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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 produced using the ancestral method, also known as *Pét-Nats*, are gaining more and more market share these days. However, scientific information on these wines is very scarce. The aim of this study was therefore to compare the physicochemical composition and sensory characteristics of a representative selection of commercial sparkling wines produced using the ancestral and traditional methods. Ancestral sparkling wines were more heterogeneous than traditional sparkling wines, as some of them showed lower internal pressure, higher turbidity, higher color intensity and lower effervescence. These differences are probably due to the fact that the protocol for making sparkling wines using the ancestral method is not as well defined as that for traditional sparkling wines. However, the ancestral method has the advantage of being able to work with riper grapes and a lower sulfur dioxide dosage.

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

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1. Introduction

The sparkling wines market is a growing worldwide industry, which reached USD 39.3 billion in 2023 [1,2]. This huge market volume can be explained through the changes in the social perception of sparkling wines and the increase in the diversity offered within the sparkling wines category [3]. While the market is still led by Champagne and Prosecco, ancestral sparkling wines—also known by their French name *Pét-Nats*, are one of these "new" products that are gaining popularity among consumers who are looking for wines with a lower alcohol content and for wine insiders who like to try particular products directly from grape growers [4,5].

The effervescence of high-quality natural sparkling wines must be obtained through 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. The traditional method, used for Champagne, Cava or Franciacorta, and the *Charmat* method, used for Prosecco, are the most well known [2,8]. Both methodologies follow a second fermentation over a low-alcohol 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 favor the sedimentation and compact the lees at the neck of the bottle (only in the case of the traditional method). The main difference between these two methodologies is that the second fermentation in the traditional method is performed in closed individual bottles while the second fermentation in the *Charmat* method is carried out in isobaric fermentation tanks, called autoclaves, which retain the generated pressure [9].

By contrast, the ancestral method, which seems to be the oldest procedure to elaborate sparkling wines, consists of only one fermentation [10]. The alcoholic fermentation starts in a fermentation tank until the fermenting must's sugars level is low enough (approximately 18 g/L). The fermenting must is then bottled to allow the fermentation to finish on sealed bottles [11] adding or not adding 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 wine-growing regions, it 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 ancestral sparkling wine production by creating its own special categories, like "Effervescent du monde®" since 2010, "Barcelona Rosé" awards since its creation in 2022, or 50 Great Sparkling Wines contest since 2022 [12–14]. Other contests have not incorporated a specific category for this kind of wine but it can be found that between 2015 and 2018, some important prizes have started to be given to ancestral sparkling wines [15–17].

However, the process for producing ancestral sparkling wine is not as well defined as that for the traditional method, and this is probably the reason why the old sparkling wines available on the market show great heterogeneity in terms of their parameters such as turbidity, effervescence, aroma or color [18]. Even though ancestral wines should theoretically have a clear appearance 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 to the difficulty in 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 yeast population in the bottle could make the riddling process difficult and consequently cause excess turbidity in the bottle and even the appearance off-flavors (reduction taint) [20,21]. In fact, these difficulties were the main reason for the progressive abandoning of the method centuries ago in favor of the traditional method [22,23].

In Spain, ancestral sparkling wines, are mainly produced in zones with an old sparkling wine culture, like the Catalan Cava region and its surroundings. This increasing phenomenon has led Protected Designation of Origins (PDOs) such as PDO Penedès and PDO Tarragona to include and regulate ancestral sparkling wine production [24,25].

Ancestral sparkling wines are not just a growing elaboration trend but are also a way to obtain sparkling wines under warm climate conditions, as 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 performed later than in the case of grapes for the traditional method. This later harvest enables obtaining 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 need for sulfite addition to protect against oxidation or microbiological spoilage is 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 sulfites have been shown to have adverse effects on human health [27] and on the environment [28].

Because of its recent popularity, the scientific literature on ancestral sparkling wines is very scarce and only a 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 wine-growing 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 the traditional method and 11 by the ancestral method, were collected from different wine-specialized shops. The ancestral sparkling wines were all without a 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 a cork or with a crown cap and some of them were presented in transparent bottles while traditional sparkling samples were all presented with a cork cap (mandatory) and in green opaque bottles. All traditional sparkling wines were from the 2022 vintage while ancestral wines 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 and 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 and 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 contains Xarel·lo, Macabeu and Parellada wines while Blend 2 contains Macabeu and Parellada coupage.

2.2. Sample Preparation

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

2.3. Analysis of General Wine Parameters

The internal CO_2 pressure of the bottles was measured using a non-invasive Laser Sensor (L.Sensor CO_2, FTSystem, Alseno, Piacenza, 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 according to the methods recommended by the OIV [38]. The total sulfur 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. Color 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., Walthman, MA, USA). Data were processed using MSCV® software (http://www.unirioja.es/color/descargas.shtml, accessed on 7 October 2024). Total Polyphenol index (TPI) was determined by the dilution 1:10 of each sample and the measurement at 280 nm via spectrophotometer. The color 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 analyzed using the methodology described by Canals et al. [40]. A value of 15 mL of each sample was concentrated in triplicate following two-step dialysis in tubes with a molecular weight cutoff of 3.5 kDa (Spectrum Laboratories Inc., Rancho Dominguez, Los Angeles, CA, USA). The first step lasted 48 h 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 48 h. The dialyzed samples were subsequently lyophilized and preserved at −20 °C. The soluble fractions were analyzed 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, CA, 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.

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) for 10 min, 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 µm acetate cellulose filters (Merck Millipore, Darmstadt, Germany). Then, 100 µL was injected into the chromatographic system. The analyses were carried out in an HPLC Agilent 1200 Series system (Agilent Technologies Inc., Santa Clara, CA, 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.

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 $(NH_4)_2SO_4$ 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, CA, 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).

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, 9 men and 6 women aged between 24 and 60. For each sample, the tasters were required to evaluate the intensity of eight sensory attributes (color, 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's comparison tests were carried out using the XLSTAT software version 2022.5.1 (Addinsoft, Paris, France). The sensorial analysis results were analyzed also with the PanelCheck V1.4.2 software (Nofima Mat, Technical University of Denmark and University of Copenhagen).

3. Results and Discussion

3.1. General Parameters

All figures contain only the average ± standard deviation of the various parameters of the two types of sparkling wine. The results for all the sparkling wines tested are shown in the 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 1A) and total titratable acidity (Figure 1B). Neither significant difference was found for residual sugars (Figure 1C) even though some of the traditional sparkling wine samples were classified as "Brut" and therefore contained some added sugar.

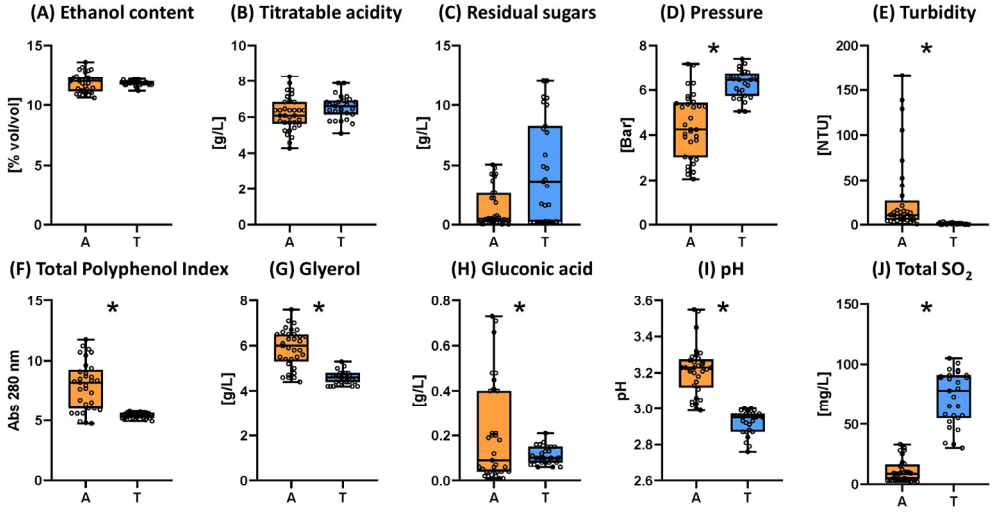

Figure 1. Box plots for standard parameters of sparkling wines elaborated by ancestral (A, $n = 11$) or traditional (T, $n = 9$) methods. The presence of an asterisk indicates the existence of significant differences at $p < 0.05$.

The average internal pressure (Figure 1D) 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 1E shows the turbidity of the different samples. The 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 1F) 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 color oxidability [45].

Glycerol (Figure 1G) content was found to be significantly higher on ancestral sparkling wines than in traditional ones. Glycerol is mainly produced by yeast throughout the 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. The last data are confirmed by the significantly higher levels of gluconic acid (Figure 1H) in ancestral sparkling wines since this acid is considered as a marker of the presence of *B. cinerea* [49].

Ancestral sparkling wines also had significantly higher pH levels (Figure 1I). 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 sulfur dioxide content (Figure 1J) was found to be significantly lower in ancestral sparkling wines, always below 30 mg/L. These lower levels of sulfur dioxide are mainly because ancestral sparkling wines are directly bottled in the adequate moment of the fermentation and therefore 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 most important acids in the various sparkling wines is shown in Figure 2. The individual results for acidic composition of all the sparkling wines tested are listed in Table S2. The levels of L-(+)-tartaric acid (Figure 2A), L-malic acid (Figure 2B) and L-lactic acid (Figure 2C) were similar in both types of sparkling wines, while the concentration of citric acid (Figure 2D) was significantly higher in traditional sparkling wines. It should be emphasized that in both groups, there were samples where malolactic fermentation was carried out and other samples where this was not the case.

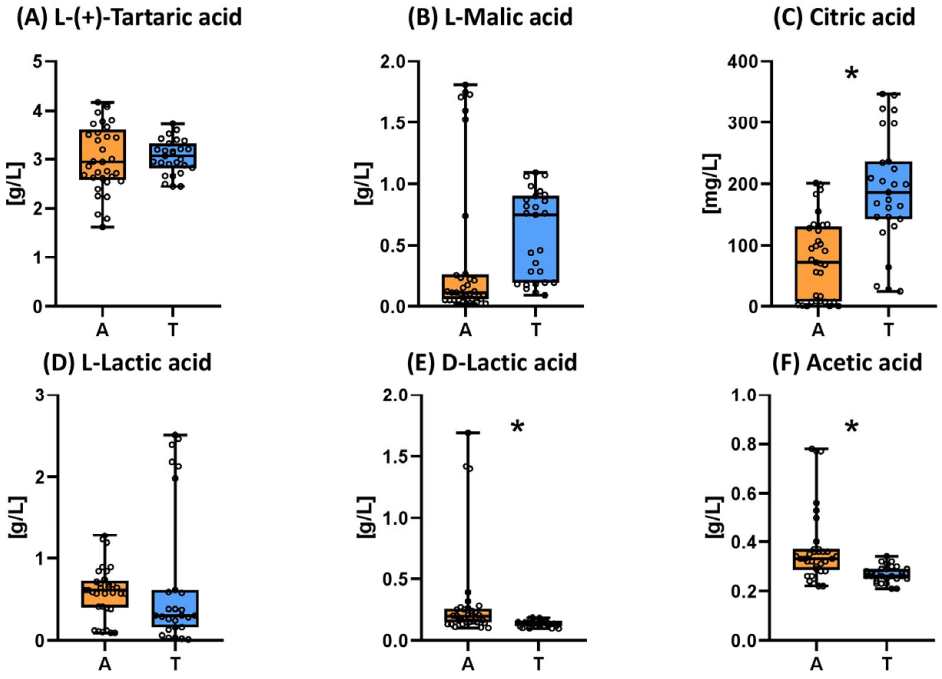

Figure 2. Box plots for organic acids of sparkling wines elaborated by ancestral (A, n = 11) or traditional (T, n = 9) methods. The presence of an asterisk indicates the existence of significant differences at $p < 0.05$.

The average content of D-lactic acid (Figure 2E) and acetic acid (Figure 2F) 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 1 g/L and acetic acid levels approximately 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. Color

Figure 3 shows the CIE$L^*a^*b^*$ coordinate values and the color appearance of the different sparkling wines. The individual results for color parameters of all the studied sparkling wines are shown in Table S3. Lightness (L^*) (Figure 3A) of ancestral sparkling wines was significantly lower and the blue-yellow component of the color (b^*) (Figure 3C) was sig-

nificantly higher than in traditional sparkling wines whereas no significant differences were found in the green-red component (a^*) (Figure 3B). These data suggest that the colors of ancestral sparkling wines were generally less clear and more yellowish than those of traditional sparkling wines, as can be seen in the picture (Figure 3D). This wider range of colors observed in ancestral sparkling wines reflects browner hues in some of these wines, which could be related to the lower use of sulfur dioxide, which could result in higher oxidation [53].

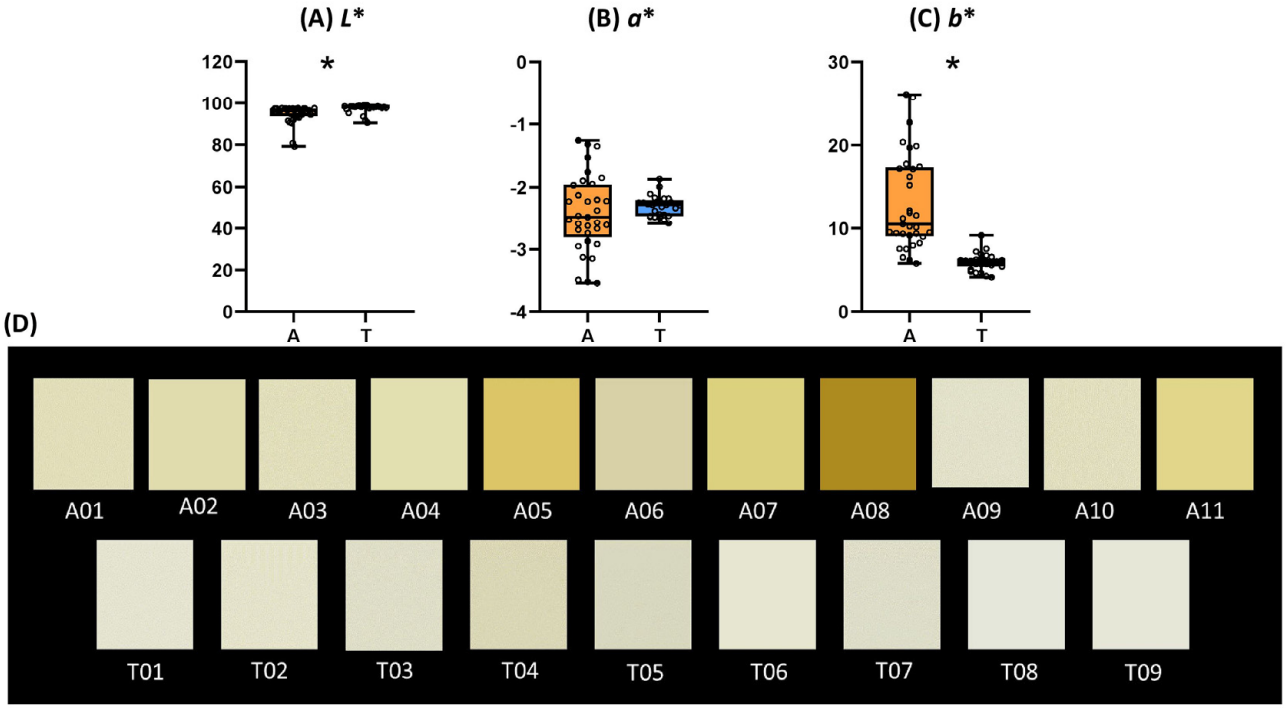

Figure 3. Box plots for color coordinates (**A**–**C**) and the palette of colors in RGB signals (**D**) of sparkling wines elaborated by ancestral (A, n = 11) or traditional (T, n = 9) methods. The presence of an asterisk indicates the existence of significant difference.

3.4. Proteins

Protein and peptide content determined via HPLC are shown 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 4A) were approximately an average of 30 mg/L in ancestral sparkling wines and approximately 20 mg/L in traditional wines. All molecular weight protein fractions (Figure 4C–E) showed a similar behavior, being high (HMW) and the low-molecular-weight (LMW) fractions were the most abundant. Peptide content (Figure 4B) was also found to be significantly higher in ancestral sparkling wines.

This higher concentration of proteins and peptides can be due to two different reasons. Firstly, the base wines of traditional sparkling wines are usually treated with bentonite for fining and riddling, which removes a significant proportion of the naturally occurring proteins in the wine [54,55]. Ancestral sparkling wines, on the other hand, are not usually fined with bentonite, and some producers do not add bentonite to promote less sedimentation during riddling. On the other hand, it is very likely that some of the ancestral sparkling wines were bottled with higher yeast populations than traditional sparkling wines, which should favor the release of proteins and peptides from yeast autolysis [56–58].

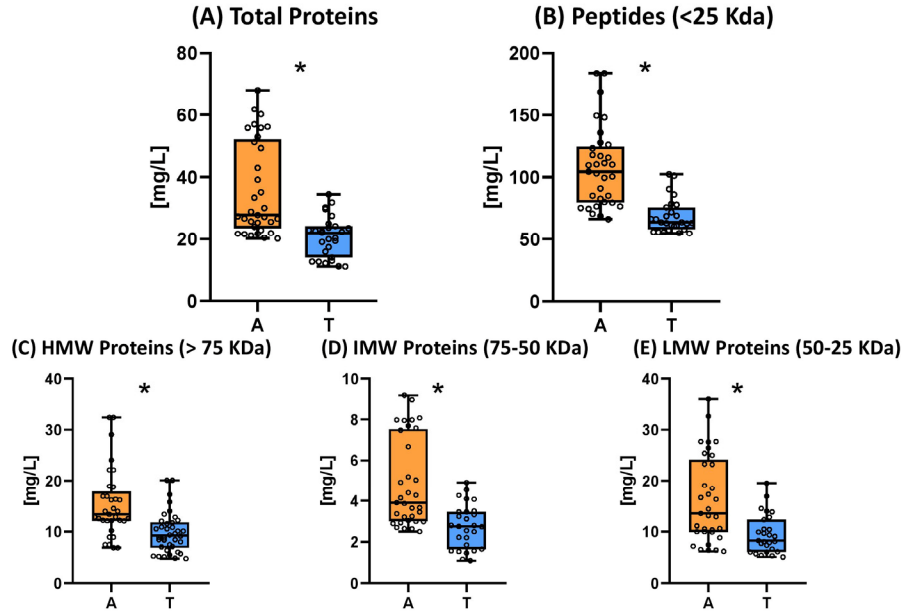

Figure 4. Box plots for proteins and peptides of sparkling wines elaborated by ancestral (A, $n = 11$) or traditional (T, $n = 9$) methods. The presence of an asterisk indicates the existence of significant differences at $p < 0.05$.

3.5. Polysaccharides

Figure 5 shows the content of polysaccharides and oligosaccharides. The individual results for the polysaccharide and oligosaccharide composition of all the sparkling wines tested are listed in Table S5. Ancestral sparkling wines showed significantly higher concentrations of polysaccharides (Figure 5A) (from 200 to 600 mg/L) than sparkling wines produced by the traditional method (all approximately 200 mg/L). The content of oligosaccharides (Figure 5B) was also significantly higher in the sparkling wines produced using the ancestral method. The reasons for the significantly higher levels of these molecules in ancestral wines are the same as those given for proteins.

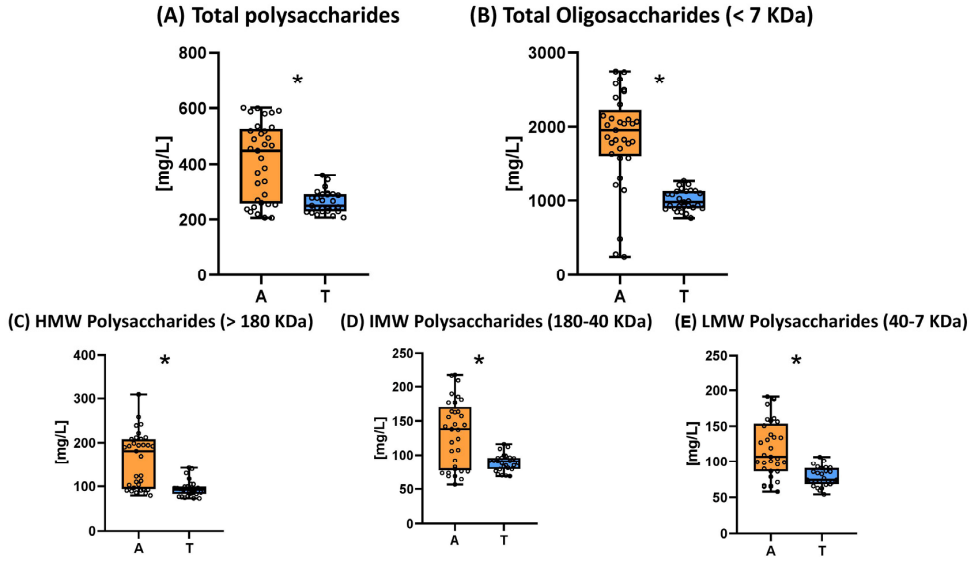

Figure 5. Box plots for polysaccharides and oligosaccharides of sparkling wines elaborated by ancestral (A, $n = 11$) or traditional (T, $n = 9$) methods. The presence of an asterisk indicates the existence of significant differences at $p < 0.05$.

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 in this figure were grouped into six families. No significant differences were found in the concentration of ethyl esters of fatty acids (Figure 6A) and total higher alcohol acetates (Figure 6B). However, ancestral sparkling wines showed higher levels of alcohol (Figure 6C) whereas traditional sparkling wines showed significantly higher levels of short- (Figure 6D), medium- (Figure 6E) and long-chain fatty acids (Figure 6F).

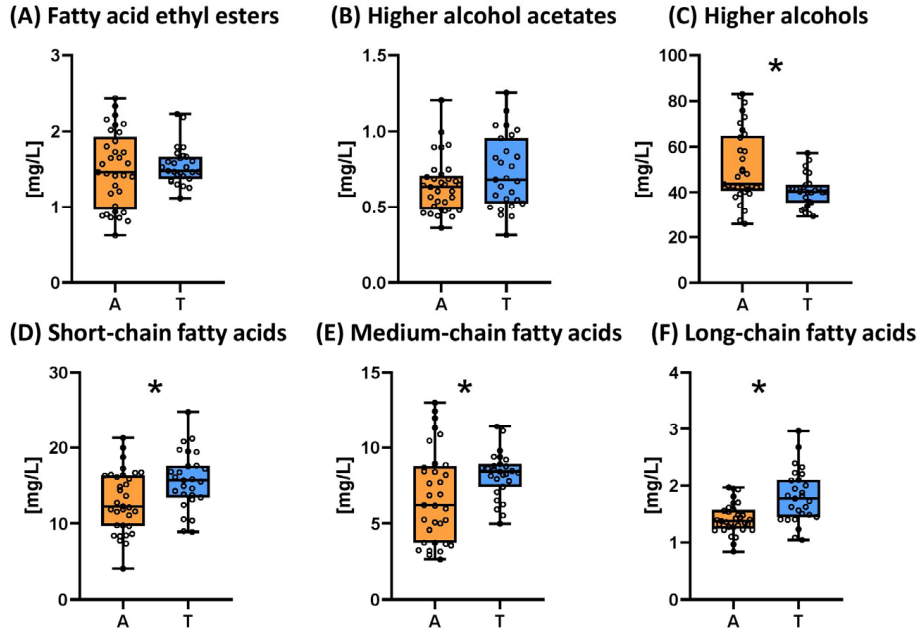

Figure 6. Box plots for volatile substances of sparkling wines elaborated by ancestral (A, n = 11) or traditional (T, n = 9) methods. The presence of an asterisk indicates the existence of significant differences at $p < 0.05$.

Higher alcohol concentrations are the result of the 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 negatively affect 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 alcohol concentrations [60]. In fact, non-settled grape musts give rise to wines with a higher concentration of higher alcohol concentrations [61]. The 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 and sometimes responsible for stuck and sluggish fermentations [63,64]. By contrast, long-chain fatty acids (LCFAs) are principal constituents of the yeast cell membrane and their 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 the refermentation of base wines, which implies 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 7A) represents the wine foamability and stable height of the foam (HS) (Figure 7B). The individual results for HM and HS of all the studied sparkling wines are shown in Table S7.

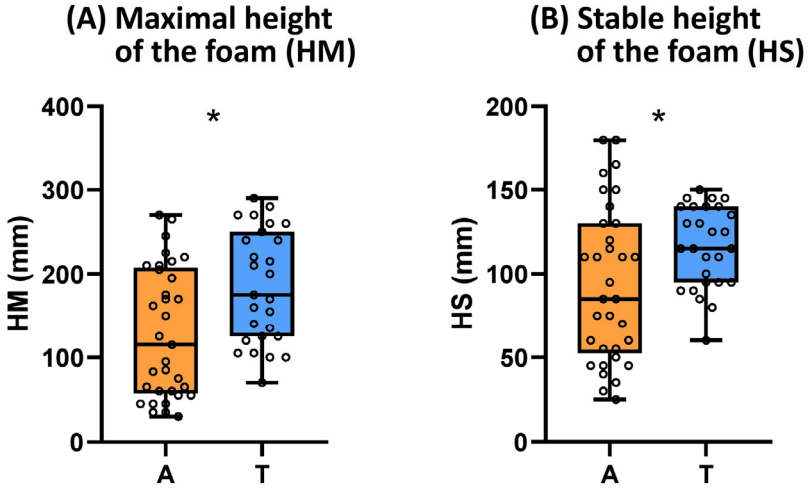

Figure 7. Box plots for the foaming properties of sparkling wines elaborated by ancestral (A, $n = 11$) or traditional (T, $n = 9$) methods. The presence of an asterisk indicates the existence of significant differences at $p < 0.05$.

In general, traditional sparkling wines had significantly higher values of HM and HS than ancestral sparkling wines although there was great heterogeneity in both groups. It has been described that peptides, proteins and mannoproteins favor 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 significantly 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 negatively affects the foamability of sparkling wines [49,69]. In addition, ancestral sparkling wines also have significantly higher concentrations of alcohols, which has been reported as detrimental to foamability [43].

3.8. Sensorial Analysis

Sensorial analysis results are shown in Figure 8 in the 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 shown as a shadowed ring surrounding it. In general, traditional sparkling wines showed greater homogeneity than ancestral sparkling wines as shown by the narrower shadowed ring. The panel scored the following sensory attributes very similarly: gas aggressivity, reduction/oxidation balance, body, acidity, tropical fruit and aniseed notes. By contrast, the panelists considered that ancestral sparkling wines showed more intense color, ageing impact and yeast-bread aroma, and less intense notes of white fruit. They also considered that ancestral sparkling wines have a 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 shown 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, Cie*L*a*b* b** parameter, pH, turbidity and total higher Alcohols. In turn, PC2 showed a positive significant correlation with gluconic acid, total proteins and pressure and a negative significant correlation with total higher alcohol acetates, total higher alcohol concentrations and turbidity. With regard to the different variables, total polysaccharides, *b**, pH, turbidity and total higher alcohol concentrations 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 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. These data indicate that traditional sparkling wines have higher pressure and sulfur 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 alcohol concentrations 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. These data confirm

that some of the ancestral sparkling wines were very similar to traditional sparkling wines while others were quite different.

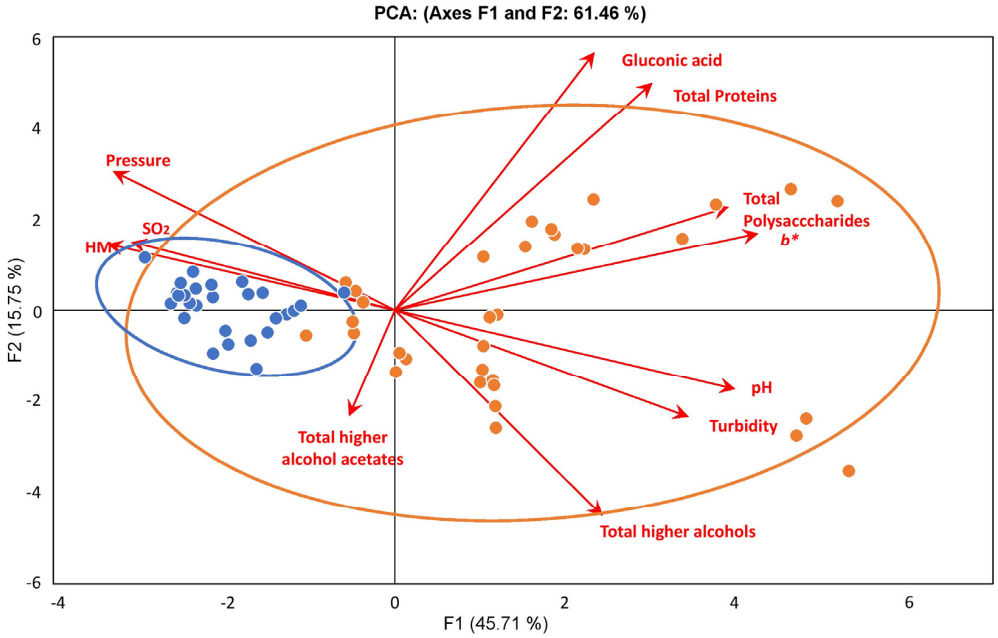

Figure 9. Plot of varimax-rotated principal component analysis for the different sparkling wines. Orange points: ancestral sparkling wines. Blue points: traditional sparkling wines.

4. Conclusions

The physicochemical and sensory analyses of various sparkling wines produced by the traditional or ancestral methods show some differences between them. Traditional sparkling wines are much more homogeneous than ancestral sparkling wines and generally have higher pressure and sulfur dioxide levels and better effervescence. In contrast, the ancestral sparkling wines were much more heterogeneous and generally had higher levels of gluconic acid, total proteins and polysaccharides, a more intense yellow color, higher pH and higher turbidity. However, these differences are mainly due to the fact that the ancestral method is not as precisely defined as the traditional method, so the quality of the product can sometimes be significantly affected. In particular, the control of sugar concentration and yeast population at bottling are critical points that need to be better defined in the production protocols of ancestral wines, as they determine the final internal pressure and probably the turbidity of the product. This lack of definition in the ancestral sparkling wine production process could be the cause of the greater heterogeneity of these wines and the fact that they even have some taints in some cases. However, the ancestral method makes it possible to work with riper grapes, which is a great advantage in view of climate change. Another advantage is that the ancestral method allows working with a lower dose of sulfur dioxide. These advantages make the ancestral method an interesting alternative to the traditional method of producing sparkling wines.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/beverages10040103/s1, Table S1: General parameters of the studied wines; Table S2: Acidic composition of the different wines; Table S3: CIE*L*a*b** coordinates of the different wines; Table S4: Protein fraction of the different wines; Table S5: Polysaccharide fraction of the different wines; Table S6: Volatile substances of the different wines; Table S7: Foaming properties of the different wines.

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supervision; F.Z., supervision, methodology, investigation, writing—original draft, and data curation. All authors have read and agreed to the published version of the manuscript.

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