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Article

# 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.

**Keywords:** sparkling wines; ancestral method; *Pét-Nat*; traditional method; physicochemical composition; sensory quality

## 1. Introduction

Sparkling wines market is a growing worldwide industry which at 2023 reached the US\$ 39.3 Billion [1,2]. 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 [3]. 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 [4,5].

The effervescence of high quality natural sparkling wines must be obtained through an alcoholic fermentation in sealed vessels (bottle or tank fermentation) [6,7] 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 [2,8]. 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 [9].

By contrast, ancestral method, which seems to be historically the oldest procedure to elaborate sparkling wines, consists only in one fermentation [10]. 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 [11] 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 [12–14]. 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 [15–17].

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 [18]. Even though theoretically ancestrals should have a clear appearance and without fermentation faults [19], 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) [20,21]. In fact, these difficulties were the main reason that explains the progressive abandon of the method centuries ago in favour of the traditional method [22,23].

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 [24,25].

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 [26]. 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 [27] and for environment [28].

Because of its recent popularity, the scientific literature on ancestral sparkling wines is very scarce and only few scientific articles can be found [29–31]. By contrast, traditional sparkling wines [32–35] and other natural sparkling wine methods [9,36,37] 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.

2. Materials and Methods

2.1. 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 & Parellada coupage.

2.2. Sample Preparation

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

2.3. Analysis of General Wine Parameters

The internal CO<sub>2</sub> pressure of the bottles was measured using a non-invasive Laser Sensor (L.Sensor CO<sub>2</sub>, FTSsystem, 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 [38]. 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).

#### 2.4. Colour Parameters

The CIEL\*a\*b\* coordinates were determined following the method described by Ayala et al. [39] using a Helios Alpha UV VIS spectrophotometer (Thermo Fisher Scientific Inc., Waltham, 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).

#### 2.5. Quantification of Proteins by HRSEC-DAD

The samples were processed and analysed using the methodology described by Canals et al. [40]. 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  $\geq 98.0$  % (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  $\mu$ L of ammonium acetate solution (300 mM) and centrifuged (12,000 g for 5 min). The supernatant was filtered through 0.22  $\mu$ m acetate cellulose filters (Merck Millipore, Darmstadt, Germany) and then 100  $\mu$ L 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  $\times$  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.

#### 2.6. Polysaccharide Extraction and Determination by HRSEC-RID

The samples were processed using a variation of the methodology described by Ayestarán et al. [41]. Briefly, 1 mL of sample was frozen to  $-20$ °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 ( $70$ °C). The pellet was resuspended in mL of 50 mM ammonium formate  $\geq 99.0$  % (Sigma-Aldrich, Madrid, Spain) and filtered through 0.22  $\mu$ m acetate cellulose filters (Merck Millipore, Darmstadt, Germany). Then 100  $\mu$ L 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  $\times$  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.

### 2.7. Volatile Compound Analysis by Gas Chromatography

Volatile compounds were extracted using a modification of the methodology described by Ortega et al. [42]. the volatile compounds were liquid/liquid extracted with 400 µL of dichloromethane in presence of 2.5 g (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 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-phenylethanol 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).

### 2.8. Measurement of Foaming Properties

The foam properties were measured using the Mosalux method (Station Oenotechnique de Champagne, Epernay, France) according to the procedure described by Maujean et al [43]. Two parameters were measured: HM, the maximum foam height, and HS, the stable foam height. HM represents foamability while HS represents foam stability.

### 2.9. 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.

### 2.10. Statistical Analysis

The data shown are the arithmetic means of triplicates with the standard deviation for each parameter. One-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).

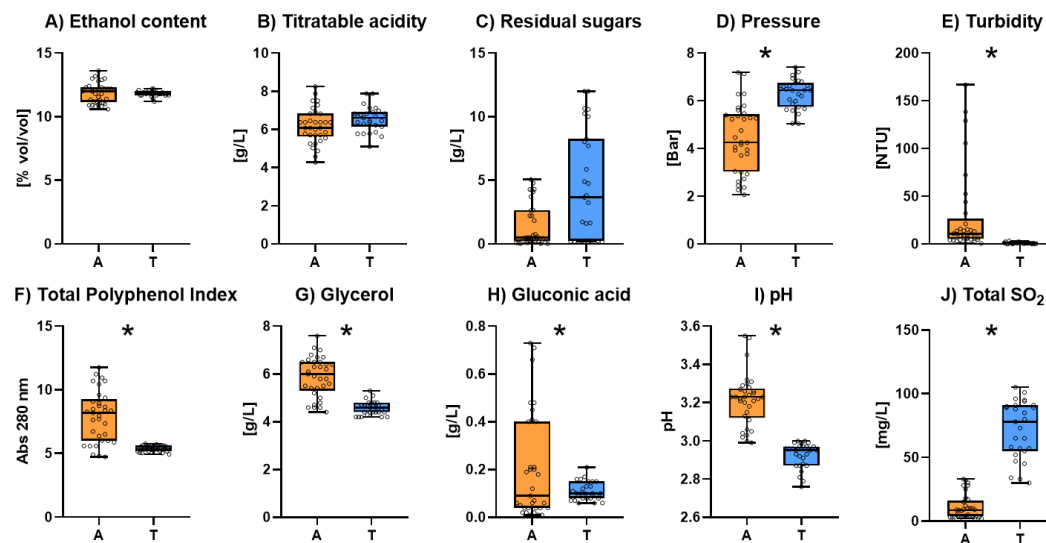
## 3. Results and Discussion

### 3.1. 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 Figure 1. The individual results for general parameters of all the studied sparkling wines are shown in Table S1. No significant differences were found between ancestral and traditional sparkling wines for ethanol content (Figure 1.A) and total titratable acidity (Figure 1.B). Neither significant difference was found for residual

sugars (Figure 1.C) even though some of the traditional sparkling wine samples were classified as “Brut” and therefore contained some added sugar.



**Figure 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 (Figure 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 [6] 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.

Figure 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 [44].

The total polyphenol index (TPI) (Figure 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 [45].

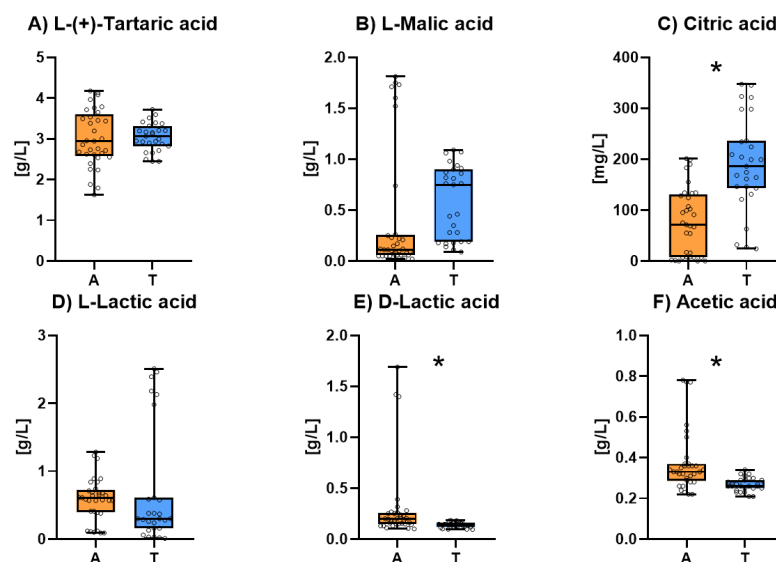
Glycerol (Figure 1.G) content was found to be significantly higher on Ancestral sparkling wines than in Traditional ones. Glycerol is mainly produced by yeast throughout glyceropyruvic pathway [46]. It has been described that glycerol is in part produced by yeast to compensate the osmotic pressure of the fermenting matrix [47]. 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* [48]. 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 (Figure 1.H) in Ancestral sparkling wines since this acid is considered as a marker of the presence of *B. cinerea* [49].

Ancestral sparkling wines had also significant higher pH levels (Figure 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 (Figure 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 [50].

### 3.2. Acidic characterization of sparkling wines

The concentration of the major acids of the different sparkling wines is showed in Figure 2. The individual results for acidic composition of all the studied sparkling wines are shown in Table S2. The levels of L-(+)-tartaric acid (Figure 2.A), L-Malic acid (Figure 2.B) and L-lactic acid (Figure 2.C) were similar in both types of sparkling wines whereas the concentration of citric acid (Figure 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.



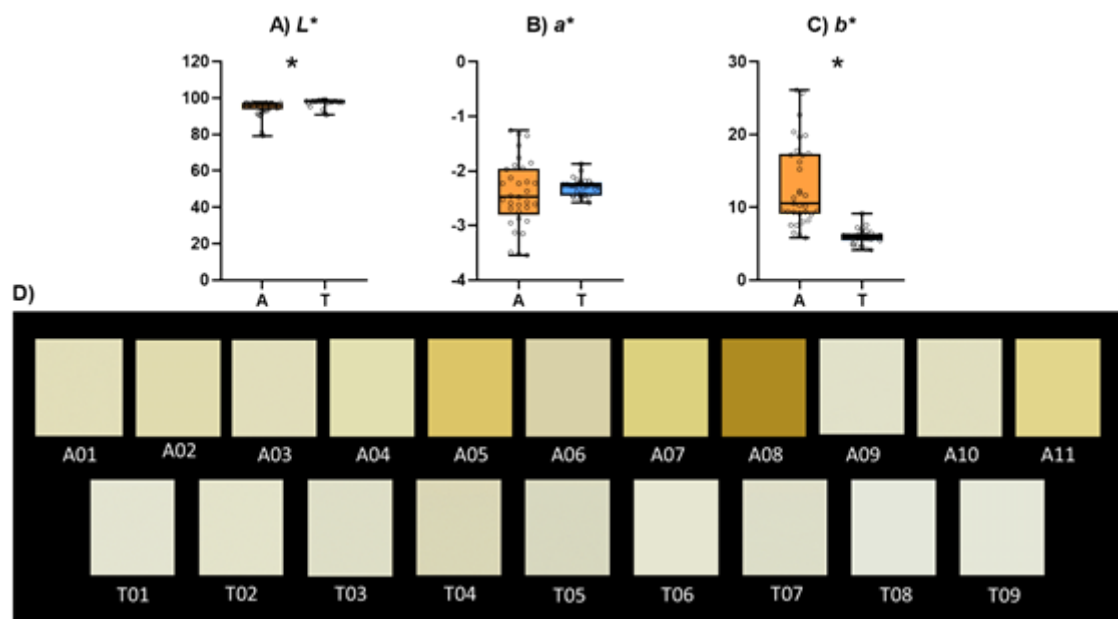
**Figure 2.** Box-plots for organic acids of sparkling wines elaborated by ancestral (A, n = 11) or traditional (T, n = 9) methods. The presence of an asterisk indicates the existence of significant differences at  $p < 0.05$ .

The average content of D-lactic acid (Figure 2.E) and acetic acid (Figure 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 [51,52].

### 3.3. Colour

Figure 3 shows the  $CieL^*a^*b^*$  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 Table S3. Lightness ( $L^*$ ) (Figure 3.A) of Ancestral sparkling wines was significantly lower and the blue-yellow component of the colour ( $b^*$ ) (Figure 3.C) significantly higher than in Traditional sparkling wines whereas no significant differences were found in the green-red component ( $a^*$ ) (Figure 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 (Figure 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 [53].

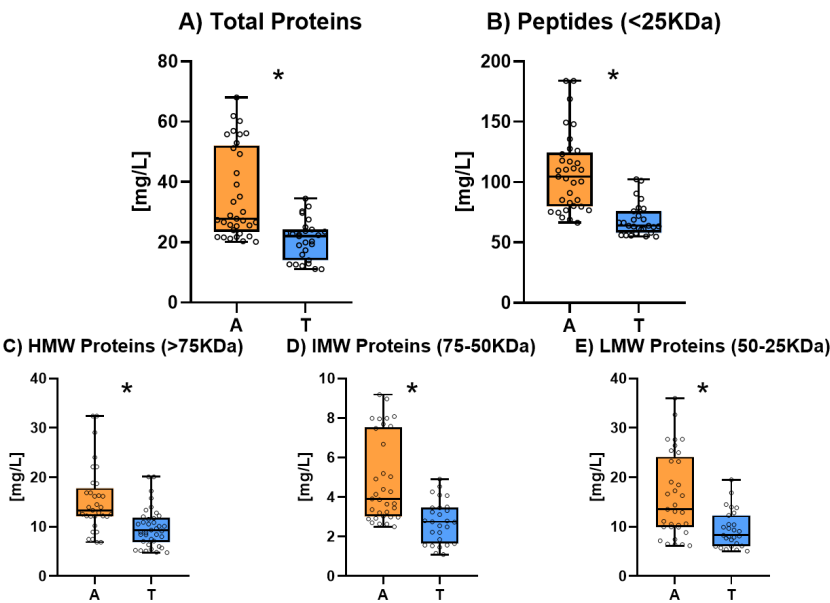


**Figure 3.** Box-plots for colour coordinates (A-C) and the palette of colours in RGB signals (D) of sparkling wines elaborated by ancestral (A,  $n = 11$ ) or traditional (T,  $n = 9$ ) methods. The presence of an asterisk indicates the existence of significant difference.

### 3.4. Proteins

Protein and peptide content determined via HPLC are showed in Figure 4. The individual results for protein and peptide composition of all the studied sparkling wines are shown in Table S4. These results show that Ancestral sparkling wines had significantly higher protein content than Traditional sparkling wines. Total proteins (Figure 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 (Figures 4.C, 4.D, 4.E) showed a similar behaviour, being high (HMW) and the low molecular weight (LMW) fractions the most abundant. Peptide content (Figure 4.B) was also found to be significantly higher in Ancestral sparkling wines

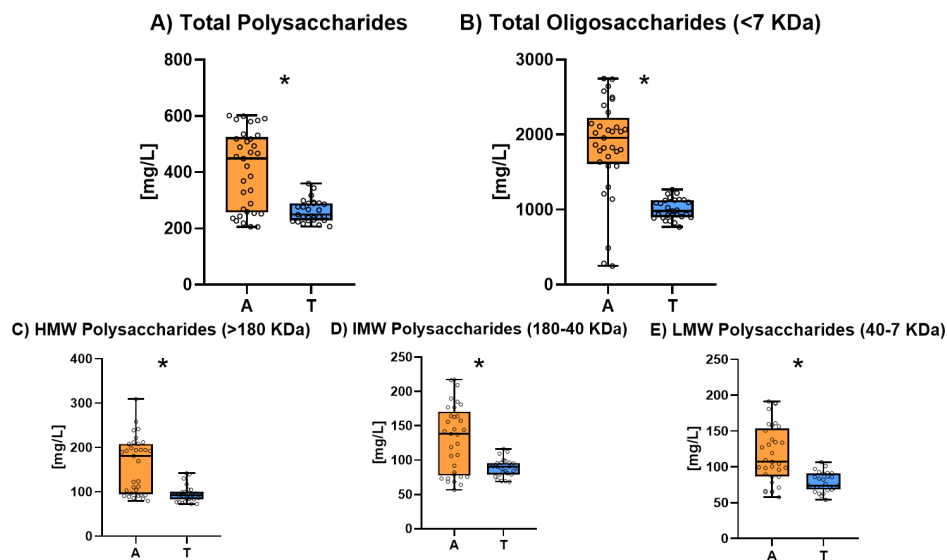
This higher concentration of proteins and peptides may be due to two different reasons. In one hand, the base wines of Traditional sparkling wines are usually treated with bentonite for fining and riddling which eliminates a substantial amount of natural wine occurring proteins [54,55]. By contrast, 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[56–58].



**Figure 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  $p < 0.05$ .

3.5 Polysaccharides

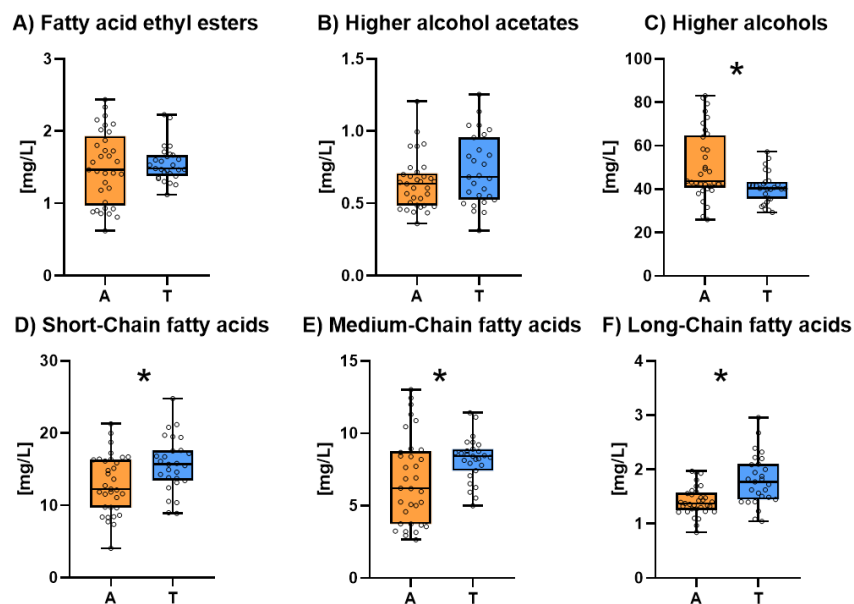
Figure 5 shows the polysaccharide and oligosaccharide content. The individual results for polysaccharide and oligosaccharide composition of all the studied sparkling wines are shown in Table S5. Ancestral sparkling wines had significantly higher concentrations of polysaccharides (Figure 5.A) (from 200 to 600 mg/L) than Traditional method sparkling wines (all around 200 mg/L). Oligosaccharide content (Figure 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.



**Figure 5.** Box-plots for polysaccharides and oligosaccharides 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  $p < 0.05$ .

### 3.6. Volatile Substances

Figure 6 shows the volatile substances composition of both types of sparkling wines. The individual results for each one of the six families of all the studied sparkling wines are shown in Table S6. The volatile substances were grouped in this figure in six families. No significant differences were found in the concentration of fatty acids ethyl esters (Figure 6.A) and total higher alcohol acetates (Figure 6.B). However, Ancestral sparkling wines showed higher levels of higher alcohols (Figure 6.C) whereas Traditional sparkling wines showed significantly higher levels of short (Figure 6.D), medium (Figure 6.E) and long-chain fatty acids (Figure 6.F).



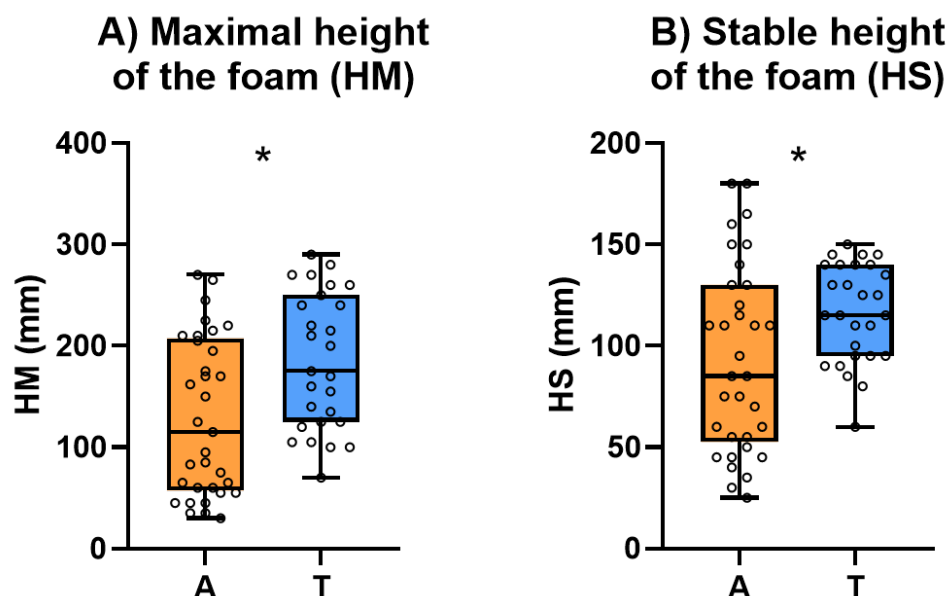
**Figure 6.** Box-plots for volatile substances of sparkling wines elaborated by ancestral (A, n = 11) or traditional (T, n = 9) methods. The presence of an asterisk indicates the existence of significant differences at  $p < 0.05$ .

Higher alcohols are the result of amino-acidic metabolism of yeasts when the nitrogen is scarce during fermentation [59]. 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 [43]. 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 [60]. In fact, non-settled grape musts give rise to wines with a higher concentration of higher alcohols [61]. 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 [62]. Short (SCFA) and medium (MCFA) chain fatty acids have been described as toxic for yeast being sometimes responsible of stuck and sluggish fermentations [63,64]. By contrast, long-chain fatty acids (LCFA) are principal constituents of the yeast cell membrane and its presence increases with aeration during fermentation [65]. 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 [64,66].

### 3.7. Foaming Properties

The maximal height of the foam (HM) (Figure 7.A) that represents the wine foamability and stable height of the foam (HS) (Figure 7.B) that represents the foam stability of both types of sparkling wines are shown in Figure 7. The individual results for HM and HS of all the studied sparkling wines are shown in Table S7.



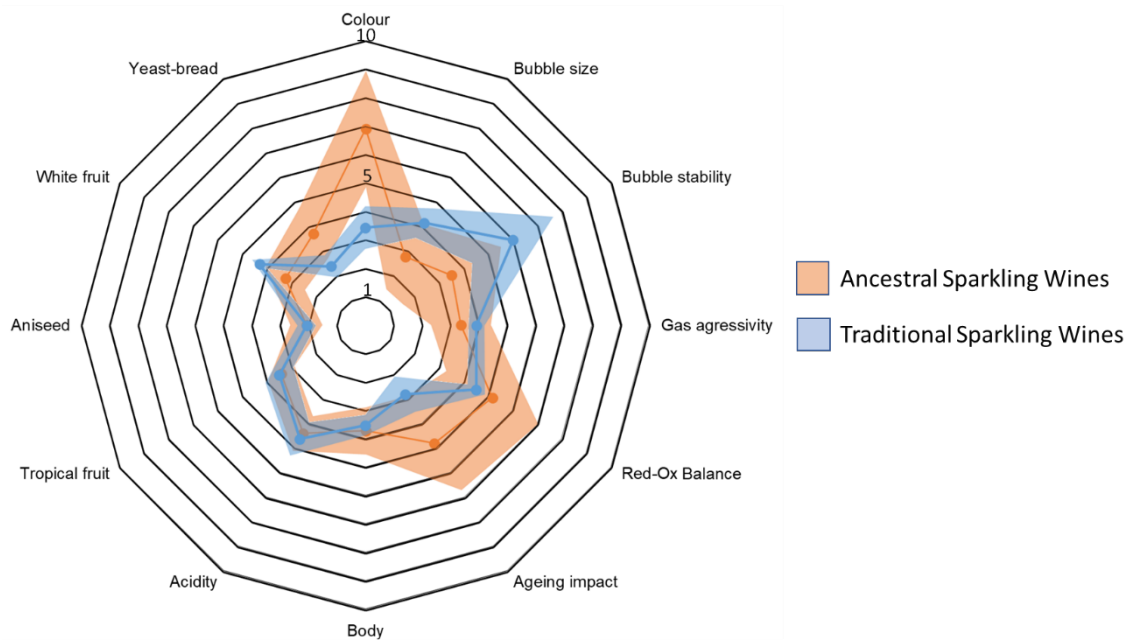
**Figure 7.** Box-plots for foaming properties of sparkling wines elaborated by ancestral (A, n = 11) or traditional (T, n = 9) methods. The presence of an asterisk indicates the existence of significant differences at  $p < 0.05$ .

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 [67,68]. 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 [49,69]. In addition, Ancestral sparkling wines also have significantly higher concentration of higher alcohols which have been reported as negative for foamability [43].

### 3.8. Sensorial Analysis

Sensorial analysis results are shown in the Figure 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.

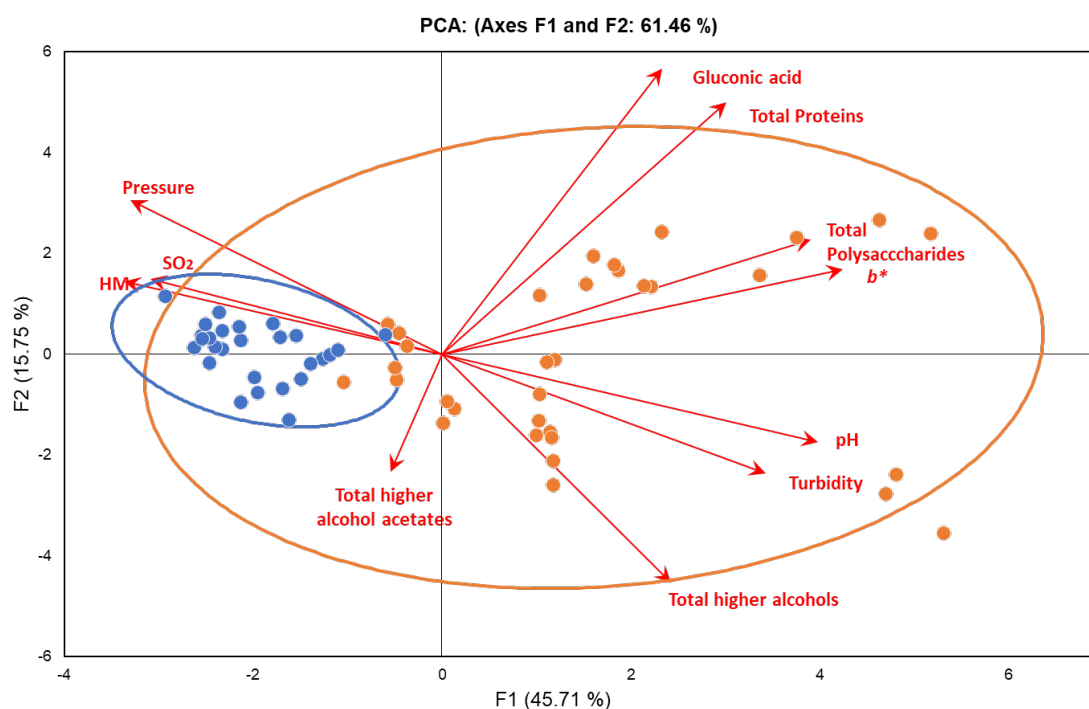


**Figure 8.** Spider web chart for the sensorial analysis results. The average value for each one of the sensory attributes is indicated by a continuous line whereas the standard deviation is showed as a shadowed ring surrounding it.

### 3.9. Principal Component Analysis

In order to better understand the differences between Ancestral and Traditional sparkling wines a principal component analysis was performed. Figure 9 shows the plot of varimax-rotated corresponding to the principal components analysis. The first component (PC1) explains 45.71% of the variance, and the second (PC2) explains 15.75% (so the explained aggregate variance was 61.46% 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, Ciel\**a*\**b*\* *b*\* parameter, 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,  $b^*$ , pH, turbidity and total higher alcohols showed a strong negative correlation with HM, SO<sub>2</sub> and pressure. In addition, gluconic acid and total proteins had a negative correlation with total higher alcohol acetates.



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

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<sub>2</sub> 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,  $b^*$ , 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.

#### 4. 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 since 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. Today these advantages make the Ancestral method an interesting alternative to the Traditional method to produce sparkling wines.

**Supplementary Materials:** The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

**Author Contributions:** A.J.-B.: methodology, investigation, writing—original draft, and data curation; M.A.-H.: methodology and research; A.G.-R.: methodology and research; M.B.: methodology and research; J.G.: methodology and research; P.C.: supervision; N.R.: supervision; J.M.C.: supervision; F.Z.: supervision, methodology, research, writing—original draft, and data curation. All authors have read and agreed to the published version of the manuscript.

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