While some studies have concluded that additions higher than 5% cause organoleptic changes in wines (Ruiz-de-Villa, Urrutia-Becerra, et al., 2023), other studies have shown that adding water could be preferable to green harvest wine (wine from non-ripe grapes) and that additions of up to 16% of water could be beneficial for wine aromas (Schelezki, Antalick, et al., 2020). However, other studies have shown that adding water decreases the quality of the wine (Piccardo, Gombau, et al., 2019).

Reverse osmosis appears to be useful for reducing the alcoholic content by lowering the sugar concentration in grape juice. This technique has some issues because the filtration can lead to the loss of other compounds related to wine complexity like colour and aroma (García-Martín, Perez-Magariño, et al., 2010).

The glucose oxidase enzyme (from *Aspergillus niger)* can also be used to reduce the must sugar content and therefore the final ethanol content of the wine up to 4% v/v (Biyela, du Toit, et al., 2009). However, this technique is incompatible with obtaining sensorially adequate wines because it produces an enormous amount of gluconic acid, which completely unbalances the acidity of the product. In addition, this technique requires extensive oxygenation of the grape must, which necessarily oxidizes the wine (Röcker, Schmitt, et al., 2016).

2.2.2. During fermentation

Alcoholic content can be reduced by using *Saccharomyces* yeast strains or non-*Saccharomyces* during fermentation, as their Crabtree effect is lower than in the usual commercial yeasts. Yeasts with a lower Crabtree effect (preference for fermentation metabolism when the glucose concentration is higher that a certain

threshold even in aerobic conditions) will synthesize higher amounts of other substrates during the fermentation, like glycerol, and will respirate more sugar, which leads to lower alcoholic content in the wine. The use of *Saccharomyces* species with lower fermentation capacity and under aerobic conditions is an option (Gonzalez, Quirós, et al., 2013). Other yeast species from different genera, like *Pichia* (Ciani & Comitini, 2021), *Torulospora* (Canonico, Agarbati, et al., 2015), *Candida* (Di Maio, Genna, et al., 2012), *Hanseniaspora* (Venturin, Boze, et al., 1994), and *Metschnikowia* (Hranilovic, Gambetta, et al., 2020), inoculated in the first steps of fermentation, reduced the ethanol content in the final wines and in most cases added aromatic complexity. In all cases, fermentations were finished by *Saccharomyces cerevisiae* because the other yeasts were unable to finish the fermentation.

Finally, the procedure with which naturally sweet wines are produced can also be an optimal solution to obtain low alcohol sweet wines. The procedure forces the fermentation to stop before it finishes by adding sulphur dioxide (SO_2) or pasteurization (Ribéreau-Gayon, Dubourdieu, et al., 2006).

2.2.3. Post-fermentation

Post fermentation techniques can be divided into evaporative thermal or membrane-based methodologies. Both types are more expensive than the other techniques shown before because they need the use of machinery with high maintenance costs, have high acquisition prices or high temperatures in thermal processes to partially dealcoholize wine. On the other hand, they are also the most evolved techniques, so they are the most used in wineries for partially or totally dealcoholizing wines in big batches (M. Müller, Bellut, et al., 2017; Zamora, 2016).

Evaporative thermal methods like rectification, falling film evaporator or spinning cone columns are usually carried out under vacuum conditions to avoid heating

the wine to high temperatures that could provoke aromatic loss (M. Müller et al., 2017).

There are other methods that do not involve heating to partially dealcoholize wines, and they are generally based on membranes. Membrane-based techniques like nanofiltration (Gonçalves, Ribeiro, et al., 2013), reverse osmosis (Gil, Estévez, et al., 2013), pervaporation (Takács, Vatai, et al., 2007) and dialysis (Calvo, Asensio, et al., 2022) are, in fact, the most used by wineries (Zamora, 2016).

2.3. Oenological techniques for increasing acidity and reducing pH

The chemical way, which involves adding organic acids that are permitted by the OIV (malic, lactic, L-(+)-tartaric and citric), is a very useful tool and is probably the most used procedure. Fumaric acid is also authorized, but currently only for the inhibition of malolactic fermentation in wines (OIV, 2023a). Fumaric acid has the advantage of inhibiting malolactic fermentation and making a double acidification effect: Its own acidification effect and the maintenance of L-malic acid by preventing its degradation by lactic bacteria (Morata, Bañuelos, et al., 2020). However, adding excessive acid can lead to unbalanced wines, unstable tartaric precipitation, second malolactic fermentation if malic acid is used or difficulty in dissolving in the case of fumaric acid. In addition, all these organic acids exert a poor effect on pH reduction (Payan, Gancel, et al., 2023).

Other chemical procedures that can help to improve the wine acidity include using metatartaric acid, carboxymethyl cellulose and mannoproteins. Nevertheless, these techniques cannot mitigate the loss of acidity provoked by the effects of climate change because they only prevent the loss of acidity by the crystallization of tartaric acid salts (Lasanta & Gómez, 2012).

Microbiological acidification of wines can be achieved by using some strains of *Saccharomyces* yeasts or with other yeast species in the grape must. The *Saccharomyces* genera have been found to increase malic acid concentration in wine (Vion, Muro, et al., 2023). Genetically engineered *Saccharomyces* species have been developed to increase other acid concentrations like lactic acid (Dequin, Baptista, et al., 1999) or succinic acid (Ito, Hirasawa, et al., 2014). However, the use of certain strains of *S. cerevisiae* can only produce moderate acidification.

Non-Saccharomyces yeasts, especially *Lachancea thermotolerans,* have been studied due to their capacity to reduce pH levels by synthesizing lactic acid from 1 to 9 g/L (Benito, 2018). However, *L. thermotolerans* has some disadvantages related to a limited fermentation capacity and therefore must be used in co-inoculation or sequential inoculation with *S. cerevisiae*. In addition, *L. thermotolerans* has the drawback of being very sensitive to free sulphur dioxide (Payan et al., 2023).

Finally, different physical acidification techniques have also been developed, but only cation exchange resins and electrodialysis are authorized by the OIV.

2.3.1. Cationic exchange

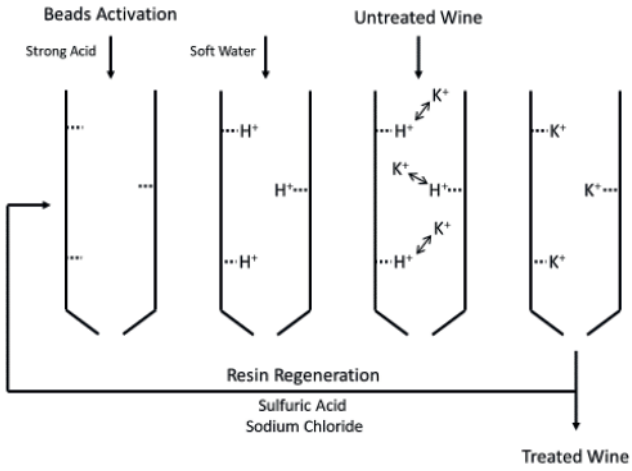

Figure 5: Cationic exchange diagram (Payan et al., 2023).

Cationic exchange was authorized by the OIV in 2012 (OIV, 2012, 2013) and by the European Union in 2012 (European Commission, 2013) as a technique for acidifying the grape must and wine (not more than 54 meq/L) and reducing its pH. With the reduction of the concentration of potassium cations, tartrate precipitation is also limited.

Cationic exchange resins enable the interchange between protons (H^+) and cations, generally potassium (K^+), from the grape juice, which leads to an acidified and sometimes tartarically stabilized grape juice (**Figure 5**). This process is carried out by using strong acids to activate a resin column for attaching the cations present in wine. The wine is passed through the column and the potassium cations from the wine are changed for the protons present in the resin.

Cationic exchange resins are not potassium specific, which also leads to the loss of other cations like calcium, magnesium, and iron (Mira, Leite, et al., 2006). This non-specificity could also lead to future uses of the technique due to the problems of increasing calcium instability related to the pH increase provoked by climate

change (Australian Wine Research Institute, 2023; Ponce, Mirabal-Gallardo, et al., 2018).

While in red wines some authors have confirmed that the cationic exchange is also responsible for a certain loss of phenolic compounds (Ibeas, Correia, et al., 2015; Mira et al., 2006). The loss of phenolic compounds in red wines could be problematic due to the loss of colour that it can provoke; however, on the other hand, a decrease in pH has also been shown to be related to an increase in colour intensity (Walker, Morris, et al., 2002). Ibeas et al. (2015) also showed that, sensorially, red wines treated with cationic exchange were less preferred to control wines due to the loss of quality parameters like body, equilibrium, and intensity.

In white wines the loss of phenolic compounds is not considered to be a possible problem. Furthermore, it is seen as possibly a good way to avoid browning related to the oxidation of the phenolic compounds present in the white wine. Mira et al. (2006) observed a decrease in absorbance at 420 nm in the cationic exchange treated wine, which is positive since the absorbance at 420 nm is related to oxidation in white wines.

Finally, in sparkling wines, some authors have seen a decrease in volatile compounds in highly treated wines (Cisilotto, Rossato, et al., 2019). Further studies related to the impact of cation exchange on the ageing of sparkling wines need to be carried out.

The main disadvantages of this process are the large amount of water used, and the residues generated (Lasanta & Gómez, 2012).

2.3.2. Electrodialysis

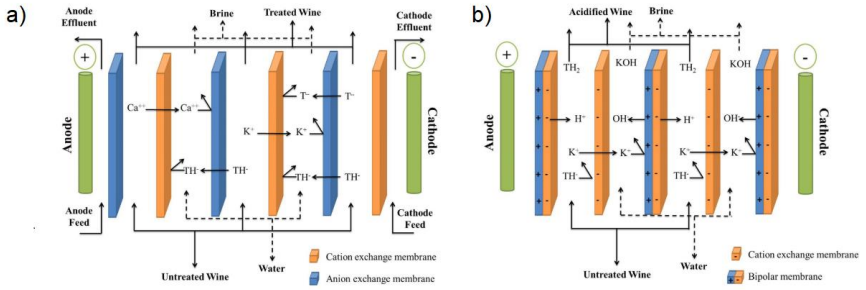

Figure 6: Electrodialysis procedure diagrams: a) membrane disposal by tartaric stabilization; b) membrane disposal by acidification (El Rayess & Mietton-Peuchot, 2016).

Electrodialysis is a similar technique to cation exchange, but in this case the ions dissolved in the wine are moved using an electrical field (**Figure 6**). The polymer-based membranes are positively or negatively charged, and they can be cation-exchange (only permeate cations) or anion-exchange (only permeate anions). This technique is permitted by the OIV for must and wine acidification (OIV, 2010) (**Figure 6 b**) and tartaric stabilization (OIV, 2011) (**Figure 6 a**).

For tartaric stabilization, membranes are arranged forming bipolar cells with a cation exchange membrane on one side and an anion exchange membrane on the other. Cells with wine flowing and with water flowing are intercalated to allow the exchange of the unwanted cations and anions with the aqueous solution (Gonçalves, Fernandes, et al., 2003). This technique also allows other problematic cations like calcium to be eliminated.

For acidification (or deacidification), cation and anion selective membranes are attached forming bipolar membranes, alternated with cationic exchange membranes forming bipolar cells where water circulates, and positively charged cells through which the wine flows (El Rayess & Mietton-Peuchot, 2016; Romanov & Zelentsov, 2007).

There have been few studies on the possible sensory impact of electrodialysis treatments of wines. Moutounet *et al.* (2005) reported that electrodialysis treated wines compared to tartaric added wines were less harsh. Granès *et al.* (2008) also reported that there was no loss of must sugar, alcohol content or phenolic compounds during the treatment. There seems to be a certain lack of consensus on the effect of electrodialysis on the aromatic profile of wines. While Forsyth (2010) reported no aroma loss during the process, other authors found a certain loss of aroma for wines with more than 30% treated grape juice (Gómez Benítez, Palacios Macías, et al., 2003).

Further studies on the electrodialysis of sparkling wines and their ageing should be performed to determine the applicability of this technique in this sector; however, electrodialysis has some large disadvantages that stop it from being widely used and thus make studying it less interesting. These disadvantages include a large water consumption (Halama, Kotala, et al., 2015) and higher cost if it is compared with cationic exchange (Lasanta & Gómez, 2012).

3. A brief history of sparkling wines

Wine production has accompanied humanity for a long time. Archaeological excavations carried out in the Kvemo Kartli province in South Georgia, revealed the presence of grape seeds in clay vessels, called *qvevri*, dating back to the sixth millennium BC (Glonti, 2010; McGovern et al., 2017).

However, sparkling wine production seems to be somewhat more recent and probably related to refermentations of the wine once it had been closed in a recipient. Virgil in the Aeneid quotes "*Spumantem plateram et pleno se proluit auro"* (poured out a foaming plate and full of gold), referring to the effervescence of these wines when they are served.

Sometime later, at 1530, monks from the Benedictine monastery at *Saint Hilaire* (Languedoc) bottled and cork capped wine that, due to the cold winter temperatures, seemed to have finished alcoholic fermentation (Borrull Riera, 2016). This happened because the variety cultivated in the Limoux area was generally *Mauzac*, a late ripening variety that was harvested in late autumn. Furthermore, although it is in a Mediterranean climate zone the Limoux area is a lot higher in altitude, so the temperatures at the end of autumn were really cold (Robinson & Harding, 2015). The warmer temperatures of the following year's spring caused the yeasts to become active again inside the bottle and enabled them to consume residual sugars inside the bottle. This process generated CO_2, which accumulated inside the bottle as it could not escape because of the cork cap (Revel, 2007). In our modern era this method has been called the ancestral method.

In parallel, in the Champagne region, monks had the same problem due to the continental climate in that region, which is characterized by long cold winters and soft springs. Bottles tended to explode because the glass of that time was not strong enough to withstand the overpressure (Buxaderas & López-Tamames, 2012). In addition, bottles used to be capped with a kind of porous material that allowed the CO_2 to escape from the bottle, so it was not until cork started to be used that the fizzy wines started to be important in these winegrowing regions with cold climates.

The fizziness and other common problems in vitiviniculture, seen as a problem by wine producers, led the Hautvillers monk **Pierre Pérignon** (1638-1715) (**Figure 7**) to suggest some viticultural and oenological practices in a book entitled *"The art of tending vineyards and the wines of Champagne"* published three years after his death. Based on this book and other works on understanding the vine life cycle and improving wine production, Dom Pérignon is now considered to be the father of

modern vitiviniculture. Some authors attribute the cork cap invention to Dom Perignon, and therefore he is also considered the father of Champagne and sparkling wines.

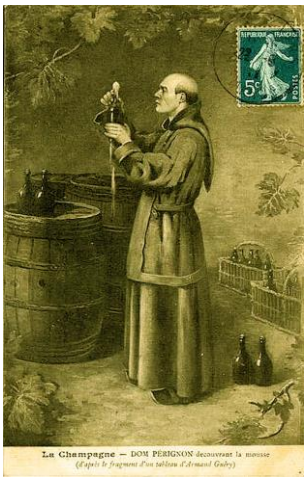

Figure 7: Dom Perignon monk

The improvements in manufacturing glass bottles during the first half of the 17th century developed by the Englishmen Sir Kenelm Digby and Sir Robert Mansell allowed wine makers to bottle wine without the fear of it exploding during refermentation and without any drop in pressure (Stephen-Skelton, 2021). These improvements lead to a popularity rise of this wine among the English aristocracy. They introduced the wine to the other European aristocracy, and it began to be seen as something pleasing rather than a mistake (Liger-Belair & Rochard, 2008).

The demand for sparkling wine in England and the interest in controlling the *prisse de mousse* process increased enough that in 1662, the doctor and naturalist Christopher Merret was the first to add sugar to a bottle to make a second fermentation as it is done in the traditional method. From then on this method was adapted, domesticated, and optimized over the years to become what we know as the classic or traditional method (Liger-Belair & Rochard, 2008).

Later technological updates such as the discovery of riddling agents (19th century by Madame Clicquot), the invention of the *Martinotti-Charmat method* and further acknowledgements of the yeasts metabolizing sugar, led the sparkling wine industry to become a highly mechanized sector and also highly understood (Buxaderas & López-Tamames, 2012).

4. Sparkling wines

Sparkling wines, as defined by the OIV-Code of Oenological Practices and the European Regulation 491/2009, are a group of special wines characterized by the production of effervescence once uncorked. The resultant effervescence must come from the release of endogenous carbon dioxide. Bottles of sparkling wine must have an excess pressure of at least 3 bar at 20^{o}C (European council, 2009; International organisation of vine and wine, 2021).

Other wines, in which carbon dioxide comes from an exogenous source, are classified by the European regulation as aerated sparkling wines or gasified wines and are considered to be low- and poor-quality wines and therefore they are not discussed in this introduction.

Sparkling wines, in the European regulations can be designated as “quality sparkling wines” if the internal pressure is greater than 3.5 bar at 20^{o}C; “quality aromatic sparkling wines” if they are derived from specific grape varieties; or “semi-sparkling wines” if the internal pressure is between 1 and 2.5 bar at 20^{o}C.

The endogenous carbon dioxide in natural sparkling wines comes from the alcoholic fermentation totally or partially produced in bottles or sealed tanks. The type of tank, the number of fermentations, the ageing time, and the presence or not of lees will determine the final product. There are a few different oenological practices or procedures that can be carried out to obtain sparkling wines. In

winegrowing regions usually under Geographical Indications or Controlled Appellations of Origin, products must be obtained according to rules established by the region, for example in the cases of Cava, Champagne, Prosecco, Franciacorta, Sekt, Clairette de Die, Asti and Colfondo. **Figure 8** illustrates the main sparkling wine production methods.

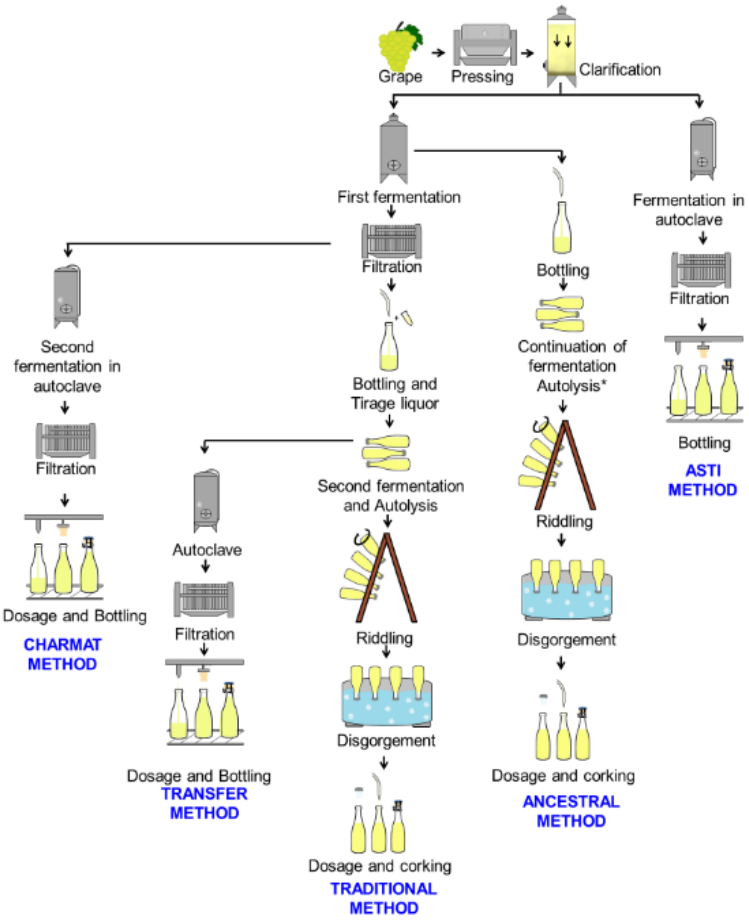

Figure 8: Main sparkling wine production methods (Welke, Dachery, et al., 2022).

4.1. Traditional method

The most well-known and top-quality sparkling wines, like Champagne, Cava and Franciacorta, are obtained following the traditional method or *méthode champenoise* for Champagne wines only. This method has two main steps. The first step is known as the first fermentation and involves the total consumption of sugars in an alcoholic fermentation of a grape juice to obtain a base wine characterized by high acidity and a low degree of alcohol (not more than 10.5-11.0% ethanol). These requirements can be problematic in warm winegrowing regions, such as in the Cava area, where the harvest starts before the grapes are "mature". This problem is not found in cooler climates like Champagne, which can have the opposite problem in which optimum sugar concentrations are not achieved.

The second main step is the second fermentation. Base wines are blended if necessary, then sucrose (around 22 g/L) and adapted yeasts are added to the base wine. The mix is sealed and capped (usually with a crown cap). The second fermentation is complete after several months, depending on the preservation conditions, in which time all the added sugar will have transformed into ethanol and CO_2. The bottle is placed lying horizontally until the disgorgement. The bottle position is fundamental for enhancing the contact between the lees and the wine so that ageing occurs.

Once the ageing is complete the lees are removed in the disgorging process in which the lost liquid is replaced with *liqueur d'expedition*. The recipe of the *liqueur d'expedition* depends on the winery.

4.1.1. Cava

Sparkling wine arrived to the Spanish market from Champagne in the 19th century. At first it was a luxury product that could only be bought by the monarchy and nobility due to the high importation costs. Penedes wine growers saw at this point the opportunity to reproduce the *champagne* procedure, and in 1879 the first sparkling wines produced in the Penedes region arrived on the market (Buxaderas & López-Tamames, 2012). Catalan sparkling wines became more popular after the phylloxera crisis affected the vines in France and consequently the French wine industry was not able to fulfil the demand for champagne. These sparkling wines were called "Xampany" due to their origin; however, French producers complained and it became necessary to choose another name. The name "Cava" was chosen, which is the word for a household wine cellar, usually underground, for keeping the wine cool and stored. This was implemented in 1972 to constitute the "Consell Regulador de Vins escumosos" (DO Cava, 2022a).

The Appellation of Origin (DO Cava) established its region in 1986 with the aim of protecting and working to develop a modern quality product. It includes territories from the Penedes and surrounding regions and certain territories from other parts of Spain that also have a sparkling wine culture (DO Cava, 2022c).

Cava can be white or rosé and must be elaborated with authorized varieties, which are the traditional varieties. For white Cava the authorized cultivars are the autochthonous Macabeu, Xarel·lo and Parellada. Chardonnay and Malvasia Riojana are also authorized. Four red varieties, Garnatxa, Monastrell, Trepat and Pinot Noir, are authorized for rosé and *blanc de noir* wines. Wines must have an alcoholic content between 10.8 and 12.8% vol., a pH between 2.8 and 3.4, a minimum titratable acidity of 5 g/L expressed as tartaric acid and an overpressure not inferior to 3.5 bar at 20^{o}C (DO Cava, 2022c).

Cava must be sold disgorged after a minimum time of ageing of nine months. Ageing time will be taken into account for labelling: "*Guarda*" for nine months ageing, "*Guarda superior-Reserva*" if ageing is at least 18 months, "*Guarda superior-Gran reserva*" if ageing is at least 30 months and the label "*Guarda superior-Paraje qualificado*" for certain special products aged for at least 36 months (DO Cava, 2022b) and which the grapes come from a particular recognized state.

Cava is mainly produced in Catalonia, but other Spanish regions also produce it (**Figure 9**). Therefore, in 2022 the region was divided into four subzones: "*Comtats de Barcelona"* for the production area in Catalunya; "*Valle del Ebro"* for wine produced in the authorized villages in the regions of Aragon, Navarra, La Rioja and Euskadi; "*Viñedos de Almendralejo"* in the Extremadura region and "*Requena*" in the Valencia region (DO Cava, 2022b).

Other labels are required that refer to residual sugars: *Brut Nature* if they are from 0 to 3 g/L and no sugar is added with the *liqueur d'expedition*; E*xtra Brut* for 0 to 6 g/L; *Brut* less than 12 g/L; *Extra sec* 12-17 g/L; *Sec* 17-32 g/L; *Semi-sec* 32-50 g/L; and *Sweet* if the concentration is higher than 50 g/L.

Figure 9: Different DO Cava subzones (DO Cava, 2022b).

4.2. Transfer method

The transfer method is a variation of the traditional method. Bottles in which the second fermentation is done are stored horizontally during ageing, exactly like in the traditional method, until the chosen ageing time has finished. When it is time for the disgorgement, the bottles are cooled to minus 5ºC. At this temperature carbon dioxide is more soluble so it is harder to lose pressure. Then the bottles are opened and emptied completely into isobaric tanks that lead the product to isobaric filters and isobarically fill new bottles that will be corked. Therefore, the traditional lengthy procedure of riddling is avoided (Buxaderas & López-Tamames, 2012).

Although this method requires more technology than the traditional method it has some very good aspects, including that the turbidity is completely removed and a more homogenous product is obtained. It also appears to be a good method for bottles of low volume (Ribéreau-Gayon, Dubourdieu, et al., 2006) in which riddling is more complicated.

4.3. Martinotti-Charmat method

The Martinotti-Charmat method, bulk method or Granvas is a procedure for obtaining sparkling wines in a more economical way than the traditional method. It was developed by the oenologist Federico Martinotti during the last years of the 19th century (Martinotti, 1895) and then improved by the French oenologist Eugène Charmat, who designed the tanks that are still used today (Leder, 2018).

The Charmat method starts with the fermentation of a base wine in the same way as in the traditional method. Once the first alcoholic fermentation is completed and the base wine has been stabilized and clarified, the *coupage* of the different wines is carried out. The blending, which contains the *liqueur de tirage (*adapted

yeasts, sugars, and nutrients for the second fermentation), is transferred into a special tank called an autoclave. The tank is pressure sealed so that the overpressure generated during the second fermentation remains inside the tank. Fermentation will stop naturally when yeasts are no longer able to ferment, and residual sugars may or may not be left in the tank. Autoclaves have agitators for mixing the yeasts with the product for the length of time that the oenologist chooses, obtaining certain notes of autolysis. Once the second fermentation and the "time of ageing" are considered complete, the wine is cold tartaric stabilized, filtered, blended with the *liqueur d'expedition* and bottled isobarically. The wines obtained with this method can be sparkling or frizzy wine depending on the carbon dioxide pressure inside the bottle (Buxaderas & López-Tamames, 2012).

The most popular wines produced using mainly this method are the Italian sparkling or *frizzante* Proseccos. Prosecco wines are a group of a different Appellations of Origin corresponding to wines produced in the Veneto and Friuli-Venezia Giulia region in northeast Italy (**Figure 10**). Prosecco includes two DOCG (Conegliano Valdobbiadene superiore and Asolo) and three DOC (Prosecco, Trieste and Treviso).

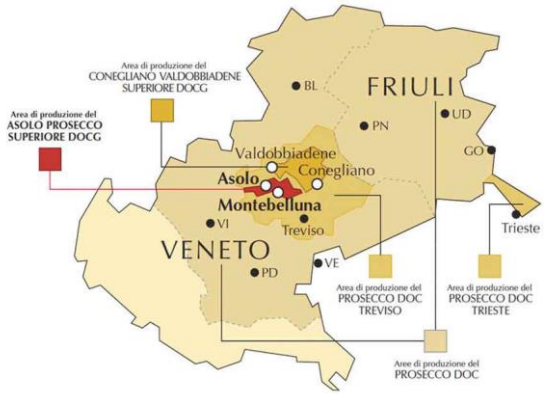

Figure 10: Map of Prosecco DOC and DOCG (Casel, 2010).

These wines are produced using at least 85% of a local variety called Glera. Due to the vigour of this variety and the high pluviometry rates where it is cultivated, large quantities of grapes with a low alcoholic rate can be obtained. *Verdiso, Bianchetta Trevigiana, Perera, Glera lunga, Chardonnay, Pinot Bianco, Pinot Grigio* and *Pinot Nero* are allowed to be under 15% of the *coupage* (Ministero delle politiche agricole alimentari e forestali, 2020).

Lambrusco is a sparkling or *frizzante* red wine. This wine is produced in the north-central region Emilia Romagna and in the Mantova province of Lombardy (**Figure 11**). The grape varieties used for this kind of wine are the Lambrusco varieties, a family of vines that is typical of the region where the wine is elaborated. Nowadays, rosé and "*blanc de noirs"* Lambruscos have also become very popular (Lombardini, n.d.).

Figure 11: Map of Lambrusco DOC and IGT (lambrusco.com).

4.4. Asti method

The Asti-Granvas method or just Asti method is based on an alcoholic fermentation of the sugars of grape juice in the autoclave. Once the residual sugar concentration has reached the desired level, the tank's temperature is decreased to stop the fermentation. As explained above, the autoclave allows the generated carbon

dioxide to remain inside the tank and therefore the sparkling wine can be filtered and bottled isobarically and remain sparkling or frizzy (Ministero delle politiche agricole alimentari e forestali, 2021).

Asti wines are a group of single fermentation wines that are obtained from *Moscato bianco*, which is a variety of the north-west region of Italy, Piedmont. Asti wines can be sparkling or *frizzante* and can be obtained through the Asti method but also through a "classical method".

Classic Asti wines are sparkling wines obtained through a single fermentation procedure. Therefore, it is a variation of the ancestral method that will be explained in the following point of this introduction.

While the sparkling wines that are obtained through the autoclave must remain at least one month in the bottle before they are sold, those obtained through the classical method must be aged for at least nine months.

4.5. Ancestral method

The ancestral or rural method differs from all the other methods because the sparklingness is obtained with just a single fermentation.

When the traditional method was fully developed, almost all winegrowers stopped using the ancestral methodology. However, some of winegrowers continued to produce sparkling and fizzy wines following this procedure. Wines produced with the ancestral method have also been improved to fulfil the buyers' requirements. All the winegrowing regions where this method is still used have adapted the method according to their climate and cultural conditions; however, the essence of the method remains the same.

Generally, before alcoholic fermentation ends, the wine is bottled and corked using a crown cap. The fermentation finishes inside the bottle, generating a CO_2 pressure

that leads to foam and bubbles forming once the bottle is opened (Robinson & Harding, 2015).

The most popular wine obtained with this method is the *Blanquette de Limoux*, but other wines such as *Clairette de Die, Gaillac méthode ancestrale, Colfondo* and *Ancestral DO Tarragona* also follow this method. Generally French ancestral sparkling wines are referred to as *pét-nat*, an abbreviation of *petillant-naturel*, which means sparkling-natural in French.

4.5.1. French ancestral wines (*pét-nat*)

In France, this procedure has been used and preserved throughout centuries by small winegrowers. *Pét-nat* wines are produced in almost all French areas; however, the most well-known are Blanquette de Limoux (Limoux, *Département* Aude), Clairette de Die (Die, *Département* Drôme) and Gaillac mousseux (Gaillac, *Département* Tarn), which are controlled under its Appellation of Origin (Ministère de l'Agriculture et de la Souveraineté alimentaire, 2009, 2011, 2023).

- **Blanquette de Limoux- méthode ancestrale:** This wine is considered the first "modern" sparkling wine of history. The AOC Limoux was created in 1938 and is nowadays one of the oldest Appellations of Origin (**Figure 12**). This sparkling wine from Occitanie must be obtained using Mauzac grapes.

Figure 12: Map of the Limoux wine region (Syndicat des Vins AOC de Limoux, 2023).

The grape juice ferments spontaneously until it reaches around 6% ethanol content. Fermentation is then stopped by filtration or cold. The process of bottling to allow the *prise de mousse,* as well as the use of *liqueur de tirage*, cannot start until the 1st of December of the same year. The fermentation inside the bottle will increase around 1 alcoholic degree or even slightly more. Once the desired ageing time is considered complete, disgorgement is carried out (Syndicat des Vins AOC de Limoux, n.d.).

The resultant wines can be sold two months after the bottling. They are clear and not dry wines that are usually "flash-bottle" pasteurized, or isobaric filtration (bottle change) is used to avoid undesired refermentations. The pasteurization process can be problematic due to a possible Maillard reaction in the product.

In the past, fermentation stops sometimes occurred after bottling, which led to the sparkling wines being too sweet and low-pressure, which was a major problem. Nowadays, most wineries work with selected yeasts, which has almost entirely solved this problem. The opposite problem of bottles exploding can also happen when the fermenting must is bottled too early,

when the concentration of the remaining sugars is still very high, or if a yeast population that is too large is added to the bottles.

- **Clairette de Die Tradition**: this is a product under the AOC Clairette de Die, which was created in 1942 in the south-east region *Vallé du Rhône* (**Figure 13**). It is produced using a variant of the ancestral method.

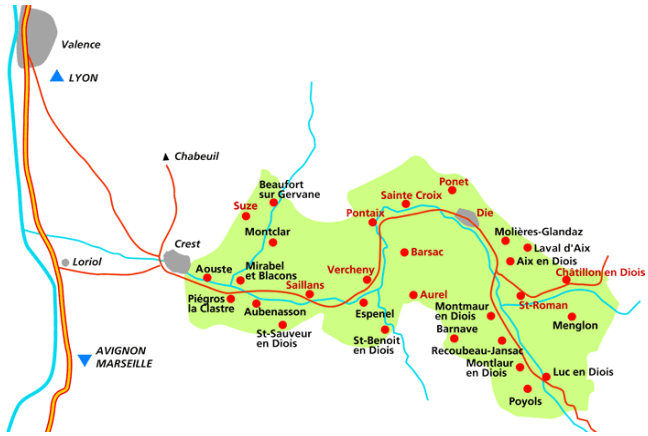

Figure 13: Map of Clairette de Die AOC (Syndicat de la Clairette, 2023).

The varieties used are *Muscat à petits grains*, which must make up at least 75%, and other varieties such as *Clairette*. The fermented grape must has to be bottled before it reaches a sugar concentration lower than 55 g/L without reinoculation of yeasts or adding more sugar. This limitation leads to products that tend to have low amounts of ethanol (7 to 9%). The ageing process must be at least four months from bottling and the residual sugars of the obtained wine must be at least 35 g/L. The removal of the lees is mandatory, and it can be carried out by disgorgement or the transfer method (Ministère de l'Agriculture et de la Souveraineté alimentaire, 2009).

- **Gaillac méthode ancestrale**: these are wines produced in the wine producing zone of south-west France in the Occitanie French region. The AOP-AOC was created in 1938 (**Figure 14**).

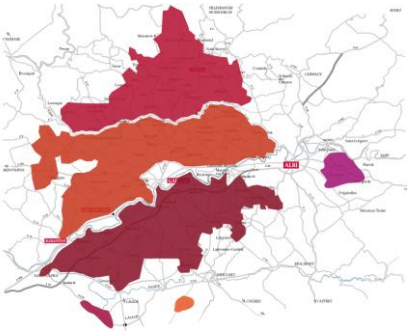

Figure 14: Map of Gaillac AOC (La Maison de la vigne et du Vin de Gaillac, 2021).

The ancestral wine from Gaillac is produced using grape juice from Mauzac extracted using the entire grape. The fermentation can lead to products that can have from 10 to 80 g/L of residual sugars. The fermentation of the must is also interrupted by cold or filtration of the grape juice before the bottling process. The fermentation in the bottle cannot be assisted by adding *liqueur de tirage*. The wines cannot be sold before ten months after bottling. It is not obligatory to eliminate the lees, but if they are removed it can be done by disgorgement or the transfer method.

Sweet ancestrals must have a minimum alcohol content of 7%, while the ones not considered sweet must have a minimum of 8% ethanol. In the market they must have an overpressure of 3 bar at 20ºC (Ministère de l'Agriculture et de la Souveraineté alimentaire, 2023).

4.5.2. Catalan ancestral wines

In recent years, in Catalonia (Spain) two Appellations of Origin have approved the elaboration of sparkling wines with this methodology (DO Tarragona and DO Penedès).

- **Ancestral DO Tarragona:** this is a single fermentation sparkling wine produced in southern Catalonia. Although wineries from this Catalan area (**Figure 15**) have been making this kind of wine throughout the ages, it was not until 2021 that this product was allowed to be produced under the Appellation of Origin.

  These sparkling wines must have a minimum of 9.5 % ethanol and less than 5.5 NTU of turbidity, which requires compulsory disgorgement. The fermentation must be stopped or slowed down before bottling. New yeasts, sugar or nutrients cannot be added in the bottling process. Wines produced with this method cannot be sold until the first day of spring of the year following the harvest (DO Tarragona, 2021).

- **Ancestral DO Penedès**: this DO, which shares a large part of its winegrowing area with DO Cava (**Figure 15**), allows its producers to carry out the following ancestral methodology. The rules are similar to those of the Ancestral DO Tarragona, but with longer ageing times. The minimum ageing time in contact with the lees is 15 months.

  This Appellation of Origin also has a category of long ageing ancestrals of four years that can be sold without disgorgement. In the two cases, the process of filling the bottles that follows the disgorgement cannot increase the alcohol level more than 0.5 % (DO Penedès, 2020).

Figure 15: Map of DO Penedès and DO Tarragona wine (Prodeca, 2020).

5. Effervescence and Foamability of sparkling wines

Sparkling wine bottles are thermodynamically altered systems supersaturated with carbon dioxide. Once they are uncorked and poured into the flute the liquid will tend to recover the natural thermodynamic state of the atmospheric pressure. CO_2 escapes from the liquid through the diffusion of the liquid surface or the formation of bubbles, which lead to the formation of foam (Jeandet, Vasserot, et al., 2011).

Foam is the main defining factor of sparkling wines. It is an agglomerate of bubbles (**Figure 16**) that come from inside the liquid and rise up until they reach the surface, where they collapse after remaining there for some time (Buxaderas & López-Tamames, 2012; Liger-Belair, 2005). How the foam is formed, the size of its constituents (bubbles), the formation of trains or rosaries (periodic sequences of bubbles that rise vertically from the bottom of the glass to the liquid surface) and the persistence of the foam will determine, for the consumer, the quality parameters that can be defined by the following characteristics (Gonzalez-Viejo, Torrico, et al., 2019; Medina-Trujillo, Matias-Guiu, et al., 2017).

- The foam must be white and compact.
- The bubbles must be small and with a continuous release.
- There must be numerous bubble trains.

- The bubbles must remain at the surface of the liquid for a certain period of time, forming a stable “crown”.

Although it might seem a subjective parameter, a correlation has been found between drinks with very tumultuous foam formed by large bubbles and a fast loss of the carbon dioxide and a sensation of aggressivity in the mouth. Moreover, small bubbles last longer, which leads to a more prolonged time of CO_2 release and therefore a creaminess and tingling sensation in the mouth (Polidori, Jeandet, et al., 2009; Vanrell, Canals, et al., 2007).

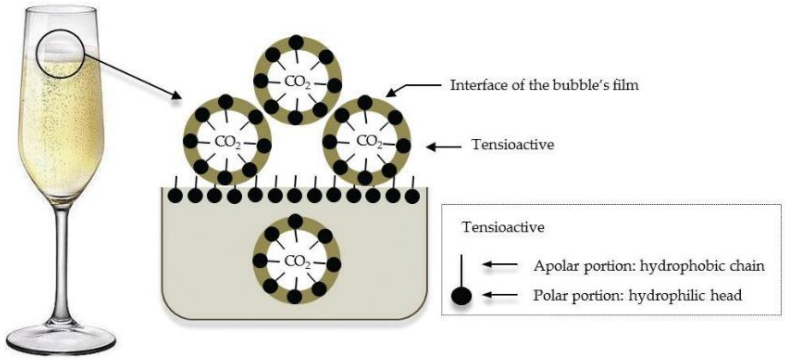

Figure 16: Drawing of bubbles as the unitary cells of the foam. The bilayer composed by surfactant substances stops the carbon dioxide from being released, and thus the foam is formed on the liquid surface (Martínez-Lapuente, Ayestarán, et al., 2017).

5.1. The Bubble

The Bubbles are the unitary components of the foam. They are described as carbon dioxide cores surrounded by a spherically shaped film. This film is made up of many wine substances, among which colloids seem to be very important due to their surfactant properties (Comelles, Bosch, et al., 1991)

Surfactant compounds, substances with the ability to reduce surface tension, are the key factor that enables the bubbles to exist. Liquids saturated with carbon

dioxide without surfactant compounds generally have a fast and non-bubbling release of this carbon dioxide; while wines, beers, or other drinks with a more complex matrix full of surfactant compounds have more difficulties in releasing the carbon dioxide due to the friction generated with the CO_2.

Surfactant compounds are amphipathic molecules whose hydrophilic part is in contact and dissolved with the aqueous phase of the wine. Therefore, the hydrophobic phase is in the internal part of the bubble in contact with the carbon dioxide (**Figure 16**). Surfactant compounds in wine (ethanol, glycerol, tartaric acid, fatty acids, and others) reduce the surface tension of the liquid, which enables bubbles to remain on the liquid's surface. Other molecules, such as polysaccharides, proteins and polyphenols, can also play a helping role in the viscosity of the bubbles, which can lead to a longer persistence of the bubble (Buxaderas & López-Tamames, 2012).

But how are bubbles generated? Bubbles are mainly generated through the accumulation of CO_2 in cavities or nucleation points (**Figure 17**). Nucleation points can be naturally present in the wine, such as in microparticles of fibre or the tartrate crystals present in the served wine. Nucleation points can also be generated artificially by the glass manufacturer by scratching certain points of the glass with the help of laser rays to allow the gas to enter at these points (Liger-Belair, 2012). The diffusion of CO_2 through the micro-cavity will go on until the forming bubble reaches a size between 10-50 micrometres. When this size is reached the bubble will detach from the microcavity to rise through the flute (Polidori et al., 2009).

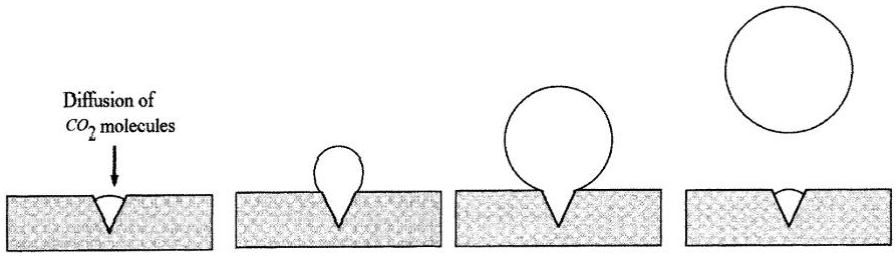

Figure 17: Representation of bubble formation from a micro-cavity (Liger-Belair, Marchal, et al., 1999).

The bubble formation process is repeated in the same cavities (heterogenous cavities), which generates trains, rosaries or bubble columns that move from the microcavity up to the liquid's surface. As they travel up, bubbles continue growing in size due to the absorption of more carbon dioxide molecules present in the liquid (Liger-Belair et al., 1999). The length and form of the glass are important in this process because the longer the distance the bubble travels, the larger it will be when it reaches the surface (Darsonville, 2001; Liger-Belair, 2012).

Once the bubbles reach the liquid's surface, they will remain on the surface, forming what is known as a crown of foam, until they disappear (Cilindre, Henrion, et al., 2021). The facility with which the bubbles pop or are retained is mainly determined by the surfactant components and the viscosity conferring molecules present in the sparkling wine (Vanrell, 2002).

Bubbles collapse through three mechanisms (Buxaderas, Riu-Aumatell, et al., 2022; Liger-Belair, 2012):

- Ostwald ripening: this is the process in which a small bubble diffuses into a bigger adjacent bubble.
- Coalescence: the thin layer that separates two adjacent bubbles breaks and a new bigger bubble is formed.

- Drainage: bubbles, due to capillarity forces, remain almost full under the liquid, which causes a deformation between the part that remains under the liquid and the part in contact with the air. This difference of shape will result in the rupture of the upper part of the membrane that is in contact with the air, and it will grow in a circular way.

When the bubble collapses through drainage (**Figure 18**), the pressure differences provoked by the release of CO_2 generate "jet drops" in which the liquid covers the empty space generated by the former bubble with enough speed to eject wine drops several centimetres above the liquid surface (Liger-Belair, Lemaresquier, et al., 2001).

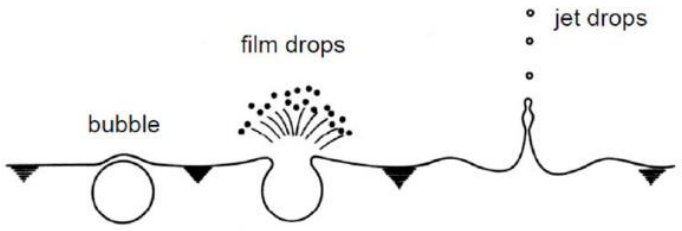

Figure 18: Process of bubble drainage and the formation of jet drops (Liger-Belair, 2012).

This process will occur when there is an overpressure of carbon dioxide in the liquid. During the time that the liquid is served in the flute its temperature increases. This heating process generates a gradient of alcohol evaporation that is higher at the centre of the glass and lower on the glass walls, which means that the bubble crowns move along the glass walls due to the lower superficial tension (as it can be seen in **Figure 19**) (Liger-Belair, 2012).

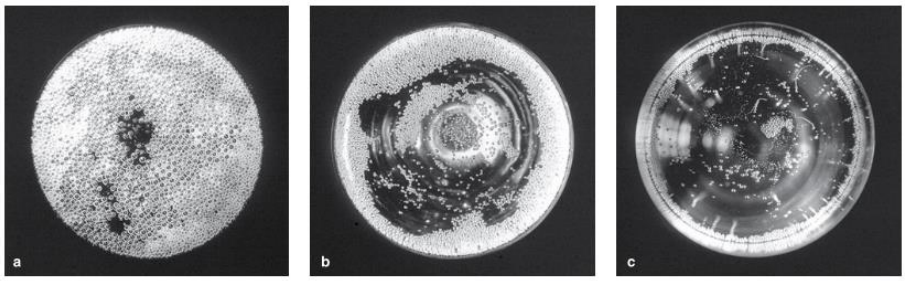

Figure 19: Top view of the crown formed in a Champagne flute. The three pictures show how the crown evolves over time from recently poured (a) and as time passes (c) (Polidori et al., 2009).

5.2. The effervescence

The effervescence is the phenomenon known as the accumulation of bubbles at the surface of the liquid when it is served. This phenomenon can be divided into two main steps.

1. **Tumultuous phase**: this starts when a bottle is opened and poured into the glass. This first phase is characterized by the formation of microbubbles during the handling, opening, and serving of the bottle. These bubbles are considered to have a homogenous nucleation because they come from single-bubble-generating points (the same liquid). During this phase there is a large increase in the foam layer that is not very long lasting (Maujean, 1989).

2. **Ordinated foam generation**: once the tumultuous phase has finished, bubbles start to rise from the microcavities forming a foam layer that is continually renewed and in which the bubbles will undergo the entire process explained in the previous part.

5.3. Influence of the wine chemical composition on foamability

Not all sparkling wines have the same foaming properties. Foam formation is intrinsically dependent on the matrix in which the CO_2 is dissolved. Wines from different varieties (Andrés-Lacueva, Lamuela-Raventós, et al., 1997) or that have been obtained through different methodologies (Culbert, McRae, et al., 2017) will have different matrixes, and therefore they will have different foaming properties. In fact, the influence of the different substances that make up the wine on the foaming properties is one of the main research subjects in the field of sparkling wines (Andrés-Lacueva et al., 1997; Cilindre, Castro, et al., 2007; Cilindre, Liger-Belair, et al., 2010; Esteruelas, González-Royo, Kontoudakis, et al., 2015; Liu, Vrigneau, et al., 2018; López-Barajas, López-Tamames, et al., 2001; Martínez-Lapuente, Guadalupe, et al., 2015; Medina-Trujillo, Matias-Guiu, et al., 2017; Vanrell et al., 2007).

5.3.1. Proteins

Proteins are large, complex biomolecules constituted by aminoacidic chains that work as the main biomolecules in the cell that maintain the homeostasis and reproduce the functions coded in the genome. These aminoacidic chains form secondary structures that can be alpha helix or beta pleated sheets that combine between them to form domains or tertiary structures. Big proteins can have various united domains that form a genome with a quaternary structure (Koshland & Haurowitz, 2023).

There is only a low concentration of proteins in sparkling wines (4-16 mg/L) (Canals, Zamora, et al., 1998; Jeandet et al., 2011; Vanrell et al., 2007) and they can have different origins. Grapes, yeasts and other fungi or bacteria that may develop in wine are the main origins; however, certain kinds of proteins may also be added

as part of the oenological practices during winemaking. The grape variety, *terroir* and the kind of fermentation are the three main factors that determine which kind and which amount of proteins are present in a wine (Marangon, De Iseppi, et al., 2022). Total nitrogen in grapes is essential for the synthesis of proteins during the maturation process, and therefore poor nitrogenous soils will generally generate poor protein musts (Ferreira, Piçarra-Pereira, et al., 2002).

Some of the proteins from the grape will precipitate during the fermentation process due to the changing conditions in the medium, which will denaturalize them or make them insoluble in the new conditions. During fermentation, yeasts synthesize a large number of proteins and protease enzymes that degrade grape and old yeast proteins into aminoacidic chains, which are used by yeasts as a nitrogenous source. At the end of fermentation, only proteins resistant to proteases and to the wine's low pH will be found in the wine medium (Ferreira et al., 2002).

The wine's pH will determine the electric charge that proteins will have at the end of fermentation. The common pH (3.00-3.50) of wine is below the Isoelectric point (Ip) of the wine proteins, which makes them positively charged in the wine medium. Oenologists use a clay silicate called bentonite to take advantage of this phenomenon and thus avoid protein haze. Bentonite has a negative charge at the pH of wine and interacts with the positively charged proteins to form insoluble bodies that precipitate. This practice has been found to negatively affect the aromatic profile and the foaming capacity of sparkling wines (Vanrell et al., 2007); therefore, alternatives like Chitosan (Marín, Riponi, et al., 2020) or zirconia (Salazar, Zamora, et al., 2010) have been studied to replace it.

Grape proteins can be divided into **Chitinases, glucanases** and **thaumatin-like proteins** (Cosme, Fernandes, et al., 2020; Esteruelas, Poinsaut, et al., 2009). They play a defence role against fungal attacks in the grape during the ripening process

(Waters, Shirley, et al., 1996) and are synthetized in a basal way even when the crop is healthy. Glucanases break the glucans from the fungal cell membrane while thaumatin-like proteins permeabilize cell membranes (Ferreira et al., 2002). Chitinases have been identified as the proteins that play the main role in heat induced haze, which causes turbidity and unstable wines (Kemp, Condé, et al., 2019).

Yeast proteins in sparkling wines gain in importance during ageing (Pons-Mercadé, Giménez, et al., 2022). **Mannoproteins** and other **glycoproteins** are released during ageing and it has been established that they have a positive effect on foaming stability (Moreno-Arribas, Pueyo, et al., 2000; Nuñez, Carrascosa, et al., 2005). There have been some studies on the use of commercial mannoprotein-based products as foam enhancers; however, no clear results have been found as yet (Medina-Trujillo, González-Royo, et al., 2017; Pérez-Magariño, Martínez-Lapuente, et al., 2015).

During fermentation and over-lees ageing, yeasts secrete proteolytic enzymes that transform proteins into peptides and free amino acids (Fornairon-Bonnefond, Camarasa, et al., 2002; Sartor, Burin, et al., 2021). While free amino acids have been shown to have clear enhancing effects on foamability, the effect of peptides is still unclear (Martínez-Rodriguez & Polo, 2003; Moreno-Arribas et al., 2000).

5.3.2. Polysaccharides

Polysaccharides are complex carbohydrates formed by more than 10 monosaccharide units that may or may not be ramified and can sometimes contain small proteinic fractions. In cells, polysaccharides play a structural role in membranes or cell walls (The Editors of Encyclopaedia Britannica, 2023). In wines they are found in concentrations that can go from a few mg/L to up to 1.5 g/L) (Guadalupe, Ayestarán, et al., 2015). There are different types of polysaccharides in

wine depending on their origin. They can come from the grape itself (Vidal, Williams, et al., 2001), from yeasts and bacteria (Caridi, 2006), from fungi that infect the grape (Francioli, Buxaderas, et al., 1999) or they can even be added to the wine in certain oenological treatments (Guise, Filipe-Ribeiro, et al., 2014).

Grape polysaccharides have less impact on still and sparkling wines because base wines are statically cold settled and usually treated with pectolytic enzymes before the first fermentation. Moreover, maceration with grape skins is not normally applied in the elaboration of sparkling wine. This process leads to the loss of a high concentration of pectins and other polysaccharides coming from vegetal cell walls, from the grape skin, and from the pulp (Gawel, Schulkin, et al., 2018; Vidal et al., 2001).

Grape skin derived pectins in the grape juice are a hetero-polysaccharide mainly constituted by galacturonic acid with 1,4-glycosidic bonds. Three major types of this polysaccharide can be identified in wine: **arabinans, rhamnogalacturonans** and **homogalacturonans** (Ayestarán, Guadalupe, et al., 2004). Other polysaccharides, like cellulose and hemicellulose, whose β-1,4-glycosidic bonds normally cannot be metabolized by living organisms can also be found (Jones-Moore, Jelley, et al., 2021).

Rhamnogalacturonan I are a chain of a disaccharide unit of (1,2)-α-D-galacturonic acid-(1,4)-α-L-rhamnose. Rhamnogalacturonan I can be ramified with other polysaccharides like arabinan, arabinogalactan I, Arabinogalactan II or galactan (Jones-Moore et al., 2021). Rhamnogalacturonan II are a sequence of galacturonic acid (1,4) linked between them. They can show different kinds of ramifications that all have glycosyl residues and glycosidic linkages. They play a minor role in the vegetal pectins and do not constitute more than 10% of the total pectin weight. Their interest lies in their capacity to form dimers between two of their molecules

that can be part of two different pectins. This confers 3D stability and strength to the pectic structures in plants (Doco & Brillouet, 1993).

Polysaccharides rich in arabinose and galactose are the other group of important grape skin derived polysaccharides. The backbone of arabinans and arabinogalactans is mainly constituted by the monosaccharide arabinose. Out of all these molecules, arabinogalactans are the most important in wine and they are usually linked to proteins to form Arabinogalactan II-protein. The backbone of this molecule is formed with (1 → 3)-D-galactose residues with substitutions of (1 → 6) linked to D-galactose residues. Small proteins that do not usually represent more than 10% of the molecule's total weight bond to this backbone through glycosidic bonds (Jones-Moore et al., 2021).

The polysaccharides of yeasts are released during alcoholic fermentation and ageing in contact with the lees (Martínez-Lapuente, Guadalupe, et al., 2013; Pons-Mercadé et al., 2022). If this contact happens to be at high temperatures (30ºC) and/or in agitation, then this process will increase. When cell death or membrane restructuration occurs, yeasts will release these polysaccharides into the medium. With the death of yeasts and cellular rupture, active glucanase enzymes are released into the medium. This enzyme will act in some glucose terminals from the polysaccharides during ageing, which leads to a change in polysaccharide composition (Ribéreau-Gayon, Glories, et al., 2006).

A total 80% of yeast polysaccharides in aged on-lees white wines are **mannoproteins**, constituted by around 90% mannose bone and 10% protein and can have a wide range of molecular weights. The other 20% are **glucomannoproteins** containing 25% glucose, 25% mannose and 50% protein. These glucomannoproteins have lower molecular weights than mannoproteins (Ribéreau-Gayon, Glories, et al., 2006).

Polysaccharides have surfactant properties, which makes them useful for enhancing the foaming properties of sparkling wines and the mouthfeel sensation when they are consumed (Buxaderas & López-Tamames, 2012). The effect of yeast derived polysaccharides on foam has already been discussed in the section on proteins. Although there have been less studies on polysaccharides derived from grapes, authors such as Martínez-Lapuente *et al.* (2015) found a good relationship between grape derived polysaccharides with arabinose and galactose residues and foam persistence.

Bacterial exopolysaccharides can also be found in wines if bacterial growth occurs. Molecules like peptidoglycans or lipopolysaccharides are constituents of bacterial cells and when cells die a part could probably be released into the medium. There has not been much study on these bacterial origin particles and therefore their possible influence on foam and other wine attributes is still unclear (Dimopoulou & Dols-Lafargue, 2021).

Botrytis cinerea in grapes during their growth and maturation, in addition to the off flavours and mushroom aromas typical of grey rot, also provokes the presence of β-glucans in the grape must. These glucans are responsible for turbidity in wines, problems during filtration and a loss of foaming properties (Cilindre et al., 2007; Esteruelas, González-Royo, Kontoudakis, et al., 2015; Marchal, Tabary, et al., 2001; Marchal, Warchol, et al., 2006).

Oenologists also add different kinds of polysaccharides to wines for different proposes, such as carboxymethylcellulose (CMC) or Arabic gum (authorized by OIV) (Martínez-Lapuente et al., 2019). CMC is used against tartaric crystallization and its use does not influence the foaming properties of sparkling wines (Crumpton, Atkinson, et al., 2018). Arabic gum was authorized to prevent colour precipitation in red wines and its use in sparkling wines also improves foaming properties (Apolinar-Valiente, Salmon, et al., 2020).

5.3.3. Lipids and fatty acids

Lipids are a diverse and heterogenous group of organic compounds, including fatty acids and oils, that share the characteristic of hydrophobicity. Lipids are generally constituted by a saturated or non-saturated hydro-carbon chain with a carboxyl group (Thompson, 2023). Fatty acids can be classified by their length: short chain (C_1-C_5), middle length chain (C_6-C_{12}) and long chain (C_{13}-C_{21}).

Lipidic content increases during the second fermentation, reaching concentrations around 300 mg/L lipids (C_{16}-C_{20}) (Troton, Charpentier, et al., 1989). Most of the lipids and fatty acids are secreted by yeasts and bacteria that grow in wine. Fatty acids are key biomolecules of cell membranes and cytosol, and their length and saturation depend on the stress situations of the microorganisms (Tesnière, 2019).

The effect of lipids on foam has been studied; however, the results are unclear. Short chain fatty acids are weak foam stabilizers (Kemp et al., 2019). Middle length chain fatty acids were found to improve the foam maximum height (HM in Mosalux parameters) but reduce foam stability (Maujean, Poinsaut, et al., 1990). Long chain fatty acids were found to have an immediate negative effect on the base wine (Vanrell, Cabanillas, et al., 2002); however, after time, and with its oxidation or esterification, the foamability of sparkling wines was recuperated (Dussaud, Robillard, et al., 1994).

5.3.4. Organic acids

Wine is a matrix with multiple organic acids with varying concentrations. Tartaric acid is the most important organic acid in wine and its concentration can vary from 6 g/L in cold climate wines to 2-3 g/L in wines produced in hotter weather (Ribéreau-Gayon, Glories, et al., 2006). Tartaric acid has been found to increase foaming properties (Pueyo E, Martín-Alvarez, et al., 1995).

L-Malic acid is the second most important organic acid in sparkling wines. Malolactic fermentation is usually avoided to preserve freshness because malic acid is stronger than L-lactic acid. Malic acid has been studied as an enhancer of foam height; however, it may affect foam stability, while the resultant lactic acid after its degradation seems to work inversely (Kemp et al., 2019).

Domizio et al. (2023) obtained interesting results when base wines were acidified with the different acids allowed by OIV (tartaric acid, malic acid, citric acid and lactic acid) after the first fermentation. All of them caused a decrease in foam height persistence after on-lees ageing.

Finally, Esteruelas et al. (2015) studied the impact of gluconic acid, which is used as a marker for grape rot (*B. cinerea* grow), in sparkling wines and found a negative correlation between its presence and foaming properties.

5.3.5. Ethanol

Ethanol has been studied as a size-limited surfactant that governs the liquid-gas interface in sparkling wines. This phenomenon is key for understanding how ethanol concentration can affect foam behaviour (Jeandet et al., 2011). It has also been described that ethanol content has a dose-response effect on foam. Some authors have found that during the first fermentation ethanol extends the duration of foam (López-Barajas, López-Tamames, et al., 1998) and other authors have found that it also improves the foam height (Andrés-Lacueva, López-Tamames, et al., 1996). However, authors have found that after the second fermentation, the foamability of sparkling wines decreases (Dussaud et al., 1994; Esteruelas, González-Royo, Kontoudakis, et al., 2015). These dose related effects on foam have led authors to state that high ethanol concentrations in wines have a negative effect on foamability (Buxaderas & López-Tamames, 2012).

5.3.6. Other factors

Generally, the residual sugars of sparkling wines come from the second fermentation. For the *prise de mousse* process, sugar is added to the wine, which will referment in the bottle. This sugar can have different origins that lead to different organoleptic, chemical, and foaming properties (Kemp et al., 2019; Wilson, Charnock, et al., 2022). Residual sugars increase the viscosity of the product and consequently it is more difficult for bubbles to drain and coalesce; therefore, the foam is higher and more persistent (Crumpton, Rice, et al., 2018). Glycerol, like sugar, also adds viscosity to the medium and has also been found to enhance foam hight and persistence (Coelho, Rocha, et al., 2011).

Phenolic compounds are found in very low concentrations in sparkling white wines, but in rosé wines they are found in higher concentrations. No clear tendencies have been found regarding the correlation between phenolic compounds and foamability in wines (Kemp et al., 2019). Most studies have been conducted with model solutions. In other experiments, side reactions with polyphenols with other macromolecules like proteins have been found to be the ones that affect foamability.

The temperature during the second fermentation and ageing has been found to affect the foaming properties of sparkling wines, and lower temperatures (13^{o}C) are better than higher ones (20^{o}C) (Cilindre et al., 2021; Esteruelas, González-Royo, Gil, et al., 2015).

pH is considered to have an impact on foamability because it can change the structural form and charge of proteins; however, it can also change the structural form of other surfactant compounds and the solubility of other foaming agents or antagonists (Buxaderas & López-Tamames, 2012).

Other factors that the oenologists cannot control are involved in the foam characteristics, such as the serving temperature. The colder the wine the more soluble the carbon dioxide and therefore the longer the time the foam persists. How the glass is cleaned and dried, its shape and how the bottle is opened and served also affect the foam produced (Kemp et al., 2019).

However, all these data should be considered with precaution because most of the analyses are only correlation studies. Although there is extensive literature on the subject, as Kemp et al. (2019) and Martínez-Lapuente et al. (2015) show in their reviews, the published data are sometimes inconclusive and some authors report data that contradict findings from other studies (Medina-Trujillo, Matias-Guiu, et al., 2017).

5.4. Methods for measuring foam

Analytical methodologies for measuring foam are relatively new. Bikerman (1938) proposed the first methodology for characterizing foam of foods, which consisted in sparging the liquid in a glass tube and observing the height reached by the foam. This methodology was adapted by different authors until the Mosalux method was proposed (Maujean et al., 1990). Mosalux is currently the most used analytical method for determining foaming properties in wines; however, it has some limitations. We will discuss it at more length later.

Other techniques based on image analysis have also been proposed. Recent technological improvements have made image analysis faster and it can quantify characteristics undetectable by the human eye. This is the case of **CAVE (Computerized Artificial Viewing Equipment)** (Machet, Robillard, et al., 1993).

CAVE consists of two video cameras that capture large and small-scale views to quantify foam collapse. The room temperature is controlled at 20 ^{o}C. This method

makes it possible to determine the foam height and foam stability with a constant liquid pouring time. The methodology was improved to avoid the variable "pouring way" with a pouring robot programmed to pour a specific foam quantity into a dark glass (Jeandet et al., 2011). Other modifications have been made to the methodology to obtain the collar behaviour data (Cilindre et al., 2010)

Ellipsometry and Brewster Angle Microscopy (BAM) was proposed to study foam at a molecular level (Abou-Saleh, Aguié-Béghin, et al., 2007). The adsorption layer of foam shows different responses to BAM if it is in the liquid phase or in the gaseous phase of the served liquid. The difference makes it possible to characterize the ellipse of the bubble layer, the lifetime of the bubbles and the ratio area covered by the bubbles.

5.4.1. Mosalux

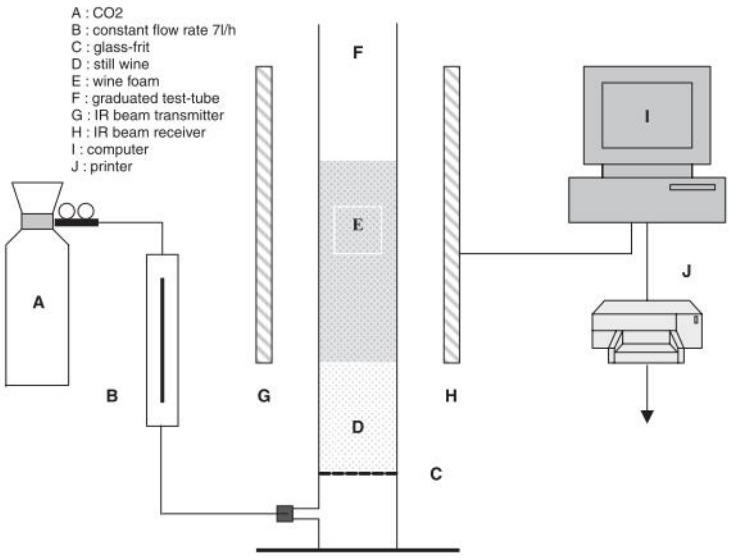

Figure 20: Diagram of the Mosalux device (Jeandet et al., 2011)

The Mosalux device (**Figure 20**) (Maujean et al., 1990; Poinsaut, 1991) is produced by the Station Oenotechnique de Champagne, Epernay, France. It uses carbon

dioxide under pressure and flow-controlled conditions (7 L/h) and at 1 or 2 bar depending on the wine. The carbon dioxide is conducted into a glass column. The glass column contains 100 mL of sample and the carbon dioxide source separated by a cellulose membrane. The CO_2 injection across the membrane allows the gas to be dispersed homogenously through the sample. The device has an automatic reader that generates a live graphic of the foam height, which allows the user to determine the maximum height of foam (HM), the stable height of the foam (HS) and the time of foam duration (Ts). This technique is currently used to determine different foaming properties between different experimental groups of the same experiment.

However, as mentioned above, this technique has some limitations, such as the possible lack of correlation with the results and the sensory attributes perceived by the consumer (Cilindre et al., 2010), or the difficulties to reproduce the same foaming way for every sample.

6. Ageing of sparkling wines

Ageing on-lees in sparkling wines is the process in which the wine is left in contact with the lees after the second fermentation. The compounds released by the lees during this time will have a sensory impact on wines that is positively perceived by the consumer. Each Appellation of Origin will determine minimum ageing times for its products to preserve its quality standards (Fornairon-Bonnefond et al., 2002).

Ageing on-lees is performed with the bottle in a horizontal position to provide maximum lees-liquid contact. It is carried out in darkness at low temperatures to change the organoleptic features of the product while avoiding the photo-oxidative effect. Ageing enhances the pastry and bakery aromas and assists the integration of carbon dioxide into the product. Sparkling wines that are not aged will have more floral and fruity aromas (Pons-Mercadé, 2021; Ribéreau-Gayon, Dubourdieu,

et al., 2006). The organoleptic features of sparkling wines made with the Martinotti-Charmat method are changed sometimes by "ageing" in the autoclave with the lees before the isobaric filtration and bottling (Buxaderas & López-Tamames, 2012).

Not all strains from *Saccharomyces cerevisiae* are appropriate for performing second fermentations in the bottle. Strains that are usually selected for second fermentations are those that can perform fermentation in mediums that already have a high ethanol content and low pH, the presence of sulphur dioxide, low fermentation temperatures, low nutrient concentrations and high amounts of CO_2 (Cisilotto, Scariot, et al., 2021). Strains that can flocculate easily or can affect foam positively and release other compounds into the medium to make the product more distinctive are also desirable (Pons-Mercadé, 2021).

6.1. Yeast autolysis

During ageing the main process that takes place inside the sparkling wine bottles is autolysis of the yeast cells (lees). When the second fermentation ends, yeasts start their process of death and secretion of different components into the wine. This process depends on the pH, temperature, ethanol, and yeast strain (Martínez, Delso, et al., 2018).

Firstly, a short phase called **excretion** takes place from the third to the sixth month of ageing. Yeast death starts a passive exorption of wine molecules and a passive liberation of amino acids and other small molecules. The actual process of autolysis starts after this short period.

The **autolysis** process is a eukaryote cell process in which the internal structures of the yeast cell are destroyed by its own enzymes. During this process, membrane polysaccharides, mannoproteins, peptides, amino acids, lipids, and many other

cytoplasmatic and membrane components are released into the wine, and therefore have a sensorial impact on the product (Robinson & Harding, 2015).

Intracellular enzymes start irreversible cell degradation due to adverse conditions. The protease and glucanase activity inside the cell reaches a stage where the resultant products are small enough to pass through the cell wall by diffusion. At a certain point, the cell membrane will also start to degrade. In the winery these processes do not take place in optimal conditions (low pH and temperature) so they are very slow (Alexandre, 2019; Feuillat & Charpentier, 1982).

Microscopy imagery has shown that despite the enzymatic action of proteases and glucanases, the cell wall does not break completely. Pons-Mercadé et al. (2022) used scanning electron microscopy to show that after some months, the yeasts have lost part of the turgor and begin to show wrinkles and folds. After five years of ageing the cell is completely flattened, and after seven years the cell centre is completely crumbled and fully wrinkled. The experiment ended at 9 years of ageing, when the lees can be seen to be completely collapsed (**Figure 21**).

Although the lees have an autolysis role, they also play a protective role against the oxygen permeability that the crown cap and bidule or the cork can have. The ability of the lees to consume oxygen tends to decrease over time, which can condition the ageing capacity of the sparkling wine for very long times (Pons-Mercadé et al., 2022).

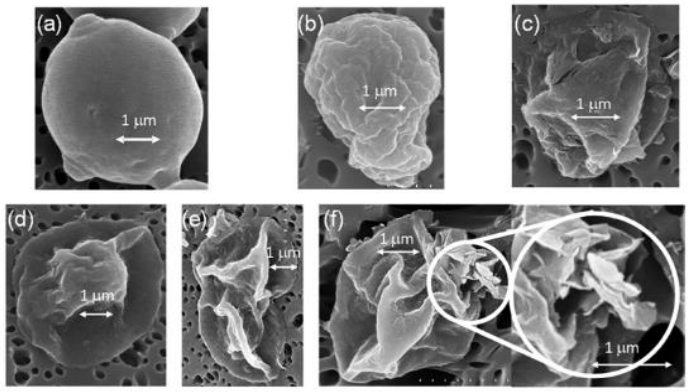

Figure 21: Process of yeast cell degradation during sparkling wine ageing, (a) yeast starter culture at (b) 1 year, (c) 3 years, (d) 5 years, (e) 7 years and (f) 9 years (Pons-Mercadé et al., 2022).

6.2. The autolysis effect on the chemical composition of sparkling wine

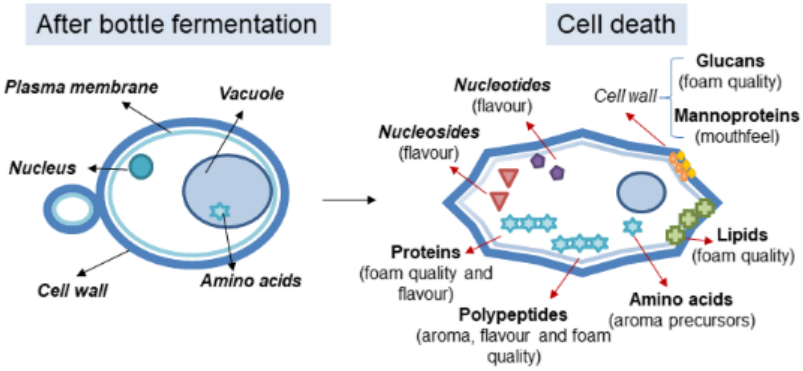

Figure 22: Important compounds released during the autolysis process (Welke et al., 2022).

As it can be seen in the **Figure 22**, during the autolysis process a lot of yeast compounds are released into the wine. These compounds can have a higher or lower sensorial impact on the wine.

Nitrogen compounds in wines are mainly proteins, peptides, and amino acids. During ageing on-lees, the peptide concentration increases due to the residual protease activity. This effect is noticeable around 12 months of ageing (Moreno-Arribas, Pueyo, et al., 1996) and from that point on its concentration increases and the mean size of peptides decreases as they degrade (Martínez-Rodríguez & Polo, 2000).

The amino acidic composition in sparkling wines is low at the end of the second fermentation and increases during ageing due to the release of free amino acids from the protease activity that occurs during autolysis (Moreno-Arribas et al., 1996). When amino acids are decarboxylated or deaminated they generate aroma precursors; therefore, an increase in amino acids is related to aromatic enrichment (Feuillat & Charpentier, 1982). Furthermore, as mentioned above, nitrogen compounds are good foaming agents.

Polysaccharides from the cell membrane and wall are liberated through glucanase activity. Their release is determined by the yeast strain, the conditions in which the fermentation takes place and the ageing time (Kemp et al., 2019). It has been shown that the polysaccharide content increases quickly during the first months of ageing; however, part of these polysaccharides will precipitate due to the ethanol content of the wine (Charpentier, 2000; Martínez-Lapuente, Apolinar-Valiente, et al., 2018). Mannoproteins are also liberated with polysaccharides. Mannoproteins are associated with the mouthfeel sensation, decreased astringency, and bitterness because of the interaction with phenolic compounds and the persistence of aromas. They also avoid protein haze and the crystallization of the tartrate salts by forming soluble colloids (Martínez-Lapuente et al., 2019; Snyman, Nguela, et al., 2023).

Pons-Mercadé et al. (2022) found that in a nine-year ageing the polysaccharide content originating from the lees found in sparkling wines went from 3% in one

year of ageing to 11% in the ninth year. Moreover, the percentage of proteins went from 2% from the lees in the first year to 15% in the ninth year. These results confirm that the impact of ageing takes longer than would seem.

As the aromatic profile of wines is greatly determined by the grape varieties and fermentation techniques used, it is known that ageing on-lees will have an aromatic impact on wine. During autolysis there are many chemical reactions, including esterification, hydrolysis, reduction and oxidation, which change the aromatic profile of the wines (Torrens, Rlu-Aumatell, et al., 2010).

Esters are the main family of **volatile molecules** that are released during autolysis. Higher alcohol acetates and ethyl esters start to be released in the first autolysis stages (3-9 months), which leads to fruity aromas (Francioli, Torrens, et al., 2003; Pozo-Bayón, Hernández, et al., 2003).

There are also aromatic precursors, such as monoterpenes, sesquiterpenes and c13-norisoprenoids, that accumulate as precursors in the ripening pulp and skin. This release of precursors depends on the glycosidase activity from autolysis (Riu-Aumatell, Bosch-Fusté, et al., 2006; Welke et al., 2022). The release of other aromas is related to this glycosidase activity, such as TDN (1,1,6-trimethyl-1,2-dihydronapthalene), diethyl succinate and vitispirane, which also accumulate during ageing (Francioli et al., 2003).

In long aged sparkling wines, it has been found that acetate esters lose importance in favour of other ester families. **Higher alcohols** and the **terpenic alcohol** concentration increase after longer ageing periods (Caliari, Panceri, et al., 2015). In relation to terpenic alcohols, farnesol and nerolidol are liberated (green, soft, floral-woody aroma) (Alexandre, 2019). Higher alcohols, like Isoamyl alcohol, can also be oxidized during ageing, resulting in methyl-3-butanal (perceived negatively as a grassy odour) (Chung, 1986).

Furans are also found after the ageing process in sparkling wines. These molecules give bread, caramel or toasted aromas to the wine and are the result of sugar degradation. They are considered to have a positive impact on the product (Torrens et al., 2010).

Some authors have found that after the second fermentation and ageing on-lees there is a loss in the aromatic-grape relationship, which demonstrates the large aromatic changes that take place during this process (De La Presa-Owens, Schlich, et al., 1998).

6.3. Evolution of foaming properties throughout the ageing process

Foaming properties depend on the chemical composition of the wine as it has been discussed above. During ageing this chemical composition is altered by the autolysis process, oxidation, precipitation processes and also by the use of riddling agents.

Mannoproteins and polysaccharides have a well-known positive effect on foam and, at the same time, they are the molecules most released during long ageing (Pons-Mercadé et al., 2022). Polysaccharides, which include mannoproteins, are related to more foam stability (HS and Ts) (Pueyo E et al., 1995) due to their qualities for increasing viscosity (Martínez-Lapuente et al., 2015).

Protein release has a more uncertain impact because a large part of the protein is absorbed by the riddling agent (Vanrell et al., 2007) and it is also hydrolysed by proteolytic enzymes (Moreno-Arribas et al., 1996). It has been reported that the proteolysis products are more related to the foamability (HM) than the foam stability (Martínez-Lapuente et al., 2015).

Andrés-Lacueva et al. (1997) summarized the effects of ageing on foam as a general decrease in HM and a maintenance or improvement of HS and Ts. Although there seems to be a consensus on the stability of HS and Ts, other authors have found an increase in HM until 30 months of ageing (Pérez-Magariño, Ortega-Heras, et al., 2015) and studies with samples aged for longer showed a decrease in HM after 2-3 years (Pons-Mercadé et al., 2022).

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Hypothesis and Objectives

UNIVERSITAT ROVIRA I VIRGILI
ANCESTRAL SPARKLING WINES; COMPARISON WITH TRADITIONAL SPARKLING WINES AND PROCEDURES TO IMPROVE THEIR QUALITY
Arnau Just Borràs

The oenological industry is a sector in continuous upgrade. Achieving better wine qualities, adapt to new and challenging conditions that climate change brings with it or to satisfy the new trends and demands that the consumer asks for are the main factors that make the wineries to constantly look for improvements. In this sense there is an uprising scenario where new consumers are asking for less alcoholic and "classic-new" styles of wine that had been forgotten as a "funky" way to consume wine. In addition, climate change is an growing problem for oenologists because the sugar concentration and pH are increasing faster which cause the advance of the harvest.

In this context, sparkling wine industry should take advantage of this increasing trend of consuming new and diverse sparkling wines, like sparkling wines elaborated by ancestral method (*Pét-nats)*.

Ancestral sparkling wines could also be seen as a potential resilient oenological tool to fight against climate change. It is clear that under the current conditions of climate change it is needed to maintain adequate levels of acidity because it brings freshness and ageing capacity to sparkling wines. In this sense, advancing grapes harvest has been the common response, but it this advance can sometimes affect the grape-balance originating the presence of herbaceous notes. Compared to traditional method sparkling wines, ancestral sparkling wines can be elaborated with later harvested grapes. This delay on the harvest can be considered as an advantage in the current conditions of climate change although the decrease in the acidity levels that the delay in the harvest implies. The higher pH and lower acidity can be corrected using other techniques. In addition, they may present less CO_2 fingerprint and less costs for the winery.

The hypothesis of this PhD Thesis is that ancestral sparkling wines can be an interesting procedure to obtain high quality sparkling wines especially under the current conditions of climate change. However, the lack of a well-defined protocol

to elaborate this kind of product makes necessary an optimization of the procedure.

To prove this hypothesis, the objectives of this PhD Thesis were:

1. To study the reality of sparkling wines in Catalonia elaborated using the ancestral method. The aim was to study a representative batch of commercial ancestral sparkling wines, both from the point of view of their physico-chemical composition and their sensory attributes in comparison with a representative commercial batch of sparkling wines elaborated with the traditional method.

2. To study how it can be improved the elaboration process of ancestral sparkling wines aiming to determine the critical points that condition the quality of the final product.

3. To study the influence of the yeast population introduced at bottling on the elaboration of ancestral sparkling wines.

4. To study the use of cationic exchange, applied to grape must for elaborating sparkling wines, to compensate the higher pH resulting from climate change.

Results

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Chapter 1

An approach to Ancestral sparkling wines present in the market. A comparison with traditional commercial sparkling wines

Chapter 1 shows a comparative study where commercial sparkling wines obtained through ancestral and traditional methods were analysed. The full analytic comprehended sensorial, physical, and chemical assays. This is, to our knowledge, the first study to compare these types of sparkling wines.

The first objective of this chapter was aimed to stablish a representative state of the art of the commercial products labelled under “ancestral method” in comparison with other representative ones labelled under “traditional method”. Traditional sparkling wines were useful for further comprehension of the results because they are deeply known and studied. Ancestral sparkling wines were found to be an heterogenous group in which high quality wines could be found but also faulty, turbid, or with insufficient effervescence were found. In contrast, traditional sparkling wines showed all the same style of wine with very few differences among products.

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Assessment of physicochemical and sensory characteristics of commercial sparkling wines obtained through ancestral and traditional methods

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Abstract

Sparkling wines elaborated by means Ancestral method, also called *Pét-Nats*, are nowadays gaining an increasing share of the market. However, the scientific information about these wines is very scarce. For that reason, the aim of this research was to compare the physicochemical composition and sensory attributes of a representative sampling of commercial sparkling wines elaborated by Ancestral and Traditional methods. Ancestral sparkling wines were more heterogenous than Traditional sparkling wines because some of them showed lower internal pressure, higher turbidity, higher colour intensity and less foamability. These differences would be probably because the ancestral elaboration protocol is not so well defined as in the case of Traditional sparkling wines. However, Ancestral method has the advantage of being able to work with riper grapes and with lower doses of sulphur dioxide.

Key words: Sparkling Wines, Ancestral method, *Pét-Nat*, Traditional method, physicochemical composition, Sensory quality.

Introduction

Sparkling wines market is a growing worldwide industry which at 2023 reached the US$ 39.3 Billion (Imarc, 2023; Viciana, 2023). This so large market volume can be explained through the changes in the social perception of the sparkling wines and the increase on the diversity offered inside the sparkling wines category (Vachon, 2023). While the market is still lead by Champagne and Prosecco, ancestral sparkling wines - also known by its French name *Pét-nat,* are one of these "new" products that are gaining popularity between consumers who are looking for lower alcoholic content wines and for the wine insiders who like to try particular products directly from grapegrowers (Concours Mondial de Bruxelles, 2022; Studeman, 2015).

The effervescence of high quality natural sparkling wines must be obtained through an alcoholic fermentation in sealed vessels (bottle or tank fermentation) (European council,

2009; OIV, 2023) called *prise de mousse*. Different methodologies exist depending on the way in which the vessel-sealed fermentation is performed. Traditional method, used for Champagne, Cava or Franciacorta, and *Charmat* method, used for Prosecco, are the most well-known (Bassi, Pennoni, & Rossetto, 2021; Viciana, 2023). Both methodologies follow a second fermentation over a low alcoholic base wine with an added mixture, called *liqueur de tirage,* of sugar (20-24 g of sucrose/L), preadapted yeasts (1-2 million viable cells/mL) and a riddling agent to favour the sedimentation and compact the lees at the neck of the bottle (only in the case of traditional method). The main difference between these two methodologies is that in traditional method, the second fermentation is performed in closed individual bottles while in *Charmat* method, the second fermentation is carried out in isobaric fermentation tanks, called autoclaves, which retain the generated pressure (Caliari, Panceri, Rosier, & Bordignon-Luiz, 2015).

By contrast, ancestral method, which seems to be historically the oldest procedure to elaborate sparkling wines, consists only in one fermentation (Rose, 2021). The alcoholic fermentation starts in a fermentation tank until the fermenting must's sugars level is low enough (around 18 g/L). The fermenting must is then bottled to allow the fermentation to finish on sealed bottles (Robinson & Harding, 2015) adding or not a riddling agent. Some winemakers apply a cold settling and even filter the fermenting must to reduce the yeast population and slow down the fermentation rate. In some French winegrowing regions is still preserved as part of its heritage like in AOC Blanquette de Limoux or AOC Clairette de Die.

Lately, due to its increasing popularity, some wine awards have recognized the ancestral sparkling wines production by creating its own special categories, like "Effervescent du monde ®" since 2010, "Barcelona Rosé" awards since its creation at 2022, or 50 Great Sparkling wines contest since 2022 (Forum Œnologie Association, 2023; Roset, 2024; Wine Pleasures, 2022). Other contests have not incorporated an specific category for this kind of wines but it can be found that between 2015-2018 some important prizes have started to be given to ancestral sparkling wines (Concours Mondial de Bruxelles, 2023; Decanter, 2024; International Wine Challange, 2024).

However, the procedure of elaboration of ancestral sparkling wines is not so well defined as traditional method and probably for that reason ancestral sparkling wines that can be found in the market present great heterogeneity on its parameters such as cloudiness, fizziness, aroma or colour (Asimov, 2018). Even though theoretically ancestrals should have a clear appearance and without fermentation faults (Wallace, 2020), it is common to find faulty products because of the difficulties and the precision that the process requires. The reason for that is related with the difficulty of stopping or at least slowing down fermentation kinetics before bottling at the adequate moment, and in the control of the yeast population introduced inside the bottle. The lack of control of these aspects could cause excess or underpressure in the bottles and also an excess of yeast population. An excessive population of yeast in the bottle could difficult the riddling process and consequently could cause excess turbidity in the bottle and even some times the appearance off-flavours (reduction taint) (Dubois, Flanzy, & Sablayrolles, 1998; Ribéreau-Gayon, Dubourdieu, Donèche, & Lonvaud, 2006). In fact, these difficulties were the main reason that explains the progressive abandon of the method centuries ago in favour of the traditional method. (Jeandet, Vasserot, Liger-Belair, & Marchal, 2011; Panesar, Joshi, Bali, & Panesar, 2017).

In Spain, Ancestral sparkling wines, are mainly produced in zones with old sparkling wine culture, like Catalan Cava region and its surroundings. This increasing phenomenon has lead some Protected Designation of Origin (PDO) such as PDO Penedès or PDO Tarragona to include and regulate the ancestral sparkling wines production under their normatives (DO Penedès, 2020; DO Tarragona, 2021).

Ancestral sparkling wines are not just an uprising elaboration trend but are also a way to obtain sparkling wines under warm climate conditions like it happens in the Mediterranean basin because they do not require the addition of sugar for a second fermentation. For that reason, the harvest of grapes for ancestral sparkling wines can be done later than in the case of grapes for traditional method. This later harvest allows to obtain a higher aromatic development of the berries and avoids the occasional appearance of herbaceous characters (Zamora, 2014). Furthermore, ancestral sparkling wines are usually conceived to be short aged which means that acidity levels from berries can be lower at harvest time. In addition,

the needs of sulphite addition to protect against oxidation or microbiological spoilage are much lower in ancestral sparkling wines since they do not require a stabilization time between the two fermentations. This is without any doubt a great advantage since sulphites that have been shown to have adverse effects for human health (Vally & Misso, 2012) and for environment (Stockley, 2005).

Because of its recent popularity, the scientific literature on ancestral sparkling wines is very scarce and only few scientific articles can be found (Dachery, Hernandes, Zini, Welke, & Manfroi, 2023; Makarov & Lutkov, 2021; Rossier, Maury, Gaillard, & Pfammatter, 2016). By contrast, traditional sparkling wines (Berbegal et al., 2022; Cilindre et al., 2021; Liger-Belair, 2005; Martínez-García, García-Martínez, Puig-Pujol, Mauricio, & Moreno, 2017) and other natural sparkling wine methods (Caliari et al., 2015; Cisilotto et al., 2023; Culbert et al., 2017) have been widely studied. Given this lack of information, the aim of this work was to compare the physicochemical characteristics and sensory properties of a representative amount of commercial ancestral sparkling wines elaborated in the winegrowing zone of Catalonia with young aged sparkling wines elaborated by the traditional method.

Materials and methods

Wine samples

A total of 20 white sparkling wines, 9 elaborated by traditional method and 11 by ancestral method were collected from different wine-specialized shops. The Ancestral sparkling wines were all without any PDO label. The Traditional sparkling wines were mainly from the classic blend of the PDO Cava (Xarel·lo, Macabeu and Parellada cultivars). Ancestral sparkling wines were presented with cork or with crown cap and some of them were presented with transparent bottle while Traditional sparkling samples were all presented with cork cap (mandatory) and green opaque bottle. All Traditional sparkling wines were from the 2022 vintage while Ancestrals were from different vintages. All the analyses were carried out by

triplicate using three bottles of each sample. The data from all the wines collected can be found at Table 1.

Table 1. Characteristics of studied wines

Method	Code	Variety	Prize (€)	Expedition cap	Dosification	Vintage
Ancestral sparkling wines	A.01	Garnatxa blanca	13	Crown cap	Non dosed	2022
	A.02	Macabeu	10.95	Cork	Non dosed	2022
	A.03	Xarel·lo	8.95	Crown cap	Non dosed	2022
	A.04	Parellada	7.83	Crown cap	Non dosed	2021
	A.05	Macabeu	8.96	Crown cap	Non dosed	2021
	A.06	Parellada	18.15	Crown cap	Non dosed	2022
	A.07	Macabeu & Sauvignon Blanc	4.5	Cork	Non dosed	2020
	A.08	Xarel·lo	10.85	Cork	Non dosed	2020
	A.09	Parellada	9.8	Cork	Non dosed	2022
	A.10	Macabeu & Xarel·lo	10.1	Cork	Non dosed	2022
	A.11	Xarel·lo	13.55	Crown cap	Non dosed	2022
Traditional sparkling wines	T.01	Blend 1	8.1	Cork	Brut	2022
	T.02	Blend 1	5.22	Cork	Brut	2022
	T.03	Blend 1	13.1	Cork	Brut	2022
	T.04	Blend 1	3.4	Cork	Brut Nature	2022
	T.05	Blend 1	5.5	Cork	Brut Nature	2022
	T.06	Blend 1	6.75	Cork	Brut Nature	2022
	T.07	Blend 2	6.7	Cork	Brut	2022
	T.08	Blend 1	6	Cork	Brut	2022
	T.09	Blend 1	4.95	Cork	Brut Nature	2022

Blend 1 stands for Xarel·lo, Macabeu & Parellada wines while Blend 2 stands for Macabeu and Parellada coupage

Sample preparation

All wine samples were centrifuged at 13,000xg (Biofuge Primo centrifuge, Heraeus, Hanau, Germany) for 15 min at 4ºC to obtain clear samples and to remove carbon dioxide.

Analysis of general wine parameters

The internal CO_2 pressure of the bottles was measured using a non-invasive Laser Sensor (L.Sensor CO2, FTSystem, Alseno, Italy). The ethanol content was determined by ebulliometry (GAB Analysis Systems, Moja-Olerdola, Barcelona, Spain). Turbidity was measured with a 2100N IS TURBIDIMETER (HACH, Loveland,CO, USA). Titratable acidity and pH were determined following the OIV recommended methods (OIV, 2023). The total sulphur dioxide content was determined using a commercial kit (GAB Analysis Systems, Moja-Olerdola,

Barcelona, Spain). The concentrations of residual fermentable sugars (D-glucose and D-fructose), glycerol, gluconic acid, L-(+)-tartaric acid, L-Malic acid, L and D Lactic acid, citric acid and acetic acid were determined using Y15 Autoanalyser (Biosystems, Barcelona, Spain).

Colour parameters

The CIEL*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. Total Polyphenol index (TPI) was determined by the dilution 1:10 of each sample and the measurement at 280 nm via spectrophotometer. The colour of each one of the samples was reproduced in power point software using the RGB signals after transforming the CIEL*a*b* coordinates (ColorMine.org).

Quantification of proteins by HRSEC-DAD

The samples were processed and analysed using the methodology described by Canals (1998). fifteen mL of each sample were concentrated in triplicate following a two steps dialysis in tubes with a molecular weight cutoff of 3.5 kDa (Spectrum Laboratories Inc., Rancho Dominguez, CA, USA). The first step lasted 48h with 0.3 M ammonium acetate ≥ 98.0 % (Sigma–Aldrich, Madrid, Spain) solution with a rate of 1:10 (sample:solution) and constant agitation. The second step was carried out with water for another 48h. The dialyzed samples were subsequently lyophilised and preserved at −20 °C. The soluble fractions were analysed by high-resolution size-exclusion chromatography (HRSEC) in order to determine the molecular distribution and quantify the proteins obtained from the samples. The lyophilized samples were resuspended in 0.6 µL of ammonium acetate solution (300 mM) and centrifuged (12,000 g for 5 min). The supernatant was filtered through 0.22 µm acetate cellulose filters (Merck Millipore, Darmstadt, Germany) and then 100 µL of

supernatant was injected into the chromatographic system. The analyses were carried out in HPLC Agilent 1200 Series system (Agilent Technologies Inc., Santa Clara, USA) equipped with a G1311A quaternary pump, a G1316A column oven, a G1329A autosampler (Agilent Technologies, Santa Clara, CA, USA) and with a diode array detector (G1315D - DAD) to monitor output at 230 and 320 nm. Separation was carried out at 20 °C using an S 165 Shodex gel permeation HPLC column 210 (OHpak 166 SB-803 HQ, 300 mm × 8 mm i.d.; Showa Denko, Tokyo, Japan). The mobile phase consisted of an aqueous solution of 300 mmol/L ammonium acetate applied at a constant flow of 0.6 mL/min for 70 min.

Polysaccharide extraction and determination by HRSEC-RID

The samples were processed using a variation of the methodology described by Ayestarán et al., (2004). Briefly, 1 mL of sample was frozen to -20^{o}C and then freeze-dried using a lyophilizer (Telstar LyoQuest HT40, Barcelona, Spain). The pellet was resuspended with 0.2 mL of ultra-pure water and 1 mL of cold acidified ethanol (hydrochloric acid 0.3 M in absolute ethanol) and kept for 24 h at 4°C to allow soluble polysaccharides precipitation. Samples were centrifugated (14,000 RPM) 10 minutes, the supernatant was discarded and the pellet was dried using heating block (70^{o}C). The pellet was resuspended in mL of 50 mM ammonium formate ≥ 99.0 % (Sigma–Aldrich, Madrid, Spain) and filtered through 0.22 µm acetate cellulose filters (Merck Millipore, Darmstadt, Germany). Then 100 µL were injected into the chromatographic system. The analyses were carried out in an HPLC Agilent 1200 Series system (Agilent Technologies Inc., Santa Clara, USA) equipped with a G1311A quaternary pump, a G1316A column oven, a G1329A autosampler (Agilent Technologies, Santa Clara, CA, USA) and with a refractive index detector (G1362A - RID). Separation was carried out at 20 °C using two Shodex gel permeation HPLC columns (OHpak SB-186 803 HQ and SB-804 HQ, 300 mm × 8 mm I.D.; Showa Denko, Japan). The mobile phase consisted of an aqueous

solution of 50 mM ammonium formate applied with a constant flow of 0.6 mL/min for 60 min, and the cell RID temperature was 35 °C.

Volatile compound analysis by gas chromatography

Volatile compounds were extracted using a modification of the methodology described by Ortega et al., (2001). the volatile compounds were liquid/liquid extracted with 400 µL of dichloromethane in presence of 2.5 g $(NH_4)_2SO_4$ using 4-methyl-2-pentanol (0.8 g/L), heptanoic acid (0.7 g/L) and heptadecanoic acid (0.7 g/L) as internal standards.

The organic phase was extracted and 2 µL was injected in split mode (10:1, 30 mL/min) into a gas chromatograph (Agilent Technologies Inc., Santa Clara, USA) with a FFAP column of 30 m × 0.25 mm × 0.25 µm. All aromatic volatile compounds were identified and quantified by comparison with standards. They included fatty acids ethyl esters (ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate and ethyl dodecanoate), fusel alcohols (cis-3-hexen-1-ol, 2-phenylethanol and 1-hexanol), acetate esters (isoamyl acetate, hexyl acetate, 2-phenyletanol acetate), short-chain fatty acids (propionic, isobutyric, butyric, 3-methyl butanoic and valeric acids), medium-chain fatty acids (hexanoic, octanoic, decanoic and dodecanoic acids) and long-chain fatty acids (myristic acid, palmitic acid, stearic acid and oleic acid).

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, Poinsaut, Dantan, Brissonnet, & Cossiez (1990). Two parameters were measured: HM, the maximum foam height, and HS, the stable foam height. HM represents foamability while HS represents foam stability.

Sensory analysis

All the samples were tasted by a trained panel of 15 tasters, nine males and six females aged between 24 and 60. For each sample, the tasters were required to evaluate the intensity of eight sensory attributes (Colour, Bubble size and stability, reduction/oxidation balance, ageing impact, gas aggressivity, body, and acidity) and 4 aromatic attributes (tropical fruit, aniseed, white fruits, yeast/bread) on a scale of 1 to 10 (1 = "slight intensity", 10 = "maximum intensity". In the case of Reduction/oxidation balance, the scale goes from evident reduction notes (1) to high oxidation notes (10). The value of each descriptor was expressed as the average of all tasters. The sensory analysis was performed by triplicate in order to avoid random results.

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 version 2022.5.1 (Addinsoft, Paris, France). The sensorial analysis results were analysed also with the PanelCheck V1.4.2 software (Nofima Mat, Technical University of Denmark & University of Copenhagen).

Results and discussion

General parameters

All figures include only the average ± standard deviation of the different parameters of both types of sparkling wines. The results for all the studied sparkling wines are shown as Supplementary Tables.

The results corresponding to the general parameters are shown in Fig. 1. The individual results for general parameters of all the studied sparkling wines are shown in Supplementary Table 1. No significant differences were found between ancestral and traditional sparkling wines for ethanol content (Fig 1.A) and total

titratable acidity (Fig.1.B). Neither significant difference was found for residual sugars (Fig 1.C) even though some of the traditional sparkling wine samples were classified as “Brut” and therefore contained some added sugar.

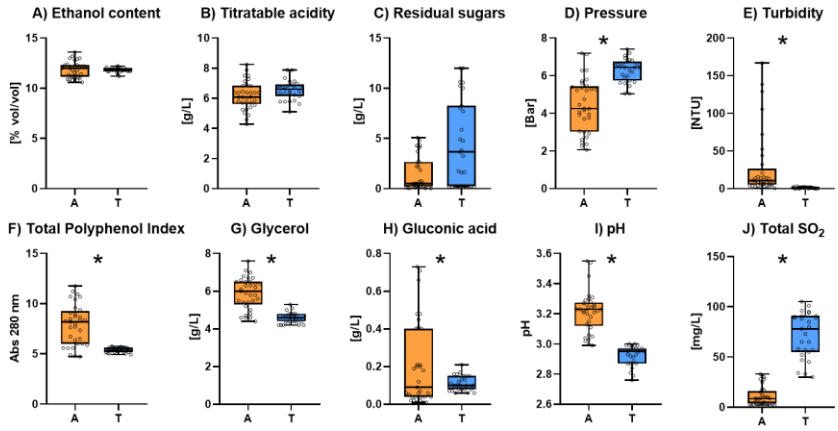

Fig. 1. Box-plots for standard parameters of sparkling wines elaborated by ancestral (A, n = 11) or traditional (T, n = 9) methods. The presence of an asterisk indicates the existence of significant differences at $p < 0.05$.

The average internal pressure (Fig 1.D) of Traditional sparkling wines was significantly higher than that of ancestral sparkling wines. In addition, all the Traditional sparkling wines accomplished with the normative (European council, 2009) having a homogeneous internal pressure above 3.00 bar whereas Ancestral sparkling wines showed greater heterogeneity being some of the samples below the minimal value.

Fig 1.E shows the turbidity of the different samples. Turbidity of Traditional sparkling wines was always below 5 NTU which should be considered as a clear appearance for the consumers. In contrast, the turbidity of Ancestral sparkling wines was more heterogenous, and the average was significantly higher than that of Traditional sparkling wines. Some of the Ancestral sparkling wines were also below 5 NTU but others had turbidity levels over 100 NTU which could lead to unstable and faulty wines. It should be highlighted that although turbidity is not regulated by OIV, a high turbidity is considered a fault (Jackson, 2009).

The total polyphenol index (TPI) (Fig 1.F) was also significantly higher in Ancestral sparkling wines than in Traditional ones. This higher value can be related to the higher turbidity of these sparkling wines probably because of the lack of cold settling before fermentation or because the lower use of fining agents according with the minimal intervention philosophy of most of Ancestral sparkling wine producers. A high TPI in white wines is also related to a loss of sensorial attributes in wines and higher colour oxidability (Gutiérrez-Escobar, Aliaño-González, & Cantos-Villar, 2021).

Glycerol (Fig 1.G) content was found to be significantly higher on Ancestral sparkling wines than in Traditional ones. Glycerol is mainly produced by yeast throughout glyceropyruvic pathway (Zamora, 2009). It has been described that glycerol is in part produced by yeast to compensate the osmotic pressure of the fermenting matrix (Remize, Roustan, Sablayrolles, Barre, & Dequin, 1999). A possible explanation of the higher levels of this metabolite may be therefore related with the fact that Ancestral sparkling wines have got only one alcoholic fermentation step with an initial total sugar concentration higher than Traditional sparkling wines. Glycerol can also be produced when the grapes are infected by *Botrytis cinerea* (Nieuwoudt, Prior, Pretorius, & Bauer, 2017). Consequently, another possible explanation may be related with the fact that grapes for Ancestral sparkling wine production are usually harvested later than those for traditional sparkling wines increasing the risk of appearance of this filamentous fungus. This last data is confirmed by the significantly higher levels of gluconic acid (Fig 1.H) in Ancestral sparkling wines since this acid is considered as a marker of the presence of *B. cinerea* (Esteruelas et al., 2015).

Ancestral sparkling wines had also significant higher pH levels (Fig 1.I). This data could be easily explained because of the harvest date of the grapes for ancestral sparkling wines is generally later than for traditional sparkling wines. Total Sulphur

dioxide content (Fig 1.J) was found to be significantly lower in Ancestral sparkling wines, always below 30 mg/L. These lower levels of sulphur dioxide are mainly because Ancestral sparkling wines are directly bottled in the adequate moment of the fermentation and therefore, they do not need the addition of this additive to protect them during the stabilization period (Cisilotto et al., 2021).

Acidic characterization of sparkling wines

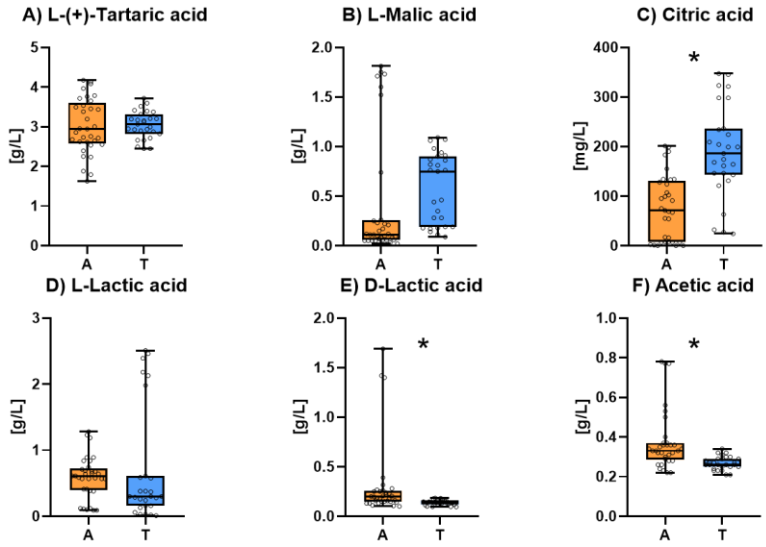

Fig. 2. Box-plots for organic acids of sparkling wines elaborated by ancestral (A, n = 11) or traditional (T, n = 9) methods. The presence of an asterisk indicates the existence of significant differences at $p < 0.05$.

The concentration of the major acids of the different sparkling wines is showed in **Fig. 2**. The individual results for acidic composition of all the studied sparkling wines are shown in Supplementary Table 2. The levels of L-(+)-tartaric acid(Fig 2.A), L-Malic acid (Fig 2.B) and L-lactic acid (Fig 2.C) were similar in both types of sparkling wines whereas the concentration of citric acid (Fig 2.D) was significantly higher in Traditional sparkling wines. It should be highlighted that in both groups there were samples in which malolactic fermentation was carried out and other samples in which it was not.

The average content of D-lactic acid (Fig 2.E) and acetic acid (Fig 2.F) were significantly higher in Ancestral sparkling wines. Nevertheless, this difference was mainly due to one of the Ancestral samples in which lactic taint seems to have been produced (D-lactic acid levels over 1g/L and acetic acid levels around 0.8 g/L). Excessive levels of D-lactic acid are related to the metabolism of D-Fructose produced by an uncontrolled growth of lactic acid bacteria in the presence of sugars. Acetic acid is also generated when heterofermentative lactic acid bacteria metabolize D-fructose (Bartowsky, 2009; Delfini, Schellino, Minetto, Pagliara, & Ambro, 2002).

Colour

Fig. 3 shows the Cie*L*a*b** coordinates values and the colour appearance of the different sparkling wines. The individual results for colour parameters of all the studied sparkling wines are shown in Supplementary Table 3. Lightness (*L**) (Fig 3.A) of Ancestral sparkling wines was significantly lower and the blue-yellow component of the colour (*b**) (Fig 3.C) significantly higher than in Traditional sparkling wines whereas no significant differences were found in the green-red component (*a**) (Fig 3.B). These data indicates that the colours of Ancestral sparkling wines were in general less clear and more yellowish than in Traditional sparkling wines as it can be seen in the picture (Fig 3.D). This larger palette of colours observed in Ancestral sparkling wines reflects more brownish tonalities in some of these wines which could be related to the lower sulphur dioxide usage that could entail a higher oxidation (Giménez et al., 2022).

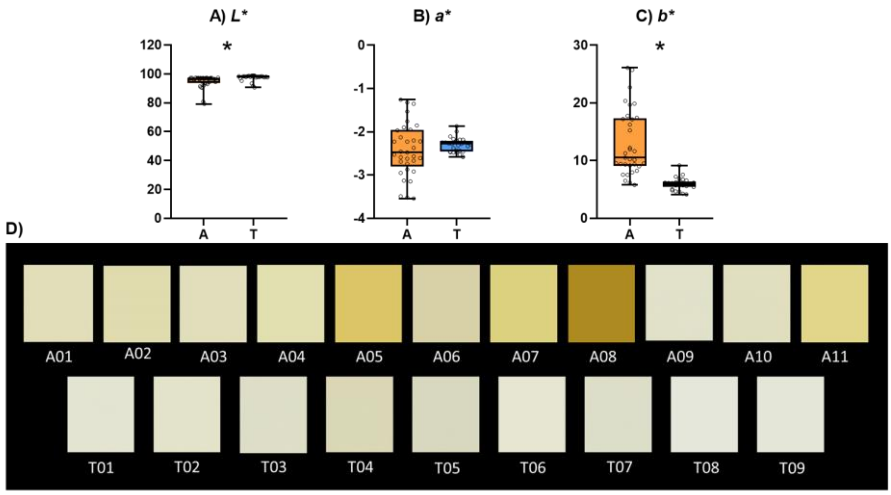

Fig. 3. Box-plots for colour coordinates (**A-C)** and the palette of colours in RGB signals **(D)** of sparkling wines elaborated by ancestral (A, n = 11) or traditional (T, n = 9) methods. The presence of an asterisk indicates the existence of significant differences at $p < 0.05$.

Proteins

Protein and peptide content determined via HPLC are showed in Fig. 4. The individual results for protein and peptide composition of all the studied sparkling wines are shown in Supplementary Table 4. These results show that Ancestral sparkling wines had significantly higher protein content than Traditional sparkling wines. Total proteins (Fig. 4. A) were around an average of 30 mg/L in Ancestral sparkling wines while in Traditional wines the average content was around 20 mg/L. All molecular weight protein fractions (Fig. 4. C, Fig. 4. D, Fig. 4. E) showed a similar behaviour, being high (HMW) and the low molecular weight (LMW) fractions the most abundant. Peptide content (Fig. 4. B) was also found to be significantly higher in Ancestral sparkling wines

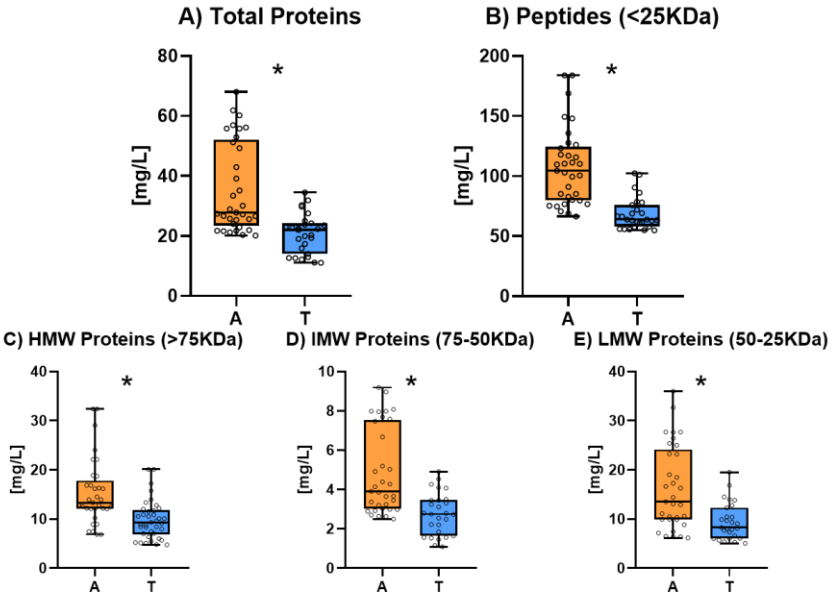

Fig. 4. Box-plots for proteins and peptides of sparkling wines elaborated by ancestral (A, n = 11) or traditional (T, n = 9) methods. The presence of an asterisk indicates the existence of significant differences at $p < 0.05$.

This higher concentration of proteins and peptides may be due to two different reasons. In one hand, the base wines of Traditional sparkling wines are usually treated with bentonite for fining and riddling which eliminates a substantial amount of natural wine occurring proteins (Salazar, Zamora, Canals, & Lopez, 2010; Vanrell et al., 2006). By contrast, Ancestral sparkling wines are not usually bentonite fined and some elaborators neither add bentonite to favour lees sedimentation during riddling. On the other hand, it is quite probable that some of the Ancestral sparkling wines were bottled with higher yeasts populations than Traditional sparkling wines which should favour the release of proteins and peptides from yeast autolysis (Fornairon-Bonnefond, Camarasa, Moutounet, & Salmon, 2002; V. Moreno-Arribas, Pueyo, & Polo, 1996; Pons-Mercadé et al., 2022).

Polysaccharides

Fig. 5 shows the polysaccharide and oligosaccharide content. The individual results for polysaccharide and oligosaccharide composition of all the studied sparkling wines are shown in Supplementary Table 5. Ancestral sparkling wines had significantly higher concentrations of polysaccharides (Fig. 5. A) (from 200 to 600 mg/L) than Traditional method sparkling wines (all around 200 mg/L). Oligosaccharide content (Fig. 5. B) was also significantly higher for Ancestral Sparkling wines. The reasons why ancestral wine has significantly higher levels of these molecules would be the same as those that have been put forward for proteins.

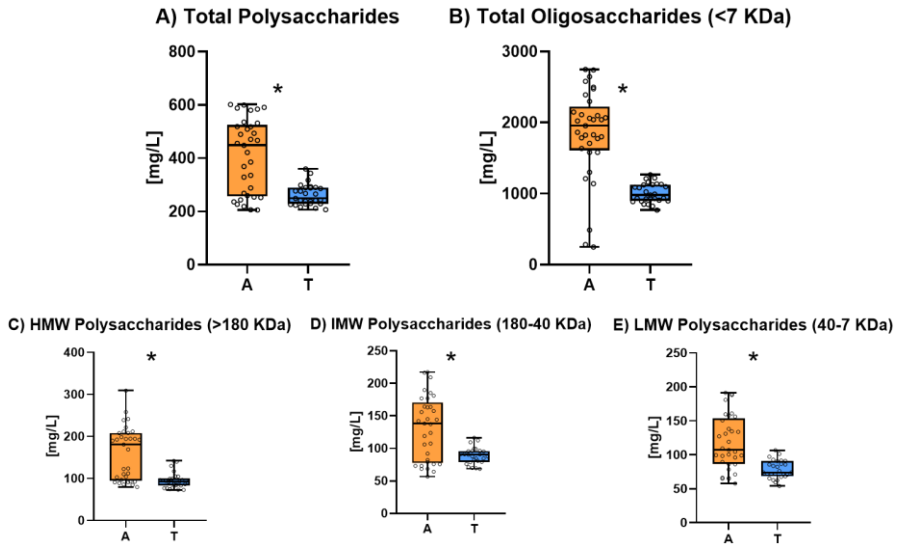

Fig. 5. Box-plots for polysaccharides and oligosaccharides of sparkling wines elaborated by ancestral (A) or traditional (T) methods. The presence of an asterisk indicates the existence of significant differences

Volatile substances

Fig 6 shows the volatile substances composition of both types of sparkling wines. The volatile substances were grouped in this figure in six families. The individual results for each one of the six families of all the studied sparkling wines are shown

in Supplementary Table 6. No significant differences were found in the concentration of fatty acids ethyl esters (Fig. 6. A) and total higher alcohol acetates (Fig. 6. B). However, Ancestral sparkling wines showed higher levels of higher alcohols (Fig. 6. C) whereas Traditional sparkling wines showed significantly higher levels of short (Fig. 6. D), medium (Fig. 6. E) and long-chain fatty acids (Fig. 6. F).

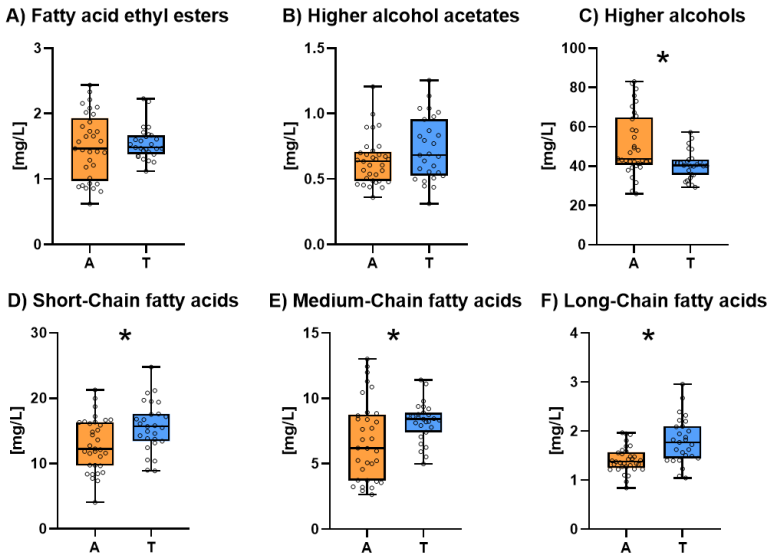

Fig. 6. Box-plots for volatile substances of sparkling wines elaborated by ancestral (A) or traditional (T) methods. The presence of an asterisk indicates the existence of significant differences.

Higher alcohols are the result of amino-acidic metabolism of yeasts when the nitrogen is scarce during fermentation (Ciani & Comitini, 2019). They are considered as not pleasant aromas once they achieve concentrations above their threshold (300 mg/L) and some authors described them as foam antagonists in sparkling wines (Maujean, Poinsaut, Dantan, Brissonnet, & Cossiez, 1990a). Nevertheless, their concentration in Ancestral sparkling wines was always below 100 mg/L, which means it should not affect negatively the aromatic profile of the wines but their presence could affect its foamability. It should be also highlighted that there is a close relationship between turbidity during alcoholic fermentation

and higher alcohols concentration (Nicolini, Moser, Román, Mazzi, & Larcher, 2011). In fact, non-settled grape musts give rise to wines with a higher concentration of higher alcohols (Bertrand, 1978). This data suggest that the grape must of some of the ancestral sparkling wines was not well settled.

Fatty acids generally are mainly produced by yeast during alcoholic fermentation but can also be released from grape skin (Rid, Markheiser, Hoffmann, & Gross, 2018). Short (SCFA) and medium (MCFA) chain fatty acids have been described as toxic for yeast being sometimes responsible of stuck and sluggish fermentations (Bisson, 1999; Lafon-Lafourcade, Geneix, & Ribéreau-Gayon, 1984). By contrast, long-chain fatty acids (LCFA) are principal constituents of the yeast cell membrane and its presence increases with aereation during fermentation (Restrepo, Espinoza, Ceballos, & Urtubia, 2019). A possible explanation of the higher levels of fatty acids in Traditional sparkling wines could be that these wines have been elaborated by a refermentation of a base wines which implies a higher stress whereas Ancestral sparkling wines have been obtained with only one fermentation. Another possibility is that ancestral sparkling wines, which have a greater population of yeasts than Traditional wines, have absorbed more fatty acids (Lafon-Lafourcade et al., 1984; Martín-Garcia, Abarca-Rivas, Riu-Aumatell, & López-Tamames, 2023).

Foaming properties

The maximal height of the foam (HM) (Fig 7.A) that represents the wine foamability and stable height of the foam (HS) (Fig 7.B) that represents the foam stability of both types of sparkling wines are shown in Fig. 7. The individual results for HM and HS of all the studied sparkling wines are shown in Supplementary Table 7.

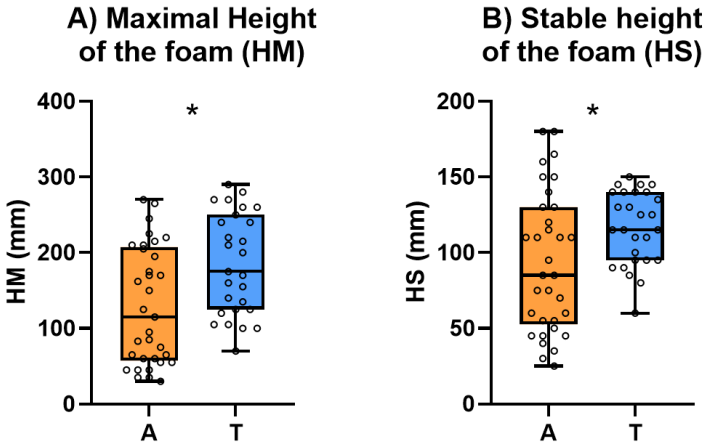

Fig. 7. Box-plots for foaming properties of sparkling wines elaborated by ancestral (A, n = 11) or traditional (T, n = 9) methods. The presence of an asterisk indicates the existence of significant differences at $p < 0.05$.

In general, Traditional sparkling wines had significantly higher values of HM and HS than Ancestral sparkling wines although there was a great heterogeneity in both groups. It has been described that peptides, proteins and mannoproteins favour the integration of carbon dioxide improving foamability (Kemp et al., 2019; Martínez-Rodríguez, Carrascosa, Barcenilla, Angeles Pozo-Bayón, & Carmen Polo, 2001). Consequently, the higher HM and HS of Traditional sparkling wines is an unexpected result because their peptide, protein and polysaccharide concentration were significantly higher than in Ancestral sparkling wines. However, it must be highlighted that Ancestral sparkling wines have significant higher levels of gluconic acid than Traditional sparkling wines. Gluconic acid is an indicator of the development of *B. cinerea* in the grapes and it is well known that their presence affects negatively the foamability of sparkling wines (Cilindre, Castro, Clément, Jeandet, & Marchal, 2007; Esteruelas et al., 2015). In addition, Ancestral sparkling wines also have significantly higher concentration of higher alcohols which have been reported as negative for foamability (Maujean, et al, 1990a).

Sensorial analysis

Sensorial analysis results are shown in the Fig. 8 in form of a spider web chart in which the average value for each one of the sensory attributes is indicated by means of a continuous line whereas the standard deviation is showed as a shadowed ring surrounding it. In general, Traditional sparkling wines showed greater homogeneity than Ancestral sparkling wines as it can be noticed by the narrower shadowed ring. The panel scored very similarly the following sensory attributes: gas aggressivity, reduction/oxidation balance, body, acidity, tropical fruit and aniseed notes. By contrast, the panellists considered that Ancestral sparkling wines showed more intense colour, ageing impact and yeast-bread aroma, and less intense notes of white fruit. They also considered that Ancestral sparkling wines have smaller bubble size and lower bubble stability.

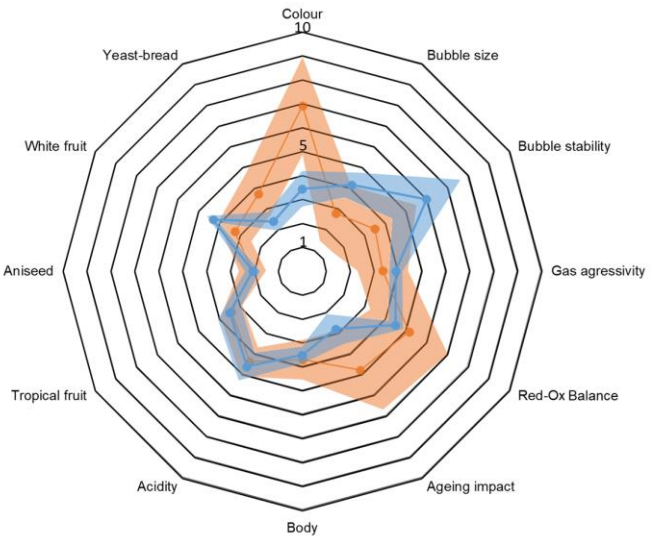

Fig. 8. Spider web chart for the sensorial analysis results. The average value for each one of the sensory attributes is indicated by a continuous line whereas the standard deviation is showed as a shadowed ring surrounding it. Ancestral sparkling wines are marked in orange. Traditional sparkling wines are marked in blue.

Principal component analysis

In order to better understand the differences between Ancestral and Traditional sparkling wines a principal component analysis was performed. Fig. 9 shows the

plot of varimax-rotated corresponding to the principal components analysis. The first component (PC1) explains 44.89% of the variance, and the second (PC2) explains 15.95% (so the explained aggregate variance was 63.61% for the two components). The loadings are presented as arrows, the length and direction of which indicate the contribution made by both components. PC1 showed a significant correlation with all variables except total higher alcohol acetates. Specifically, PC1 was positively correlated with gluconic acid, total proteins, total polysaccharides, chroma (*C**), pH, turbidity and total higher Alcohols. In turn, PC2 showed positive significant correlation with gluconic acid, total proteins and pressure and negative significant correlation with total higher alcohol acetates, total higher alcohols and turbidity. With regard to the different variables, total polysaccharides, chroma, pH, turbidity and total higher alcohols showed a strong negative correlation with HM, SO_2 and pressure. In addition, gluconic acid and total proteins had a negative correlation with total higher alcohol acetates.

All the samples corresponding to the Traditional sparkling wines group were placed in a relatively small confidence ellipse (95%) which indicates the existence of a great homogeneity among the samples. By contrast Ancestral sparkling wines were more ubiquitously placed throughout the entire diagram which confirms the much greater heterogeneity among the samples.

It must be highlighted that the arrows corresponding to pressure, SO_2 and HM were directed towards the left coinciding with the location of the Traditional sparkling wines. This data indicates that Traditional sparkling wines have higher pressure and sulphur dioxide and better foamability than Ancestral sparkling wines. The arrow corresponding to total higher alcohol acetates was directed also towards the left, but downwards, indicating that Traditional Sparkling wines are richer in these volatile substances. By contrast, the arrows corresponding to gluconic acid, total proteins, total polysaccharides, chroma, pH, turbidity and total

higher alcohols were directed to the right, indicating that the samples placed there have higher levels of all these parameters. It must be highlighted that most of the Ancestral sparkling wines were located to a greater or lesser extent at the right side of the diagram. However, some of the Ancestral sparkling wines were placed on the left side, very close to the Traditional sparkling wines confidence ellipse. This data confirms that some of the Ancestral sparkling wines were very similar to Traditional sparkling wines whereas others were quite different.

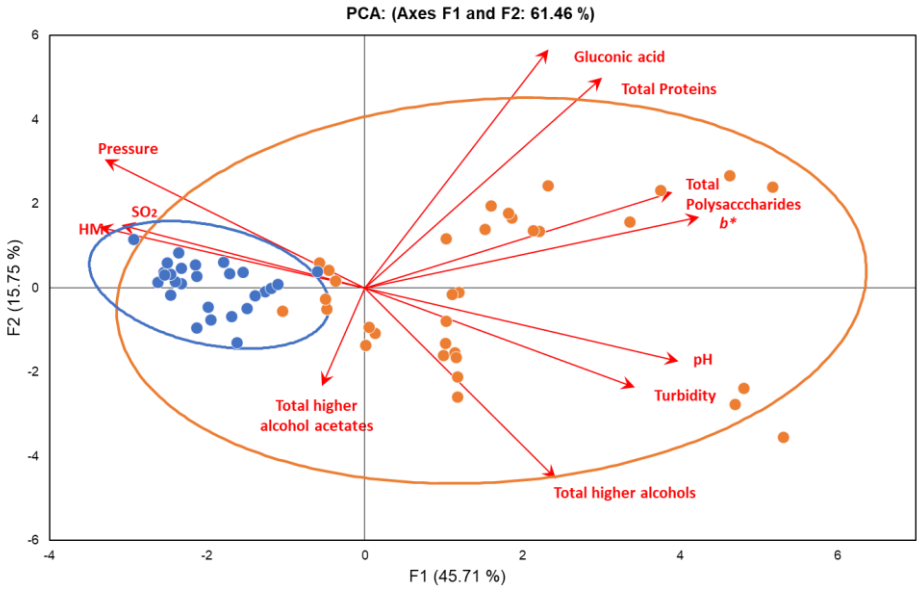

Fig. 9. Plot of varimax-rotated principal component analysis for the different sparkling wines. Orange points: Ancestral sparkling wines. Blue points: Traditional sparkling wines

Conclusions

The physicochemical and sensory analyses of different sparkling wines elaborated by Traditional or Ancestral methods reveal the existence of some differences between them. Traditional sparkling wines are much more homogeneous than Ancestral sparkling wines having in general higher values of pressure, sulphur dioxide and better foamability. By contrast, Ancestral sparkling wines were much

more heterogenous showing in general higher values of gluconic acid, total proteins and polysaccharides, more intense yellow colour, pH and higher turbidity. However, these differences are mainly due to the fact that Ancestral method is not so well defined as Traditional method making that sometimes the quality of the product can be affected seriously. More specifically, the control of the sugar concentration and yeast population at bottling, are critical points that must be better defined in the elaboration protocols of Ancestral wines because they conditionate the final internal pressure and probably the turbidity of the product. This lack of definition in the Ancestral sparkling wine elaboration procedure may be responsible of the greater heterogeneity of these wines and that even in some cases they present some taints. However, Ancestral method allows working with riper grapes which represents a great advantage in the face of climate change. An additional advantage is that Ancestral methods allows working with lower doses of sulphur dioxide. These advantages make today the Ancestral method an interesting alternative to the Traditional method for the production of sparkling wines.

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UNIVERSITAT ROVIRA I VIRGILI
ANCESTRAL SPARKLING WINES; COMPARISON WITH TRADITIONAL SPARKLING WINES AND PROCEDURES TO IMPROVE THEIR QUALITY
Arnau Just Borràs

Chapter 2

A proposal for improvement on the ancestral method; comparison with the traditional method

After finding that Ancestral sparkling method undefinition may lead to bad oenological praxis and consequently faulty products, it was necessary to find the critical points that may affect the quality of the wine (objective 2) in order to correct it. In accordance with our opinion, the chosen bottling time and the yeast population that performs the bottle fermentation are the most important critical points for ancestral sparkling wine production. It is obvious that the bottling moment conditions the fermentable sugar concentration. An excess of sugars will lead to bottle overpressure which can cause an excess of CO2 effervescence and even bottle explosions. In contrast, an insufficient sugar concentration will cause a lack of sparklingness. To our knowledge, the influence of the yeast population is less known. It can be assumed that a very high yeast population can cause difficulties in the disgorgement (high turbidity) and even reduction taint and for that reason a reduction of the total yeast population should be applied. However, no information exists about which is the best yeast population. With this aim, a study about the influence of yeast population into the bottle on the sensorial and physicochemical attributes of the wine (objective 3) was performed.

For that reason, two ancestral sparkling wines with different yeast population inside the bottle and one traditional sparkling wine were obtained from the same grape juice

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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.

Keywords: Sparkling wines; Ancestral method; Traditional method; Wine composition; Foaming properties

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, Dubourdieu, Donèche, & Lonvaud, 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, Alexandre, Robillard, & Marchal, 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).

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 & Harding, 2015).

However, the ancestral method was abandoned by most producers due to the difficulties involved in controlling this process (Jeandet, Vasserot, Liger-Belair, & Marchal, 2011; Panesar, Joshi, Bali, & Panesar, 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, Flanzy, & Sablayrolles, 1998; Ribéreau-Gayon et al., 2006). 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, White, Cooper, & Storchmann, 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 degrees more alcohol, and therefore it is necessary to harvest the grapes with a potential alcohol level not exceeding 11.0% (Esteruelas et al., 2015). 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 & 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, González-Royo, Gil, et al., 2015; Marchal et al., 2001; Kemp et al., 2015; Martínez-García, García-Martínez, Puig-Pujol, Mauricio, & Moreno, 2017; Medina-Trujillo, Matias-Guiu, López-Bonillo, Canals, & Zamora, 2017; Liger-Belair, & Cilindre, 2021 ; Wilson, Charnock, Xu, & Kemp, 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, Hernandes, Zini, Welke, & Manfroi, 2023; Makarov & Lutkov, 2021; Rossier, Maury, Gaillard, & Pfammatter, 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.

Material and methods

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

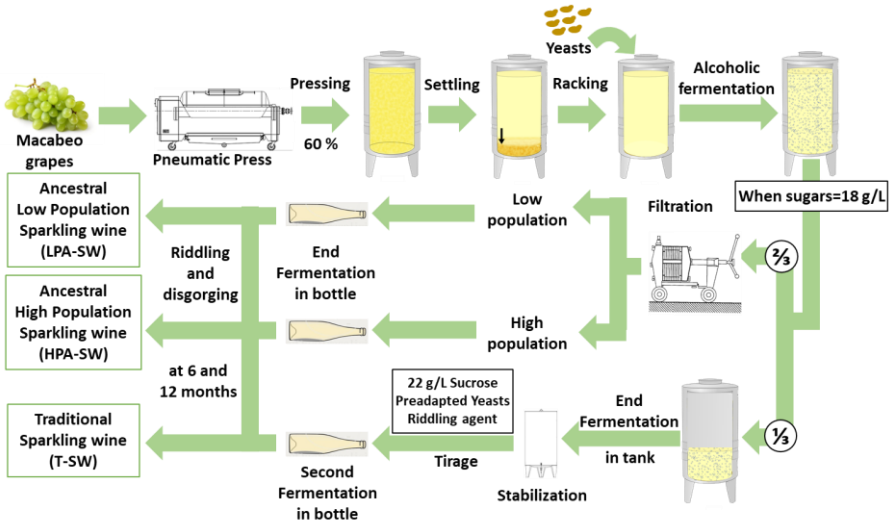
acid (purity ≥99.0%) were purchased from Agrovin (Alcázar de San Juan, Ciudad Real, Spain). Ethanol (purity ≥99.5%), hydrochloric acid (purity ≥37.0%), NaOH (purity ≥98.0%), sulphuric acid (purity ≥96.0%) and $CuSO_4 \cdot 5\ H_20$ (purity ≥99.0%) were purchased from Panreac (Castellar del Vallès, Barcelona, 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).

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-510BF, Optika, Ponteranica, Italy). The total yeast cell population was calculated considering the applied dilution factor.

Experimental design

Figure 1. 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 °Brix, 3.32 pH and a titratable acidity of 5.2 g/L (expressed as tartaric acid). Figure 1 illustrates the experimental design.

The grapes bunches were crushed (Delta E2, Bucher Vaslin, 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 hours. 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^{o}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 6.0x10^6 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 12.0x10^6 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^{o}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 4ºC 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 $2.0x10^6$ 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-$7.0x10^6$ cell/mL depending of the nitrogen content and temperature (Valade & Laurent, 1999; Martínez-Rodríguez, Carrascosa, Martín-Álvarez, Moreno-Arribas & Polo, 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 15-16ºC 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 ºC, 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 –28ºC 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.

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 4ºC 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).

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 (Δ*Eab**) was calculated according to Martínez, Melgosa, Pérez, Hita, & Negueruela, (2001).

Quantification of proteins, polysaccharides and mannose by HPLC

The protein measurement was processed and analysed by HRSEC-DAD using the methodology described by Canals, Zamora & Arola (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).

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, Poinsaut, Dantan, Brissonnet, & Cossiez (1990). Two parameters were measured: HM, the maximum foam height, and HS, the stable foam height. HM represents foamability while HS represents foam stability.

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).

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 ºC. 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 to 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.

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).

Results and discussion

Physicochemical analyses were performed at six months of ageing (minimum 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.

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, Deroover, & Stockley, 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).

Table 1. General parameters

Parameter	Traditional		Ancestral			
	T-SW		HPA-SW		LPA-SW	
CO_2 presure (bars)	5.86 ± 0.15	**B**	4.80 ± 0.17	**A**	4.84 ± 0.14	**A**
Ethanol (% v/v)	12.2 ± 0.2	**B**	10.7 ± 0.1	**A**	10.7 ± 0.1	**A**
Glycerol (g/L)	7.68 ± 0.15	**A**	6.90 ± 0.65	**A**	7.01 ± 0.40	**A**
Residual sugars (g/L)	0.35 ± 0.02	**B**	0.25 ± 0.01	**A**	0.24 ± 0.04	**A**
Total SO_2 (mg/L)	39 ± 1	**B**	28 ± 1	**A**	27 ± 1	**A**
Titratable acidity (g of tartaric acid/L)	6.15 ± 0.01	**C**	5.93 ± 0.04	**A**	6.05 ± 0.02	**B**
pH	2.99 ± 0.02	**A**	2.95 ± 0.02	**A**	2.98 ± 0.02	**A**
L-(+)-Tartaric acid (g/L)	4.64 ± 0.11	**A**	4.79 0.05	**A**	4.71 ± 0.02	**A**
L-Malic acid (g/L)	0.50 ± 0.11	**A**	0.33 ± 0.09	**A**	0.43 ± 0.10	**A**
L-Lactic acid (g/L)	0.04 ± 0.01	**A**	0.20 ± 0.02	**B**	0.22 ± 0.02	**B**
Citric acid (g/L)	0.19 ± 0.02	**B**	0.08 ± 0.01	**A**	0.10 ± 0.02	**A**
Acetic acid (g/L)	0.37 ± 0.01	**A**	0.39 ± 0.02	**A**	0.39 ± 0.01	**A**
Fumaric acid (g/L)	0.08 ± 0.04	**B**	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 ($p < 0.05$).

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, 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, Tian, Qin, & Han, 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., 2006). 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 & 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, Verheijen, Heijnen, & Van Gulik, 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.

Colour parameters

Table 2. CIE*L*a*b** Coordinates

CIE*L*a*b** coordinates	Time (months)	Traditional T-SW	Ancestral HPA-SW	Ancestral LPA-SW
L*	6	99.40 ± 0.23 **A** α	99.20 ± 0.05 **A** β	99.20 ± 0.05 **A** α
	12	98.50 ± 0.50 **A** α	97.90 ± 0.40 **A** α	97.70 ± 0.80 **A** α
a*	6	-0.70 ± 0.10 **B** β	-0.88 ± 0.02 **A** β	-0.92 ± 0.03 **A** β
	12	-2.70 ± 0.60 **A** α	-3.00 ± 0.30 **A** α	-2.90 ± 0.60 **A** α
b*	6	4.49 ± 0.07 **A** α	4.35 ± 0.10 **A** α	4.38 ± 0.09 **A** α
	12	6.98 ± 0.04 **A** β	6.85 ± 0.02 **A** β	7.62 ± 0.12 **B** 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.

Table 2 shows the CIE*L*a*b** coordinates and Table 3 the total colour difference (Δ*Eab**) 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 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, Aguilera-Curiel, Riu-Aumatell, Buxaderas, & López-Tamames, 2013), confirming a fact that winemakers know well: the longer the ageing time, the more intense the yellow colour (Kanavouras, Coutelieris, Karanika, Kotseridis, & Kallithraka, 2020).

Table 3. Total colour differences (Δ*Eab**)

A. Δ*Eab** between elaboration methods

Comparison	6 months	12 months
T-SW vs HPA-SW	0.30	0.68
T-SW vs LPA-SW	0.32	1.04
HPA-SW vs LPA-SW	0.05	0.91

B. Δ*Eab** between ageing times

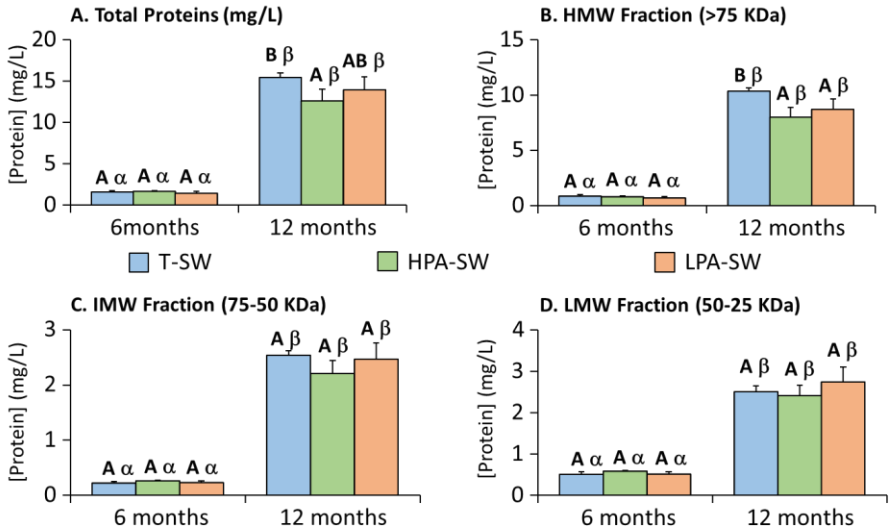
Comparison	T-SW	HPA-SW	LPA-SW
6 vs 12 months	3.32	3.53	4.08

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

Protein fraction

Figure 2 shows the protein fraction of the different sparkling wines at six and twelve months of ageing. The total protein concentration (Figure 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 (Figures 2B, 2C 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 & 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, González-Royo, Gil, 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.

Figure 2. Protein composition

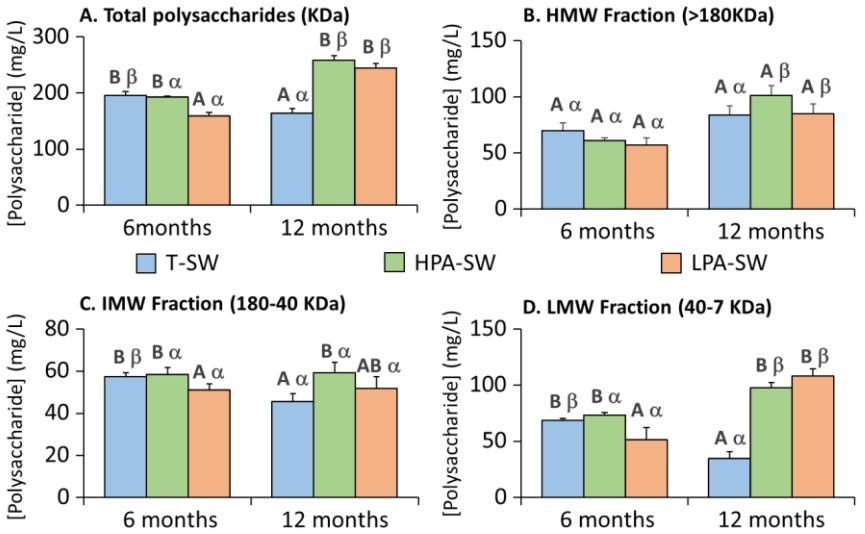


Results are expressed as mean ± SD of three replicates. Concentration of proteins is expressed as bovine serum albumin equivalents. T-SW: Traditional sparkling wine; HPA-SW: High population ancestral sparkling wine; LPA-SW: Low population ancestral sparkling wine. HMW: High Molecular weigh Fraction; 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.

Polysaccharide fraction

Figure 3 shows the polysaccharide fraction of the different sparkling wines at six and twelve months of ageing. The total polysaccharide concentration (Figure 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 (Figures 3C and 3D), 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 (Figure 3D).

Figure 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 weight 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.

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, Guadalupe, Ayestarán, Ortega-Heras, & Pérez-Magariño, 2013; Moreno-Arribas, Pueyo, Nieto, Martín-Álvarez, & Polo, 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 lower the polysaccharide release during the yeast autolysis. However, this difference is no longer significant after twelve months of ageing.

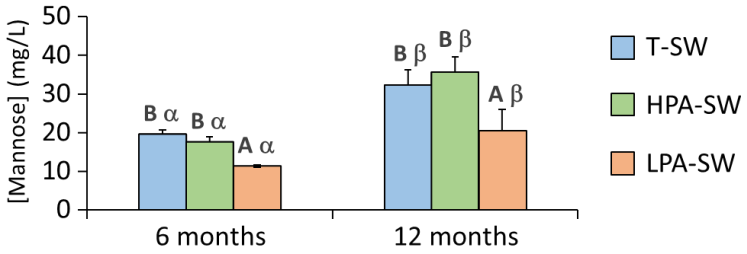
In the T-SW, an increase in the total polysaccharide concentration with the time of ageing would also be expected; however, the opposite actually happens. A possible explanation could be its higher ethanol content. It is well known that the solubility of polysaccharides decreases when the ethanol concentration increases (Bouchard, Hofland, & Witkamp, 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.

Mannose concentration after polysaccharide hydrolysis

T-SW and HPA-SW showed similar mannose concentration levels after polysaccharide hydrolysis (Figure 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, Glories, Maujean, & Dubourdieu, 2006) and are therefore also quantified as polysaccharides (Ayestarán et al., 2004).

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 & Guilloux-Benatier, 2006; Martínez-Lapuente et al., 2013).

Figure 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.

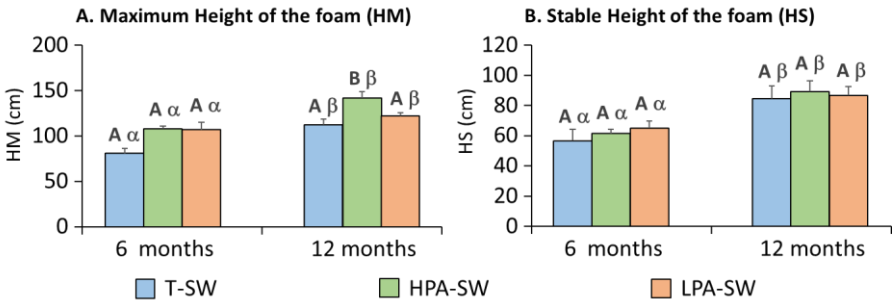
Foaming properties

Figure 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 & Guilloux-Benatier, 2006; Kemp et al., 2019; Martínez-Lapuente, Guadalupe, Ayestarán, & Pérez-Magariño, 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, Robillard, Carles, Duteurtre, & Vignes-Adler, 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, Liger-Belair, Villaume, Jeandet, & Marchal, 2010; Pérez-Magariño et al., 2015).

Figure 5. Foam properties

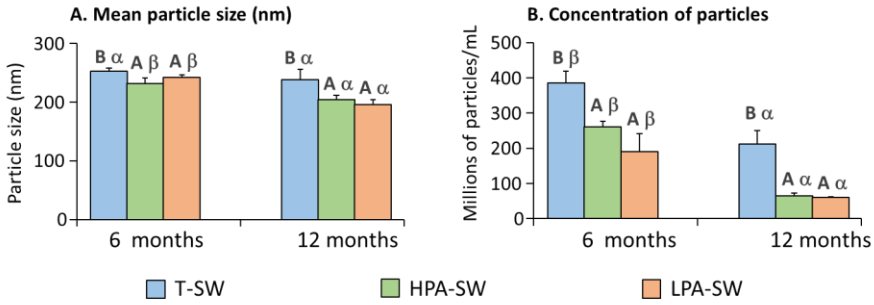


Results are expressed as mean ± SD of three replicates. T-SW: Traditional sparkling wine; HPA-SW: High population ancestral sparkling wine; LPA-SW: Low population ancestral sparkling wine. HM: maximum height of the foam; HS: stable height of the foam. Different letters indicate the existence of a statistical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

Colloidal properties

To our knowledge, there is only few information about the colloidal composition of sparkling wines (Senée, Robillard, & Vignes-Adler, 1998; Senée, Robillard & Vignes-Adler, 2001). Figure 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, Li, Smith, Blando, & Bindon, 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.

Figure 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.

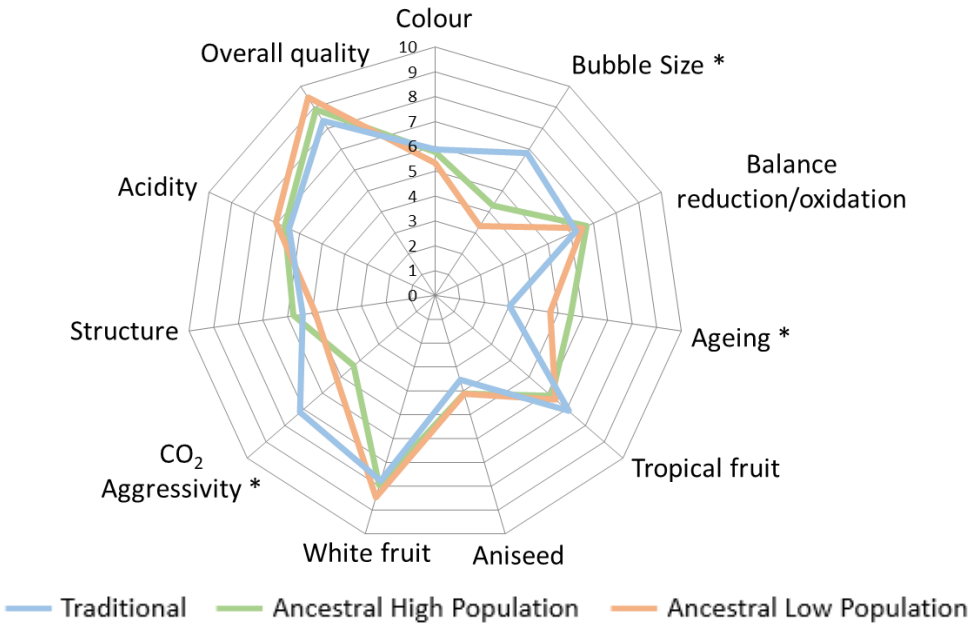
Interestingly, the analysis of the number of colloidal particles during ageing (Figure 6B) reveals that T-SW contained significantly more colloids than the two ancestral wines at both six and twelve months. Altogether, data of Figure 6 seem to indicate that the T-SW had more and bigger colloids than 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 Figure 2A), the same or less total polysaccharides (see Figure 3A), and the same or worse HM and HS parameters (see Figure 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 (Figure 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 2012).

Sensory analysis

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

Figure 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 ($p < 0.05$).

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.

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UNIVERSITAT ROVIRA I VIRGILI
ANCESTRAL SPARKLING WINES; COMPARISON WITH TRADITIONAL SPARKLING WINES AND PROCEDURES TO IMPROVE THEIR QUALITY
Arnau Just Borràs

Chapter 3

Cationic exchange: Investigating its potential impact on the characteristics of sparkling wines during ageing

Throughout the third chapter, a study is presented whose objective was to meet Objective 4. Climate change is causing some consequences on oenology being probably the higher sugar concentration and higher pH that grape musts are achieving the more noticeable. In these conditions, wineries tend to harvest earlier which can compensate the excess of sugars and the high pH, but could cause that the grapes are not ripe enough and that consequently the wines present herbaceous notes. Ancestral method can be useful in that conditions since it allows harvesting later since no sugar is added for a second fermentation. However, harvesting later provokes lower acidity and higher pH that need to be compensated for obtaining well balanced sparkling wines.

Cationic exchange resins are probably nowadays the most used technique to reduce pH of grape musts and wines and their effectiveness is widely known. However, no studies exist about the influence of the use of this technique on the physicochemical characteristics and quality of sparkling wines. The aim of this chapter was to study how the application of cationic exchange to the grape must could affect the physicochemical composition and sensory quality of sparkling wines. This study was performed using the traditional method because it was only possible to carry out in a winery of the DO Cava. However, the results should be perfectly extrapolated to ancestral sparkling wines.

UNIVERSITAT ROVIRA I VIRGILI
ANCESTRAL SPARKLING WINES; COMPARISON WITH TRADITIONAL SPARKLING WINES AND PROCEDURES TO IMPROVE THEIR QUALITY
Arnau Just Borràs

Effects of using cationic exchange for reducing pH on the composition and quality of sparkling wine (Cava)

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Abstract

Climate change is affecting vine and grape physiology and consequently wine composition, causing a decrease in titratable acidity and an increase in ethanol content and pH. These effects are especially problematic in sparkling wines that need higher acidity to maintain an adequate freshness. Therefore, the wine industry is currently using certain procedures for reducing wine pH, among which cation exchange stands out as it is probably the most widely used. To study the influence of cation exchange treatment on the composition and quality of sparkling wines, a grape juice of Macabeo (pH 3.21 and titratable acidity 5.70 g of tartaric acid/L) after settling was treated to obtain a very acidic grape juice (pH 1.9 and titratable acidity 8.70 g of tartaric acid/L). The original grape juice was then blended in different proportions (0-45 %) with a treated grape juice. These different grape juices were used for obtaining their corresponding base wines which in turn were used for elaborating their corresponding sparkling wines using the traditional method. The cation exchange treatment reduced the pH from 3.15 (Control) to 2.87 (45 % of treatment) and increased the titratable acidity from 4.61 (Control) to 7.69 (45 % of treatment). No significant effects were observed on the concentration of any of the protein or polysaccharide fractions and the foaming properties of the base wine or young sparkling wines were not affected; however, cation exchange caused a decrease in foamability and persistence of the foam in older sparkling wines, especially when the proportion of treated grape juice was higher. A trained panel only found clear sensory differences in the acidity of the sparkling wines without the rest of the attributes being affected by the treatment.

Keywords: Sparkling Wines; Cationic Exchange; pH; Titratable Acidity; Potassium

Introduction

Cava is the name of an AOC of sparkling wines produced in Spain by the traditional method that comprises two fermentation steps (Ministerio De Agricultura y medio ambiente, 2018). The grape juice is transformed into a base wine during the first fermentation of a standard winemaking process. After blending and stabilisation, the base wine is

transformed into sparkling wine in a second fermentation called prise de mousse. This second fermentation takes place inside the bottle. The sparkling wines age for some time in contact with the lees to benefit from the autolysis process (Maujean, 1989).

The maturity and the healthiness of the grape berries plays a very important role in the final composition and quality of base wines and their corresponding sparkling wines (Cilindre et al., 2007). It has been reported that an excess of maturity can seriously affect some factors that are important for sparkling wine quality, such as foamability, titratable acidity and especially pH (Esteruelas et al., 2015; Liu et al., 2018). For this reason, sparkling wine producers consider low sugar concentration, high titratable acidity and low pH as the main criteria for determining the harvest dates (Jones et al., 2014). High acidity and low pH are needed to maintain the necessary sensory freshness of sparkling wines and both parameters have been reported to be key factors for guaranteeing the correct evolution during aging (Zoecklein, 2002).

In recent years, the increase in temperature and the changes in rainfall distribution caused by climate change are affecting vine and grape physiology and are consequently impacting wine composition and quality (Jones et al., 2005; Santos et al., 2020; Schultz, 2000). As a consequence of global warming, the grape pulp ripens faster, and the pH and sugar concentration become too high and titratable acidity too low (Godden et al., 2015; Mira de Orduña, 2010; Schultz, 2016). Therefore, grapes reach a very high potential alcoholic degree and pH sooner than usual. This phenomenon causes harvest dates to be earlier and makes it much more difficult to pinpoint proper aromatic and pulp maturity, which leads to unbalanced wines (Zamora, 2014). This is an increasing problem in the case of AOC Cava (Esteruelas et al., 2015; Ramos, 2017). Therefore, wineries are very interested in knowing how they can mitigate the effects of climate change on grape and wine composition.

In this new situation, oenologists are looking for strategies to counteract these effects. There seems to be only two possibilities: they can harvest when alcoholic degree and pH are at the correct level and accept that the grapes will not have the correct aromatic and phenolic maturity; or they can wait for adequate maturity and accept that the wines will have high ethanol content and pH.

Neither of these choices is conducive to obtain high quality wines and therefore winemakers are obviously concerned about this problem. Since the lack of real grape maturity cannot be compensated for, most winemakers prefer to wait for the correct grape maturity and then later apply procedures to compensate for the disequilibrium of these unbalanced grapes (Zamora, 2014).

Several practices for reducing sugar in grape juice or ethanol in wines have been proposed, including selecting grape cultivars and clones that ripen later or adapting farming practices to this new situation (Schultz, 2000), selecting yeasts with lower sugar/ethanol transformation yields (Dequin and Barre, 1994), reverse osmosis (Gil et al., 2013) or partial evaporation of ethanol from the wine (Takács et al., 2007). For more information on these procedures for reducing alcohol, the following reviews are recommended: Saha et al. (2013) and Zamora (2016).

The problem of the low titratable acidity of grape juices and wines can be easily solved by adding authorised acids, such as L-(+)-tartaric, citric, lactic or malic acids. Nevertheless, all these organic acids have pK values that are relatively high and therefore they are not efficient enough for lowering the pH. Furthermore, the use of mineral acids is strictly forbidden (International Organisation of Vine and Wine, 2021). In fact, there are only two techniques authorised by OIV for reducing pH: electrodialysis and cationic exchange (International Organisation of Vine and Wine, 2021).

Electrodialysis makes it possible to extract ions, mainly potassium cation and hydroxyl anion, through selective ion exchange membranes under the influence of a continuous electric field (El Rayess and Mietton-Peuchot, 2016; Gonçalves et al., 2003; Romanov and Zelentsov, 2007). In contrast, cationic exchange makes it possible to interchange cations, mainly potassium, with protons using cationic exchange resins (Lasanta and Gómez, 2012). Both techniques are very effective and are being increasingly used by wineries; however, cation exchange is probably being more widely used due to its lower cost (Lasanta and Gómez, 2012; Walker et al., 2004).

Several studies have been reported about the use of cationic exchange in grape juice and wine and its effects on wine composition and quality (Ibeas et al., 2015; Mira et al., 2006;

Ponce et al., 2018). However, to our knowledge, only few of them refer to base wines (Cisilotto et al., 2020; Cisilotto et al., 2019) and none of them have focused on the effect on sparkling wines produced using the traditional method. Therefore, the aim of this work is to study how applying cationic exchange to the grape juice influences the composition and quality of the base wines and their corresponding sparkling wines after 11 and 20 months of aging. This study was performed by blending treated grape juice with non-treated grape juice in different proportions in order to determine which treatment percentage was the most adequate for obtaining the most balanced sparkling wine.

Materials and methods

Experimental design

The experiment was carried out using Macabeo grapes (VIVC Prime name: Viura; VIVC Variety number: 13127) from the 2018 vintage. The grapes were from Juvé & Camps SL vineyards in Sant Sadurní d'Anoia (AOC Cava, Barcelona, Spain; 41° 26' 47.42'' N and 1° 49' 0.63'' E). The grapes were harvested when the maturity level was adequate for sparkling wine production. Specifically, the maturity parameters of the obtained grape juice were: total sugar concentration: 159 g/L (corresponding to 16.6 °Brix), titratable acidity: 5.7 g/L (expressed as tartaric acid) and pH: 3.21. The grapes were immediately pressed in a pneumatic press to obtain a yield of 0.6 L/kg of grape juice. The grape juice was immediately sulfited with 100 mg/L of $K_2S_2O_5$ and pectinolytic enzymes (20 mg/L) were added to facilitate settling. After 18 h, clean grape juice (around 70 NTUs) was racked into a stainless steel tank.

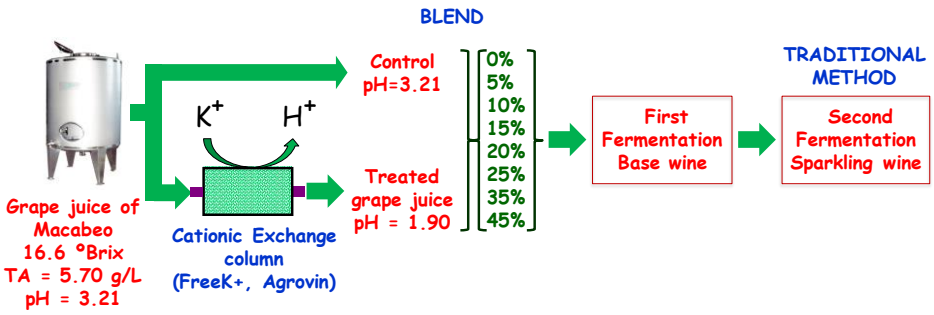

Figure 1. Experimental design

Around 2,000 L of this grape juice were divided into two parts, and 1,000 L were treated with a cationic exchange column (FreeK+ column, Agrovin, Ciudad Real, Spain) and the other 1,000 L were kept without any treatment. The pH and titratable acidity of the initial grape juice were 3.21 ± 0.01 and 5.70 ± 0.02 respectively. In contrast, the final pH and titratable acidity of the treated grape juice were 1.90 ± 0.02 and 8.70 ± 0.05 respectively. Subsequently, both grape juices, treated and non-treated, were blended in different proportions in order to obtain a set of grape juices with different pH levels. Specifically, the different blends were obtained with the following proportions of treated grape juice: 0, 5, 10, 15, 20, 25, 35 and 45 %. All the different blends were placed in 8-litre alimentary plastic tanks to perform the first fermentation. All the tanks were immediately inoculated with 200 mg/L of selected yeasts (*Saccharomyces cerevisiae*, Lalvin EC1118™, Lallemand, Inc., Montreal, Canadá). The fermentation kinetics were monitored using a digital densimeter (Mettler Toledo-PortableLabTM). All alcoholic fermentations were performed at 16-18 °C. Once alcoholic fermentation was finished, the base wines were racked, sulfited (40 mg/L of $K_2S_2O_5$) and cold stabilised. The entire process was carried out in triplicate.

Once the base wines were stable they were used for producing sparkling wine (Cava) following the traditional method. All the base wines were supplemented with 22 g/L of sucrose, 0.2 mL/L of a liquid riddling agent (Inoclair, Institut

Oenological de Champagne, Epernay, France) and $2x10^6$ cells/mL of pre-adapted yeast culture (*Saccharomyces cerevisiae* - IOC 18-2007; Institut Œnologique de Champagne, Epernay, France). The wines were then bottled in standard green glass bottles (750 mL), crown sealed and stored at 12-15 ^oC until disgorgement. Eleven and twenty months later, four bottles of each experimental group were disgorged. Three bottles were used for chemical and physical analyses and one bottle was used for the sensory analysis. The second fermentation was monitored by measuring CO_2 pressure accumulation in each bottle following a non-destructive method (L.sensor CO_2-FTSYSTEM). No important differences were found in the internal pressure kinetics during the second fermentation (Data not shown). Only a very small delay was observed in the case of the more acidic samples (35 and 45 %) at the beginning of the second fermentation, but all the samples reached the maximal internal pressure at the same time (around two months).

Standard wine analysis

The analytical methods recommended by the OIV were used to determine the ethanol content (pycnometry), residual fermentable sugar concentration (D-glucose + D-fructose enzymatic method), pH, titratable acidity and volatile acidity (International organisation of vine and Wine, 2019). The CIELab coordinates were determined following the method described by Ayala *et al*. (1997) using Helios Alpha UV VIS spectrophotometer (Thermo Fisher Scientific Inc., Waltman, MA, USA), and the data were processed using MSCV® software.

Sample preparation

All the samples, grape juices, base wines and sparkling wines were centrifuged at 17.000 g (Biofuge Primo centrifuge (Heraeus, Hanau, Germany)) for 15 min in order to obtain limpid liquids and when necessary to eliminate all carbon dioxide.

Measurement of the foaming properties

A Mosalux device (Station Oenotechnique de Champagne, Epernay, France) was used to measure HM, the max height of the foam after CO_2 injection through the glass frit, and HS, the stable height during CO_2 injection. HM represents foamability (the wine's ability to foam) while HS represents foam stability (the persistence of the foam collar or the wine's ability to produce a stable foam). HM and HS are expressed in millimeters.

Base wine and sparkling wine samples were tempered at 18°C for 24 h before analysis. The foam properties were measured using the Mosalux method (Maujean *et al*., 1990). A glass cylinder placed on a glass frit was filled with 100 mL of the sample. CO_2 was then injected into the glass cylinder through the glass frit with a constant gas flow of 115 mL/min under a constant pressure of 1 bar in the case of base wines and of 2 bar in the case of sparkling wines. Sparkling wines were measured at 2 bars in order to improve the method's sensitivity, because their values are noticeably lower than in base wines.

Calibration of Mosalux was performed using a standard solution composed of absolute ethanol 96% vol. (17 % v/v), tartaric acid 99.5 % (4 g/L), glycerol 99.5 % (6 g/L) (all purchased from Panreac (Barcelona, Spain)), diethyl phthalate 99.5% (0.5 % v/v), and bovine serum albumin ≥98 % (10 mg/L) (both purchased from Sigma–Aldrich (Madrid, Spain)). Sodium hydroxide ≥98 % was used to adjust pH to 3.00. The foaming parameters (HM and HS) of this calibration solution were measured at 1 and 2 bar in order to determine a compensatory coefficient to refer all the measurements to 1 bar of pressure. Therefore, the foaming properties of the base wine and sparkling wines can be correctly compared. All measurements were determined in triplicate.

Potassium determination

The potassium concentration of the different base wine samples was determined by means of Flame Atomic Emission Spectroscopy (UNICAM969 AA SPECTROMETER) according to an adaptation of the method described by Aceto *et al*. (2002).

Acid composition

All the main wine acids were analysed using commercial kits provided by r-Biopharm (Darmstadt, Germany) following the instructions provided by the kit manufacturer. L-Malic, L-Lactic, Acetic and Citric acids were analysed according to the enzymatic methods recommended by the OIV (International organisation of vine and Wine, 2019). Specifically, the following enzymatic kits were used for each acid: Art. No. 10139068035 for L-Malic, Art. No. RCS4260 for L-Lactic, Art. No. RCS4226 for Acetic , and Art. No. E1214 for Citric. Succinic acid was also enzymatically analysed according to the method described by Michal *et al* (1976) using the commercial kit Art. No. 10176281035. Tartaric acid was analysed according to Hill and Caputi (1970) using the colorimetric kit Art. No. E3100.

The main wine acids were analysed using commercial kits provided by r-Biopharm (Darmstadt, Germany) following the instructions provided by the kit manufacturer. Specifically, the following enzymatic kits were used for each acid: Art. No. 10139068035 for L-Malic, Art. No. RCS4260 for L-Lactic, Art. No. RCS4226 for Acetic, Art. No. 10176281035 for Succinic and Art. No. E1214 for Citric. Tartaric acid was analysed using colorimetric kit Art. No. E3100).

Polysaccharide extraction and determination by HRSEC-RID

The samples were processed using the methodology described by Ayestarán *et al*. (2004). Briefly, 10 mL of sample in triplicate were concentrated to a final volume of 2 mL using a vacuum evaporator (Univap 148 100ECH; Progen Scientific, London, UK). Total soluble polysaccharides were precipitated by adding 10 mL of cold acidified ethanol (hydrochloric acid 0.3 M in absolute ethanol) and kept for 24 h at 4°C. The samples were then centrifuged (10,000 g for 15 min) and the supernatants discarded. Finally, the precipitates were dissolved in 1 mL of ultra-pure water, frozen to −20°C and freeze-dried using a lyophilizer (Telstar LyoQuest HT40, Barcelona, Spain). The soluble fractions were analysed by high-resolution size-exclusion chromatography (HRSEC) in order to determine the molecular distribution and quantify the polysaccharides obtained from the samples. The lyophilized samples were resuspended in 1 mL of 50 mM ammonium formate ≥99.0 % (Sigma–Aldrich (Madrid, Spain)) and filtered through 0.22 µm acetate cellulose filters (Merck Millipore, Darmstadt, Germany). Then 100 µL were injected into the chromatographic system. The analyses were carried out in an HPLC Agilent 1200 Series system (Agilent Technologies Inc., Santa Clara, USA) equipped with a G1311A quaternary pump, a G1316A column oven, a G1329A autosampler (Agilent Technologies, Santa Clara, CA, USA) and with a refractive index detector (G1362A - RID). Separation was carried out at 20°C using two Shodex gel permeation HPLC columns (OHpak SB-186 803 HQ and SB-804 HQ, 300 mm × 8 mm I.D.; Showa Denko, Japan). The mobile phase consisted of an aqueous solution of 50 mM ammonium formate applied with a constant flow of 0.6 mL/min for 60 min, and the cell RID temperature was 35°C. The molecular weight distribution of the wine fractions was monitored by calibration with a Shodex P-82 pullulan calibration kit (P-5, MW = 5.9 kDa; P-10, MW = 11.8 kDa; P-20, MW = 22.8 kDa; P-50, MW = 47.5 kDa; P-100, MW = 112 kDa; P-200, MW = 212 kDa; P-400, MW = 404 kDa; and P-800, MW = 788 kDa) purchased from Waters (Barcelona, Spain) and four dextrans

(BioChemika; 12, 25, 50 and 80 kDa) purchased from Fluka (St. Louis, MO, USA). The polysaccharides were quantified according to the peak area for each fraction using the external standard method with pectin and dextran commercial standards (Sigma–Aldrich, Saint Louis, MO, USA) in a range between 0 and 2 g/L ($r2 > 0.99$).

Determination of proteins by HRSEC-DAD

The samples were processed and analysed using the methodology described by Canals *et al*. (1998). Fifteen mL of each sample were concentrated in triplicate following a two steps dialysis in tubes with a molecular weight cutoff of 3.5 kDa (Spectrum Laboratories Inc., Rancho Dominguez, CA, USA). The first step lasted 48h with 0.3 M ammonium acetate ≥98.0 % (Sigma–Aldrich (Madrid, Spain)) solution with a rate of 1:10 (sample:solution) and constant agitation. The second step was carried out with water for another 48h. The dialyzed samples were subsequently lyophilised and preserved at −20°C.

The soluble fractions were analysed by high-resolution size-exclusion chromatography (HRSEC) in order to determine the molecular distribution and quantify the proteins obtained from the samples37. The lyophilized samples were resuspended in 0.6 µL of ammonium acetate solution (300 mM) and centrifuged (12 000 g for 5 min). The supernatant was filtered through 0.22 µm acetate cellulose filters (Merck Millipore, Darmstadt, Germany) and then 100 µL of supernatant was injected into the chromatographic system. The analyses were carried out in HPLC Agilent 1200 Series system (Agilent Technologies Inc., Santa Clara, USA) equipped with a G1311A quaternary pump, a G1316A column oven, a G1329A autosampler (Agilent Technologies, Santa Clara, CA, USA) and with a diode array detector (G1315D - DAD) to monitor output at 230 and 320 nm. Separation was carried out at 20°C using an S 165 Shodex gel permeation HPLC column 210 (OHpak 166 SB-803 HQ, 300mm× 8mm i.d.; Showa Denko, Tokyo, Japan). The mobile phase consisted of an aqueous solution of 300 mmol/L ammonium acetate

applied at a constant flow of 0.6 mL/min for 70 min. The proteins were quantified according to the peak area for each fraction using the external standard method with bovine serum albumin (Sigma–Aldrich, Madrid, Spain) in a range between 0 and 1 mg/mL ($r2 > 0.99$).

Sensory analysis

All sensory analyses were performed in the tasting room of the Faculty of Oenology of Tarragona (University Rovira i Virgili), which was designed in accordance with UNE 87004.197 (AENOR, 2010). Tasting was carried out using ISO official tasting glasses (ISO-3591, 1997). All the samples were tasted by 10 trained panelists. This panel was made up of seven males and three females aged between 22 and 60. For each sample, tasters were required to evaluate the intensity of six sensory attributes (Colour, Balance Reduction/Oxidation, CO_2 integration, Structure, Acidity and Global quality) on a scale of 1 to 10 (1 = 'slight intensity', 10 = 'maximum intensity'). In the case of Balance Reduction/Oxidation the scale goes from the presence of evident reduction notes (1) to high oxidation notes (10). The intensity level of each descriptor was then expressed as the mean value of all the judges. No more descriptors were used so as not to over complicate the tasting. A sensory training session was held beforehand so that the panelists could agree on the criteria for each of the different sensory attributes. Samples were served randomly to avoid the influence of the tasting order.

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).

Results and discussion

Influence of cationic exchange treatment on the general composition of grape juice, base wine and sparkling wine

Table 1 shows the effect of the treatment with the cationic exchange column on the pH. The initial pH value of the grape juice was 3.21 ± 0.01. This value can be considered as very common for Macabeo grape juice harvested for sparkling wine production in the region. This value decreased significantly in the base wine and sparkling wines of 11 and 20 months of aging. This small decrease in pH can be associated with the crystallisation of potassium hydrogen tartrate caused by the presence of ethanol. When potassium hydrogen tartrate crystallises, a proportion of hydrogen tartrate anion is removed from the tartaric acid equilibrium causing a displacement towards the release of protons (Devatine *et al.*, 2002).

TABLE 1. Influence of cation exchange treatment on pH

Percentage of treated grape juice in the blending	Grape juice	Base wine	Sparkling wine 11 months	Sparkling wine 20 months
C	3.21 ± 0.01 F b	3.13 ± 0.02 F a	3.17 ± 0.02 D a	3.15 ± 0.01 C a
5%	3.15 ± 0.01 EF a	3.06 ± 0.06 E a	3.09 ± 0.03 C a	3.11 ± 0.01 C a
10%	3.13 ± 0.01 E b	3.06 ± 0.00 E a	3.05 ± 0.01 C a	3.05 ± 0.04 BC a
15%	3.05 ± 0.04 D a	3.02 ± 0.00 DE a	3.06 ± 0.03 C a	2.98 ± 0.09 AB a
20%	3.03 ± 0.02 CD a	2.97 ± 0.03 CD a	3.05 ± 0.07 C a	2.95 ± 0.06 AB a
25%	2.98 ± 0.03 C a	2.94 ± 0.01 BC a	2.97 ± 0.00 B a	2.94 ± 0.03 AB a
35%	2.90 ± 0.06 B a	2.87 ± 0.01 B a	2.91 ± 0.01 A a	2.90 ± 0.02 A a
45%	2.77 ± 0.02 A a	2.81 ± 0.02 A a	2.88 ± 0.02 A b	2.87 ± 0.02 A b

Results are expressed as mean ± standard deviation of three replicates. Different letters in a row indicate the existence of statistical difference ($p < 0.05$). First row (capital letters) indicates the influence the percentage of cation exchange treated grape juice in the blending. Second row (lowercase letters) indicates the influence of the elaboration process.

This table also shows that the cationic exchange treatment clearly reduces the pH. In general, the higher the proportion of treated grape juice the lower the pH, and

this tendency was observed in all the samples: grape juices, base wines and both sparkling wines. It was also observed that the pH of the base wines was in general lower than in its corresponding grape juices; however, the differences was only significant for the control, and 5 and 10 % of treatment. This decrease in pH after alcoholic fermentation occurs, because the presence of ethanol reduces the solubility of potassium acid tartrate, and when this salt precipitates, it shifts the equilibrium of tartaric acid towards the release of protons. The lack of significance when the percentage of treatment was higher than 10 % could be because the removal of potassium caused by the cationic exchange treatment progressively diminishes the crystallisation of potassium hydrogen tartrate.

Table 2 shows the influence of the cationic exchange treatment on the titratable acidity (TA) of the different grape juice blends and their corresponding base and sparkling wines.

TABLE 2. Influence of cation exchange treatment on titratable acidity

Percentage of treated grape juice in the blending	Titratable acidity (g of tartaric acid/L)			
	Grape juice	Base wine	Sparkling wine 11 months	Sparkling wine 20 months
C	5.70 ± 0.00 A c	5.53 ± 0.04 A c	5.05 ± 0.11 A b	4.61 ± 0.34 A a
5%	5.93 ± 0.15 AB a	5.76 ± 0.02 B a	5.57 ± 0.15 B a	5.58 ± 0.25 B a
10%	5.93 ± 0.15 AB a	6.02 ± 0.06 C a	5.95 ± 0.14 BC a	6.10 ± 0.21 BC a
15%	6.00 ± 0.24 AB a	6.20 ± 0.05 C a	6.10 ± 0.12 C a	6.15 ± 0.27 BC a
20%	6.23 ± 0.15 BC a	6.49 ± 0.04 D a	6.35 ± 0.10 CD a	6.34 ± 0.19 C a
25%	6.38 ± 0.15 CD a	6.74 ± 0.05 E a	6.55 ± 0.18 D a	6.48 ± 0.23 C a
35%	6.68 ± 0.15 DE a	7.30 ± 0.05 F b	7.16 ± 0.16 E b	7.20 ± 0.20 D b
45%	6.98 ± 0.15 E a	7.92 ± 0.17 G b	7.61 ± 0.19 F b	7.69 ± 0.27 D b

Results are expressed as mean ± standard deviation of three replicates. Different letters in a row indicate the existence of statistical difference ($p < 0.05$). First row (capital letters) indicates the influence the percentage of cation exchange treated grape juice in the blending. Second row (lowercase letters) indicates the influence of the elaboration process.

The TA value of the control grape juice was 5.70 ± 0.02 g of tartaric acid/L, which is a very common value for Macabeo grape juice used for Cava production. In this

case, a decrease in the TA of the control base and sparkling wines was observed with respect to the control grape juice. This reduction in TA in the control samples may be related to the development of malolactic fermentation, as discussed later, and also to the previously described crystallisation of potassium hydrogen tartrate caused by the presence of ethanol in wines, which matches very well with the observed pH decrease. However, this behaviour was dampened and even inverted as the proportion of treated grape juice was increased in the final blend. In any case, the cationic exchange treatment exerted a significant effect on the TA of the grape juices since the higher the proportion of treated grape juice in the blend, the higher the TA. This effect was maintained in the base and sparkling wines.

Table 3 shows the ethanol content and residual sugars of the different base and sparkling wines of 11 months of aging.

As expected, the ethanol content of the sparkling wines was on average 1.47 % higher than in the base wines. This increase matches a normal sugar/ethanol transformation yield of the sugar added in the tirage liquor (22 g/L of sucrose) for the second fermentation.

TABLE 3. Influence of cation exchange treatment on ethanol an residual sugars

Percentage of treated grape juice in the blending	Base wine		Sparkling wine (11 months)	
	Ethanol (% v/v)	Residual sugars (g/L)	Ethanol (% v/v)	Residual sugars (g/L)
C	10.85 ± 0.05 A	0.88 ± 0.40 A	12.32 ± 0.06 C	0.26 ± 0.03 A
5%	10.83 ± 0.07 A	0.78 ± 0.29 A	12.29 ± 0.08 CB	0.23 ± 0.04 A
10%	10.84 ± 0.01 A	0.97 ± 0.09 AB	12.32 ± 0.01 C	0.30 ± 0.02 AB
15%	10.80 ± 0.07 A	0.99 ± 0.25 AB	12.27 ± 0.07 CB	0.36 ± 0.02 B
20%	10.79 ± 0.03 A	0.90 ± 0.13 A	12.26 ± 0.03 CB	0.29 ± 0.02 AB
25%	10.74 ± 0.05 AB	1.13 ± 0.42 AB	12.21 ± 0.06 BA	0.39 ± 0.06 B
35%	10.66 ± 0.02 B	1.44 ± 0.35 BC	12.15 ± 0.02 A	0.60 ± 0.03 C
45%	10.66 ± 0.02 B	1.78 ± 0.32 C	12.15 ± 0.02 A	0.69 ± 0.04 C

Results are expressed as mean ± standard deviation of three replicates. Different letters in a row indicate the existence of statistical difference ($p < 0.05$). First row (capital letters) indicates the influence the percentage of cation exchange treated grape juice in the blending. Second row (lowercase letters) indicates the influence of the elaboration process.

In general, no large differences were found in the ethanol content or in the residual sugars as a function of the proportion of the treated grape juice included in the blend. However, it seems that when the pH was very low, the yeasts had some difficulties in finishing the first and second fermentations completely since the ethanol content was slightly lower and the residual sugar concentration slightly higher. In any case, these differences were so small that they have very little practical and technical relevance. Table 4 shows the potassium concentration of the base wines.

TABLE 4. Influence of cation exchange treatment on volatile acidity

Percentage of treated grape juice in the blending	Volatile acidity (g of acetic acid/L)		
	Base wine	Sparkling wine 11 months	Sparkling wine 20 months
C	0.24 ± 0.02 A a	0.22 ± 0.02 A a	0.32 ± 0.03 A b
5%	0.26 ± 0.04 AB a	0.23 ± 0.04 A a	0.28 ± 0.05 A a
10%	0.26 ± 0.02 AB a	0.22 ± 0.02 A a	0.26 ± 0.02 A a
15%	0.26 ± 0.02 AB a	0.21 ± 0.02 A a	0.24 ± 0.04 A a
20%	0.24 ± 0.01 AB a	0.21 ± 0.01 A a	0.24 ± 0.03 A a
25%	0.26 ± 0.01 AB a	0.23 ± 0.01 A a	0.26 ± 0.02 A a
35%	0.27 ± 0.01 B a	0.24 ± 0.01 A a	0.27 ± 0.03 A a
45%	0.31 ± 0.03 C a	0.26 ± 0.03 A a	0.31 ± 0.03 A a

Results are expressed as mean ± standard deviation of three replicates. Different letters in a row indicate the existence of statistical difference ($p < 0.05$). First row (capital letters) indicates the influence the percentage of cation exchange treated grape juice in the blending. Second row (lowercase letters) indicates the influence of the elaboration process.

As expected, the potassium concentration decreased progressively as the proportion of treated grape juice in the blend increased. It should be noted that the proportion of potassium extracted decreases with a steep slope to eventually reach almost asymptotic behaviour as the proportion of treated must increases. In any case. these data confirm the effectiveness of cationic exchange treatment for removing this cation from wines.

The main acids were analysed to better understand the influence of the cationic exchange treatment on the wine acidic composition (Table 5).

TABLE 5. Influence of cation exchange treatment on acidic composition

Percentage of treated grape juice in the blending	L-Malic acid (g/L)	L-Lactic acid (g/L)	Succinic acid (g/L)	Citric acid (g/L)	Tartaric acid (g/L)
C	0.03 ± 0.00 A	0.42 ± 0.01 B	0.68 ± 0.05 A	0.02 ± 0.01 A	2.55 ± 0.08 A
5%	0.67 ± 0.00 B	0.01 ± 0.00 A	0.44 ± 0.06 A	0.12 ± 0.02 B	2.93 ± 0.12 B
10%	0.67 ± 0.01 B	0.00 ± 0.00 A	0.49 ± 0.01 A	0.13 ± 0.02 B	3.18 ± 0.14 BC
15%	0.68 ± 0.01 B	0.00 ± 0.02 A	0.50 ± 0.01 A	0.10 ± 0.05 B	3.36 ± 0.10 CD
20%	0.68 ± 0.02 B	0.02 ± 0.02 A	0.46 ± 0.11 A	0.14 ± 0.02 B	3.65 ± 0.13 D
25%	0.69 ± 0.02 B	0.01 ± 0.01 A	0.53 ± 0.01 A	0.14 ± 0.01 B	3.60 ± 0.09 D
35%	0.69 ± 0.01 B	0.01 ± 0.01 A	0.42 ± 0.17 A	0.15 ± 0.00 B	4.24 ± 0.08 E
45%	0.68 ± 0.02 B	0.00 ± 0.00 A	0.55 ± 0.10 A	0.14 ± 0.01 B	4.61 ± 0.13 E

Results are expressed as mean ± standard deviation of three replicates. Different letters in a row indicate the existence of statistical difference ($p < 0.05$).

Tartaric acid concentration increased progressively as the proportion of treated grape juice in the blend increased. The explanation for this behaviour is very simple. Briefly, the elimination by crystallisation of tartaric acid in the form of potassium hydrogen tartrate in the wine decreased as the potassium concentration of the medium became lower. This is the main reason why the tartaric acid concentration and the titratable acidity increase when the proportion of treated grape juice is increased in the final blend. These results are in agreement with other previous studies (Ibeas *et al*., 2015; Cristina Lasanta *et al*., 2013; Walker *et al*., 2004).

It should also be noted that the concentrations of the L-malic and citric acids of the control wine were almost zero and that L-lactic acid was present in a quantifiable concentration (0.42 g/L). In contrast, all the other wines maintained similar concentrations of L-malic and citric acids (around 0.68 and 0.13 g/L respectively) and the L-lactic acid concentration was negligible. It seems, therefore, that the

control wine was the only one that performed malolactic fermentation, although all the base wines were equally sulfited as soon as alcoholic fermentation was completed. This different behaviour was probably a result of the higher pH favouring the development of lactic acid bacteria and also reducing the antiseptic effectiveness of the sulfur dioxide (Liu and Gallander, 1983). These data demonstrate that a relatively high pH carries an increased risk of unwanted malolactic fermentation taking place, and that therefore the use of cation exchange is also helpful in preventing it. A significant increase in acetic acid concentration was observed in the base wines in which the proportion of treated grape juice was very high, probably because their very low pH induced higher stress in the yeasts. In any case, this increase in acetic acid did not affect the final quality. Finally, no significant differences in the succinic acid concentration were found.

Influence of cationic exchange treatment on the protein fraction of base and sparkling wines

Figure 2 shows the protein concentration of the different base wines and their corresponding sparkling wines at 11 and 20 months. As expected, the total protein concentration decreased drastically between the base wines and sparkling wines. This decrease was mainly due to the lower and intermediate molecular weight (LMW and IMW) fractions, whereas the high molecular weight (HMW) was less affected. This protein reduction between base and sparkling wines has been reported previously and has been mainly attributed to the deproteinising effect of the riddling agent bentonite (Martínez-Rodriguez and Polo, 2003; Vanrell *et al*., 2006).

Almost all wine proteins have a positive electric charge at wine pH since their isoelectric point is higher than the pH of the medium. Therefore, it is reasonable to assume that cation exchange resins can retain part of these proteins. However,

according to our results, cationic exchange does not seem to remove them since neither the concentration of the total protein nor any of its molecular weight fractions are affected by the treatment. This is a very interesting result, because proteins have been described as being foam enhancers and stabilisers (Cilindre *et al*., 2007; Maujean *et al*., 1990; Medina-Trujillo *et al*, 2017).

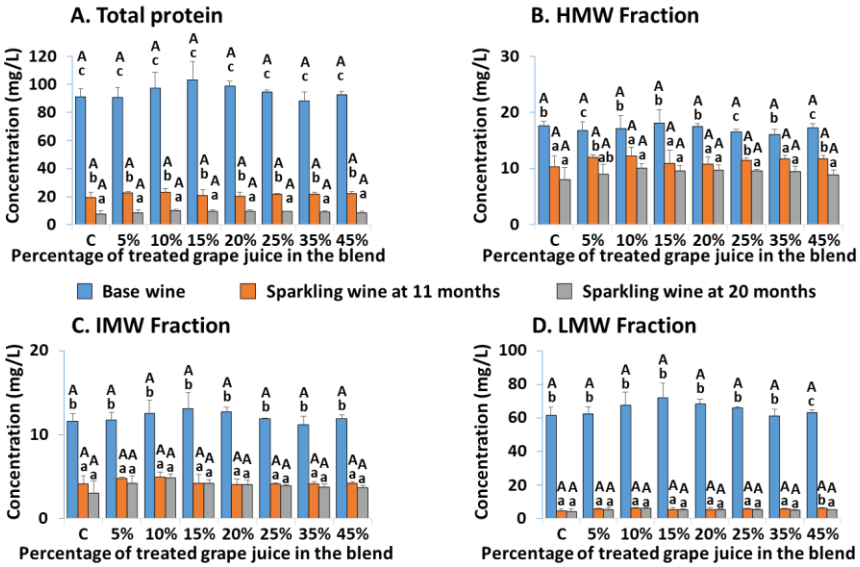

FIGURE 2. Protein composition.
All data are expressed as the arithmetic mean of 3 replicates ± standard deviation. C: Control wine. HMW: high molecular weight fraction (MW > 80 kDa); IMW: intermediate molecular weight fraction (80 kDa > MW > 60 kDa); LMW: low molecular weight fraction (MW < 60 kDa); Different capital letters indicate statistically significant differences ($p < 0.05$) between the samples in function of the percentage of cation exchange treated grape juice in the blend. Different lowercase letters indicate statistically significant differences ($p < 0.05$) between the different steps of the elaboration process.

Influence of cationic exchange treatment on the polysaccharide fraction of base and sparkling wines

Figure 3 shows the total polysaccharide concentration of the base wines and their corresponding sparkling wines at 11 and 20 months of aging time.

In this case, a small but significant increase was observed in the total concentration of the polysaccharides of the sparkling wines compared to their corresponding base wines. This higher polysaccharide content of sparkling wines

can be attributed to the release of mannoproteins and polysaccharides from yeast autolysis during the second fermentation and subsequent aging time in contact with the lees (Kemp *et al*., 2019; Martí-Raga *et al*., 2016; Martínez-Lapuente *et al*., 2013). In contrast, treatment by cationic exchange did not affect the polysaccharide concentration since no significant differences were observed between the control base wine and the blends with different proportions of treated grape juice. Similar results were obtained for the sparkling wines. Moreover, no differences were observed in any of the different molecular weight polysaccharide fractions (data not shown).

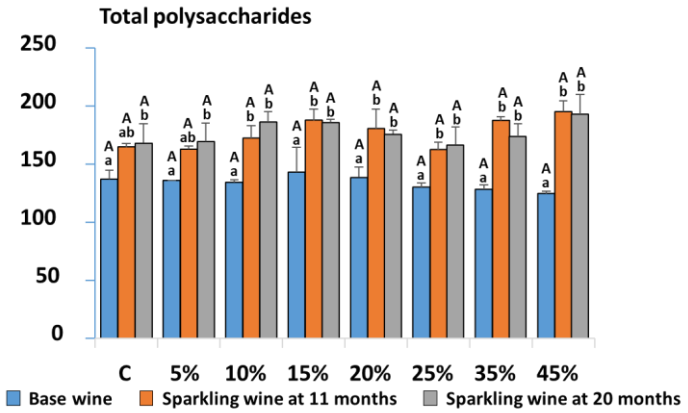

FIGURE 3. Polysaccharide composition.
All data are expressed as the arithmetic mean of 3 replicates ± standard deviation. C: Control wine. Different capital letters indicate statistically significant differences ($p < 0.05$) between the samples in function of the percentage of cation exchange treated grape juice in the blend. Different lowercase letters indicate statistically significant differences ($p < 0.05$) between the different steps of the elaboration process.

Influence of cationic exchange treatment on the foaming properties of base and sparkling wines

Figure 4A shows the maximum height of the foam (HM) of the base wines and their corresponding sparkling wines of 11 and 20 months of aging.

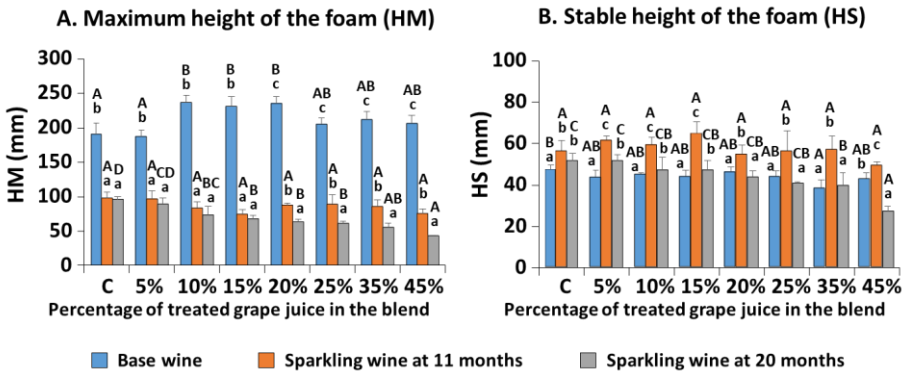

FIGURE 4. Foam parameters.
All data are expressed as the arithmetic mean of 3 replicates ± standard deviation. C: Control wine. Different capital letters indicate statistically significant differences ($p < 0.05$) between the samples in function of the percentage of cation exchange treated grape juice in the blend. Different lowercase letters indicate statistically significant differences ($p < 0.05$) between the different steps of the elaboration process.

In all cases, the HM of sparkling wines was significantly lower than in the corresponding base wines. No significant differences in HM were detected between sparkling wines aged 11 and 20 months when the percentage of grape juice treated in the blend was less than 20 %. However, when the proportion of treated grape juice was higher, a significant decrease in HM was observed in older sparkling wines. It therefore seems that high percentages of cationic exchange treatment can negatively affect the foamability of sparkling wines over time. This observed decrease in HM after the second fermentation has been described elsewhere and has two probable causes. First, the second fermentation increases ethanol content, which is negative for foam (Dussaud *et al*., 1994), and second, the use of bentonite as a riddling agent removes surface active agents like proteins (Dambrouck *et al*., 2005; Martínez-Rodriguez and Polo, 2003; Vanrell *et al*., 2006). In fact, our results confirm a drastic reduction in protein concentration in sparkling wines with respect to the base wines (Figure 2).

Figure 2B shows the stable height of the foam (HS) of base wines and sparkling wines of 11 and 20 months of aging. In general, a significant increase in HS was observed in the sparkling wines of 11 months of aging with respect to their

corresponding base wines. Other authors have described the HS of sparkling wines to be usually lower than in their corresponding base wines (Martí-Raga *et al*., 2016; Martínez-García *et al*., 2017; Vanrell *et al*., 2006). Nevertheless, similar or even increased values of HS after the second fermentation have also been reported (Cilindre *et al*., 2010; Esteruelas *et al*., 2015).

Only slight differences were detected in the foam parameters of the base wines and sparkling wines at 11 months of aging as a function of the percentage of treated grape juice included in the blend. However, the sparkling wines of 20 months showed a clear decreasing trend in both parameters, HM and HS, as the percentage of the treated grape juice included in the blend increased. This data therefore indicate that the cationic exchange treatment of the grape juice does not affect the foaming properties of the base wine and young sparkling wines, but that it can negatively affect the foaming characteristics of older sparkling wines, especially when the proportion of treated grape juice is higher. Figure 5 shows in a radar chart the results obtained from the sensory analysis with the sparkling wine samples aged for 20 months.

Influence of cationic exchange treatment on the sensory attributes of sparkling wines

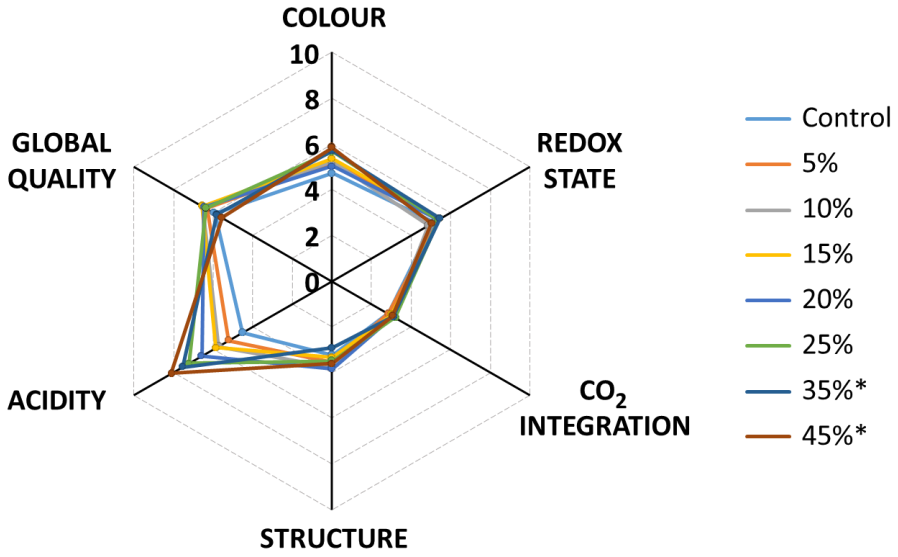

FIGURE 5. Sensorial analysis.
Influence of cationic exchange treatment on the sensory perception of sparkling wines of 20 months of aging. All data are the arithmetical average corresponding to the results of 10 tasters. The asterisk (*) indicates that the panel considered the acidity as excessive.

The results showed that the only sensory attribute in which the trained panel found clear differences was the acidity, with no differences detected in any of the other descriptors. The panel detected a clear increase in acidity of the sparkling wines as the proportion of cation exchange treatment increased. The panel also considered that the sparkling wine gained in freshness when the proportion of the treated grape juice in the blend was not too high. However, the acidity of the sparkling wines with a very high proportion of treated grape juice was considered excessive. The fact that the panel did not find any differences in the other sensory attributes indicates that the cation exchange treatment of the grape juice does not exert a negative sensory effect on the final sparkling wines.

Conclusions

The cation exchange treatment of the grape juice made it possible to increase the titratable acidity and reduce the pH of the base wines and their corresponding sparkling wines. This effect is clearly associated with the reduction of potassium levels. Its effect on other chemical parameters, such as ethanol content, residual sugars, L-malic acid, L-lactic acid, succinic acid, citric acid and acetic acids, can be considered as negligible. Moreover, no significant effects were observed on the concentration of any of the protein or polysaccharide fractions. Cationic exchange treatment of the grape juice did not negatively affect the foaming properties of the base wine or young sparkling wines; however, it caused a decrease in foamability and persistence of the foam in older sparkling wines, especially when the proportion of treated grape juice was higher.

A trained panel only found that the acidity of the sparkling wines was greater as the percentage of cationic exchanged treated grape juice increased. The higher acidity was perceived as positive when percentage of treated grape juice in the blend was relatively low, because the sparkling wines gained freshness. However, the sparkling wines with the higher percentages of treatment were considered as too acidic.

It can therefore be concluded that applying cationic exchange treatment to the grape juice is a very useful tool for reducing the pH of sparkling wines and increasing their freshness; however, the fact that excessive treatment can damage the acidity balance and negatively affect the quality of the sparkling wine should be taken into account.

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General discussion

UNIVERSITAT ROVIRA I VIRGILI
ANCESTRAL SPARKLING WINES; COMPARISON WITH TRADITIONAL SPARKLING WINES AND PROCEDURES TO IMPROVE THEIR QUALITY
Arnau Just Borràs

The main research of this thesis was to study possible technological solutions to compensate for the effects of climate change on the sparkling wine industry.

In the warm Mediterranean climate, the effects of global warming are more noticeable than in other areas. These effects can be summed up as a greater and earlier accumulation of sugars, a simultaneous premature loss of acidity and a late accumulation of aromatic compounds, which lead to unbalanced grapes. This study focuses on some possible oenological tools for mitigating the problems that the sparkling wine sector faces due to climate change. More precisely, the ancestral method was evaluated as an alternative to the traditional method for elaborating sparkling wine. In addition, the use of cationic exchange was studied as a possible solution to the lack of acidity in the wines as a result of climate change.

To our knowledge this work is the first scientific approach to the ancestral method with the aim of understanding the process and especially its limitations in order to propose improvements in the procedure to avoid faulty wines. For this work, it was first necessary to know what products elaborated using the ancestral method are on the market and how they are produced.

Therefore, ancestral sparkling wine producers in France, Catalonia, and Italy were interviewed and were asked to highlight the main problems of the ancestral method. Producers emphasized that the *prise de mousse* step is the critical point for different reasons, as it involves selecting the most appropriate time for bottling, which conditions the final internal pressure. They also considered that controlling the healthiness of the yeast population introduced into the bottle was a very important factor. An incorrect *prise de mousse* could lead to problems, such as bottles exploding, a lack of pressure and even bottles that do not ferment, faulty aromas, reduction taint or cloudiness.

Other producers highlighted the importance of short ageing periods because acidity levels of ancestral sparkling wines are usually lower. The interviews also showed the great heterogeneity of procedures that are encompassed under the name of ancestral sparkling wine. It was also emphasized that in the ancestral method, wines are bottled when the harvest is still not finished, which implies an additional amount of work at critical moments when most wineries do not have enough personal.

For this experimental approach, a representative batch of 11 commercial ancestral sparkling wines produced in Catalonia were analysed and compared with another representative batch of 9 traditional sparkling wines. As expected, the ancestral sparkling wines were a heterogenous group in which both well-elaborated and faulty products were found. In contrast, the traditional sparkling wines were found to be a homogenous group in which the analysed parameters did not vary very much among products.

Ancestral sparkling wines showed lower internal CO_2 pressures than traditional sparkling wines, which eventually leads to a less fizzy product once opened. In contrast, higher levels of turbidity, total polyphenols, glycerol, and gluconic acid were found. Ancestral sparkling wines had higher pH values. This is understandable due to the later harvest dates compared to the traditional method. Lower SO_2 levels can be used in the elaboration of ancestral sparkling wines because the wine is fully protected by the CO_2 generated in the bottling process. In contrast, traditional sparkling wines need higher amounts of SO_2 to protect the wine during the stabilization period prior to the second fermentation.

The colour of ancestral sparkling wines was significantly less clear and more intensely yellowish than that of traditional sparkling wines. It was also found that there was a larger palette of colours for ancestral sparkling wines, while traditional sparkling wines had a very similar colour.

Regarding the acidic composition, ancestral sparkling wines had higher D-lactic and acetic acid contents, which could indicate a certain uncontrolled growth of yeasts and/or bacteria related to product spoilage. It must be highlighted that only a few ancestral sparkling wines showed these altered parameters, but they had very high values.

Higher concentrations of macromolecules (proteins and polysaccharides) were found in ancestral sparkling wines. This data may be the result of the lower usage of clarifying or riddling agents and the higher yeast population present during bottling.

Although it was expected that larger amounts of proteins and polysaccharides could lead to higher foamabilities for ancestral sparkling wines, the opposite phenomenon occurred. In general, ancestral sparkling wines had lower foamability but with the higher heterogeneity previously described.

The determination of volatile compounds showed that ancestral sparkling wines had a higher concentration of higher alcohols and lower concentration of fatty acids. Higher alcohols, in the concentrations that were found, do not result in aromatic defects but they could result in a decrease in foamability. Higher alcohols are also related to non-settled grape juices and wines with high turbidity, which has been suggested by previous results.

A sensorial analysis corroborated the experimental results in terms of homogeneity/heterogeneity. The panel of tasters found that all traditional wines had similar characteristics while the ancestral sparkling wines varied quite a lot between wines.

In summary, the results show that the ancestral method can lead to a new, wider catalogue of sparkling wines that is different to what the consumer is used to. In contrast, traditional sparkling wines are produced using a precise and well-defined

methodology that leads to the products being very similar. The results also show that the ancestral method needs closer control of the process to avoid faulty wines.

Once it had been determined that faulty products could result from bad praxis at the winery, it was necessary to pinpoint the critical points of the ancestral process and highlight the importance of controlling them. Therefore, a study was carried out in which the same grape must was fermented in three different ways: one following the traditional method; and two following the ancestral method with two different initial yeast populations. This experiment also monitored the ageing-on lees of these products for one year.

Ancestral sparkling wines were bottled once the residual sugar concentration of the fermenting must was about 18 g/L. The fermenting grape must was partially filtered before bottling to obtain two initial yeast populations ($6.0x10^6$ and $12.0x10^6$ cell/mL). A disgorgement riddling agent was added in both cases to facilitate the future sedimentation of the lees. The remaining fermenting grape must finished the first fermentation in the tank, and was stabilized and used for a second fermentation according to the traditional method.

The results show that under these controlled conditions the pressure generated inside ancestral bottles was slightly lower than that in traditional bottles. It was also seen that for the same harvest time, ancestral sparkling wines always had less alcohol because no sugar was added for the second fermentation. The sulphur dioxide levels of ancestral sparkling wines were significantly lower than in traditional sparkling wines even considering the very low dose of this additive added during the stabilization period.

The foaming parameters improved in both types of sparkling wines between 6 and 12 months of ageing. In general, the foam parameters of ancestral sparkling wines

tended to be better than in traditional sparkling wines although these differences were not always significant. This behaviour is probably due to the higher alcohol content in ancestral wines compared to traditional sparkling wines.

In terms of the macromolecular composition, the protein concentration increased during ageing similarly in all sparkling wines. In contrast, polysaccharide content behaved erratically. In the traditional method the polysaccharide concentration decreased, probably due to precipitation. In contrast, the polysaccharide concentration increased in both ancestral sparkling wines, probably because their higher yeast population favours the autolysis process.

It was also seen that the colloidal composition (aggregations that occur among macromolecules like proteins, polysaccharides, and polyphenols) of the wines differed between ancestral and traditional sparkling wines. Traditional method sparkling wines had more and larger colloids than ancestral sparkling wines, which could indicate that the *prise de mousse* methodology could have an impact on these parameters and consequently on the foamability of the wines. Unfortunately, there are very few results on the colloidal composition of wines in the literature and therefore no clear justification could be found for this phenomenon.

Finally, a tasting session with a trained panel was performed at 6 and 12 months of ageing. The results showed that in these short ageing periods, the panel preferred the ancestral sparkling wine with the lower inoculated yeast population. Ancestral sparkling wines were also found to have less aggressive CO_2, smaller bubbles, and a higher impact of ageing, probably due to the higher yeast populations in the bottle during ageing.

One of the main disadvantages of ancestral sparkling wines is the higher pH and lower acidity levels in comparison with traditional sparkling wines. This is because

grapes for the ancestral method are normally harvested later than those for traditional sparkling wines. This disadvantage could be accentuated by the impacts of global warming.

Some viticultural and technological techniques have been suggested to solve this problem. Cationic exchange was studied as part of this thesis because it is currently widely used and due to its economic price and efficiency. This study was performed with the traditional method and not with the ancestral method because none of the wineries that produce sparkling wine using the ancestral method had the necessary equipment. However, these results can be extrapolated perfectly to the ancestral sparkling wine.

To perform this study a Macabeo grape must was treated with cationic exchange resins to decrease its pH and stabilize its acidic content. A final must with a pH = 1.9 was obtained. Then, blends were made with different percentages of treated and non-treated grape must and the resultant blends were vinified following the traditional method.

Results were obtained for base wine at 11 and 20 months of ageing. It was observed that as the percentage of treated grape juice increased, the acidity increased and pH decreased proportionally.

In all cases residual sugars were below 2 g/L, which means that all fermentations were performed without any problems. This indicates that the increased acidity in the media was not a problem for any group.

The macromolecular composition of the sparkling wines was determined by HPLC. The protein concentration decreased during the first ageing months, while the polysaccharide concentration increased during the ageing process. This phenomenon was due to riddling agents being used and due to the autolysis process. Riddling agents containing bentonite are used to facilitate the removal of

the lees in the disgorgement process, and they also lead to a loss of proteins. In contrast, polysaccharides are released into the wine during the ageing on-lees process, and therefore it is normal that their concentration increases over time. Regardless of the percentage of treated grape must added, no different behaviour was detected. This means that the treatment had no significant impact on the macromolecular composition of the wines.

The foaming parameters were evaluated for base wine and sparkling wines. While it was observed that additions of 10-20% of treated grape juice had a positive effect on HM (maximum height of the foam) in base wine, in sparkling wines this effect disappeared. At 20 months of ageing the maximum height of the foam diminished at high concentrations of treated grape must. The same phenomenon happened for the stable height of the foam (HS).

Sensorially, a clear preference for the wines with moderate treatments was observed. It was also seen that the trained panel was only able to distinguish the products by their acidity and were unable to differentiate the products by characteristics like aroma, structure, or colour.

It was concluded that cationic exchange is a tool that has no negative impact on the sensory and major physico-chemical parameters of the sparkling wines. It was also observed that extreme additions of treated grape juice may be negative for the foaming properties and may also not be acceptable by the consumer. Cationic exchange was found to be a reliable tool for enhancing the titratable acidity of grape musts and decreasing their pH, and these differences were maintained in the final sparkling wines.

In summary, cationic exchange was found to be a reliable, cost-effective technique that can help sparkling wine producers to deal with the high pH and low acidities that climate change is bringing with it. This technique, which can be used in low

percentages of the grape must, can be applied with the traditional or ancestral methods as it has been found that the fermentation dynamics are not affected.

These studies on ancestral sparkling wines are just the first step in a new path of research possibilities in which other characteristics and potential innovations in the methodology can be studied.

Conclusions

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1. Commercial ancestral sparkling wines show much greater heterogeneity than traditional sparkling wines, not only on a physicochemical level but also on a sensory level.

2. In general, ancestral sparkling wines have similar ethanol content, lower internal pressure, titratable acidity and sulphur dioxide, and higher pH, colour intensity, turbidity and gluconic acid than traditional sparkling wines.

3. Ancestral sparkling wines have a similar ethanol content as traditional sparkling wines because they are normally harvested later. The greater grape maturity therefore compensates for the addition of sugar in the second fermentation in the traditional method. This is an advantage of the ancestral method in relation to climate change.

4. The higher presence of gluconic acid in some of the ancestral sparkling wines is probably because the grapes of these wines are usually harvested later than those used for the traditional method. This later harvest increases the risk of *Botrytis cinerea* developing and therefore the levels of gluconic acid.

5. The lower internal pressure of some ancestral sparkling wines could be because they were bottled too late when the remaining fermentable sugars were too low.

6. Ancestral sparkling wines have a more intense yellow colour probably because some of them were not well protected with sulphur dioxide during elaboration.

7. The higher turbidity of ancestral sparkling wines is probably due to the larger yeast population introduced during bottling and also because some of them were elaborated without adding a riddling agent.

8. The lower sulphur dioxide levels of ancestral sparkling wines are due to the fermenting must being bottled directly at the correct time and therefore they do not need a supplementary dosage of this additive during the stabilization process prior to the second fermentation. This lower sulphur dioxide level of ancestral sparkling wines is another advantage of this elaboration method.

9. Ancestral sparkling wines have a significantly higher concentration of proteins and polysaccharides than traditional sparkling wines. This is probably because some of the ancestral sparkling wines have been elaborated without using bentonite for fining and/or riddling and also because they probably have higher yeast populations that would favour autolysis.

10. Ancestral sparkling wines have a higher concentration of higher alcohols and lower levels of fatty acids than traditional sparkling wines.

11. Foamability and the foam persistence of commercial ancestral sparkling wines were in general worse than in traditional sparkling wines. This could be related to their lower internal pressure and also to the higher levels of gluconic acid in some of the ancestral sparkling wines.

12. The sensory analysis confirmed the greater heterogeneity of ancestral sparkling wines. It must be highlighted that some of the ancestral sparkling wines were very similar to traditional sparkling wines whereas others were very different.

13. The greater heterogeneity of ancestral sparkling wines is probably because the ancestral method is not so well defined as the traditional method. This means that some of the ancestral sparkling wines are not elaborated correctly.

14. When ancestral and traditional sparkling wines are elaborated with the same grapes, the differences between them are lower than those observed in commercial sparkling wines.

15. Ancestral sparkling wines have a lower ethanol content because they are not supplemented with sugars for the second fermentation. Ancestral sparkling wines also have a lower sulphur dioxide concentration because they do not have a stabilization period.

16. Unlike in commercial ancestral sparkling wines, no differences were found in the protein fraction, probably because in that case bentonite was added during the elaboration process.

17. The concentration of polysaccharides was higher in ancestral sparkling wines probably because of the larger yeast population, which favours autolysis and also because traditional sparkling wines have more ethanol, which favours their precipitation.

18. Experimetnal ancestral sparkling wines present in general better foaming parameters than traditional sparkling wines probably because of its lower alcohol content. Although the differences were not always significant.

19. In general, a trained panel considered that ancestral sparkling wines have smaller bubbles and less carbon dioxide aggressivity than traditional sparkling wines. Tasters also considered that ancestral sparkling wines were more aged.

20. The ancestral sparkling wine with a low yeast population was scored the highest by the trained panel. These data, together with those observed in the previous experiment, confirm the importance of limiting the yeast population in the ancestral method.

21. The application of cationic exchange in grape must makes it possible to decrease the pH and increase the titratable acidity of sparkling wines. No significant differences were found in any of the other physico-chemical parameters.

22. A trained panel considered that relatively low percentages of treated grape must in the blend improved the freshness and quality of the sparkling wines. In contrast, an excess of treated grape must in the blend can alter the sensory quality of the product.

23. In this study, cationic exchange was applied to the grape must and not to the base wine, and therefore it shows that this technique can be used perfectly in the ancestral method.

24. All these data indicate that the ancestral method is a very interesting procedure for elaborating different sparkling wines. However, the great heterogeneity observed in commercial products, some of which were even faulty, suggests the need to develop a manual of good practices for elaborating ancestral sparkling wines, which could be adopted by the Protected Designations of Origin (PDO) that produces this type of sparkling wines.

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Appendix

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Publications derived from this PhD thesis

- Assessment of physic-chemical and sensory characteristics of commercial sparkling wines obtained through ancestral and traditional methods. (2024) *submitted to Food Research International*
- Comparison of Ancestral and Traditional methods for elaborating sparkling wines. Just-Borràs, A., Moroz, E., Giménez, P., Gombau, J., Ribé, E., Collado, A., Cabanillas, P., Marangon, M., Fort, F., Canals, J-M., Zamora, F. (2024) *Accepted on Current Research in Food Science*
- Effects of using cationic exchange for reducing pH on the composition and quality of sparkling wine (Cava). Just-Borràs, A., Pons-Mercadé, P., Gombau, J., Giménez, P., Vilomara, G., Conde, M., Cantos, A., Canals, J-M., Zamora F. (2022). OENO One, 56(2), 179–192. https://doi.org/10.20870/oeno-one.2022.56.2.5399

Publication to non-indexed journal

- Influence of cationic exchange for reducing pH on the composition and quality of sparkling wine. Just-Borràs, A., Pons-Mercadé, P., Gombau, J., Giménez, P., Vilomara, G., Conde, M., Cantos, A., Canals, J-M., Zamora F.

Contribution to national and international meetings

Oral communication

- Influencia del intercambio catiónico para reducir el pH en la composición y calidad del vino espumoso. Just-Borràs, A. in Congreso Internacional ACE de la Enología y el Cava 2023. Sant Sadurní d'Anoia.

Poster communications

- Comparison of Ancestral and Traditional methods in the elaboration of sparkling wines; Preliminary results. Just-Borràs, A., Moroz, E., Giménez,

P., Cabanillas, P., Gombau, J., Canals, J-M., Zamora, F. in II International Conference of Grapevine and Wine Sciences (2023), Logroño.

- Comparative study among sparkling wines elaborated by traditional and ancestral methods. Just-Borràs, A., Alday-Hernández, M., García-Roldán, A., Bustamante, M., Gombau, J., Cabanillas, P., Rozès, N., Canals, J-M., Zamora, F. in XVI Congreso Nacional de Investigación Enológica (GIENOL) (2024), Zaragoza.

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