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Storage Time in Bottle: Influence on Physicochemical and Phytochemical Characteristics of Wine Spirits Aged Using Traditional and Alternative Technologies

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Abstract: A few studies have investigated the influence on physicochemical and phytochemical compositions during storage in the bottle of wine spirits (WS) aged using Alternative Ageing Technology (AAT) compared it to Traditional Ageing Technology (TAT). The aim of this study was to evaluate the effect of the bottle storage over one and four years on the evolution of chromatic characteristics (CIELab method) and physicochemical characteristics (alcoholic strength, acidity and total dry extract), total phenolic index (TPI), low molecular weight compound contents (HPLC-DAD technique), antioxidant activities (DPPH, FRAP and ABTS assays) and phenolic characterization (HPLC-DAD-ESI-MS/MS technique) of the WSs aged with chestnut wood using TAT (barrels, B) and AAT (micro-oxygenation levels (MOX): O15, O30 and O60; and control (N)). The results showed that after four years of storage in the bottle, the O60 modality resulted in lower changes in physicochemical characteristics, higher preservation of the phenolic content, greater evolution of chromatic characteristics, ensuring its overall quality compared to other modalities. The antioxidant activity decreased similarly in both technologies, such as the phenolic acid content, in particular gallic acid content. According to the findings of this study, Alternative Ageing Technology might be the best alternative for wine spirit quality and the ageing process sustainability.

Keywords: spirit beverages; bottle storage; phenolic compounds; antioxidant activity; chestnut staves

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1. Introduction

The use of a sustainable processes is fundamental for the competitiveness of the beverage industry, contributing to its brand value, new market opportunities, consumers' preferences and purchase choices [1]. Integrating sustainability into industrial processes and operations, contributes to address global challenges, such as climate changes, pollution, energy consumption, depletion of natural resources, contributing to achieve the Sustainable Development Goals (SDGs) of United Nations [2,3].

In this scenario, one of the pillars of sustainability is the development of more eco-efficient processes, which will pave the way for the discovery and application of new technologies, as well as heightened awareness of environmental, economic and social challenges [4,5]. As the majority of wine spirits (WS) are produced in Europe, it is crucial to highlight the European plan for attaining sustainable production and ageing processes for spirit beverages by 2050 [6].

In particular, one of the strategies has been the use of sustainable wood-based ageing technology, which combines wood fragments (staves, chips, tablets, cubes, among others) with micro-oxygenation (MOX) flow, applied to beverages stored in stainless steel tanks, aiming to mimic the ageing process that occurs in wooden barrels [7,8]. The ageing process of the wine distillate stands out for its significant contribution to the enrichment of the beverage in phenolic and volatile compounds, given the specific positive chemical and sensory characteristics conferred by the wood, depending on the botanical species [9–12].

From a legal point of view, in many producing countries of WS, including Portugal, the legislation requires the use of "traditional practices" at this stage (Traditional Ageing Technology – TAT), not allowing the use of AATs [13]. Given this sustainable approach, TAT has strong drawbacks, such as low production efficiency, high evaporation, high cost, longer ageing time and high demand for wood, a natural resource with limited availability, contributing to a high environmental impact. For these reasons, the authors looked into the use of the sustainable wood-based ageing technology (AAT), an alternative technology for the ageing of WS that combines wood staves with various micro-oxygenation (MOX) flows [7,14–16]. Several factors have encouraged the study using AAT, aiming at a strong connection between sustainable practices and process optimisation, to produce aged WS in less time, with a lower cost, and lower environmental impact, maintaining the same or even providing better quality [17]. In fact, from a future perspective, AAT does not aim to replace TAT, but rather to provide the spirit's industry with cost-effective and sustainable solutions, especially in situations of scarcity of natural sources, such as wood.

In previous studies, comparing sustainable ageing processes, the authors showed that AAT and TAT could be distinguished based on the concentrations of the volatile phenols, specifically on the higher concentrations of guaiacol, syringol, 4-methylguaiacol, 4-methylsyringol and acetovanillone [14]. These compounds are highly appreciated by consumers, as syringol and 4-methylsyringol are associated with odour notes of wood and smoke, while guaiacol and 4-methylguaiacol confer toasted and burnt odours [9,14]. Likewise, higher total phenolic content and individual contents of low molecular weight phenolic compounds (syringic acid, ellagic acid, vanillin, syringaldehyde, coniferaldehyde and sinapaldehyde) were obtained using AAT than by TAT [7], contributing to a greater evolution of colour in the WSs. Despite these positive results on some chemical and sensory features of the WSs during the ageing process using AAT, it is crucial to assess the overall quality, including phenolic content, colour and physicochemical characteristics, of these aged WSs during their storage in the bottle.

The studies on physicochemical and phytochemical compositions during storage in the bottle of the WS aged by traditional and alternative technologies (AAT), and comparison of both technologies are scarce. A previous study evaluated how the phenolic content changed over a year in bottled wine spirits aged with chestnut wood using AAT. It showed that the total phenolic content did not reduce, and the antioxidant capacity was maintained [18]. As the WS is stored, oxygen present in bottle's headspace, and dissolved in aged WS, contributes to phenolic compounds undergo oxidation and condensation reactions [18]. These compounds stay dissolved in the aged WS, evolving and causing

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changes in the organoleptic characteristics. Furthermore, during the oxidation process in the bottle, *o*-diphenols are oxidized to *o*-quinones and semi-quinone, and free radicals may be produced, while oxygen is reduced to H_2O_2 [19]. These cascade reactions and continuous modifications in the aged WSs composition in the bottle during storage can be affected by different parameters, such as temperature, oxygen content, light exposure, humidity, bottle position, time of storage, and types of closure [20].

The main objective of this study was to investigate whether the ageing characteristics obtained by the Alternative and Traditional Technologies are retained during storage in the bottle, and to compare WSs characteristics obtained from both processes. For the first time, the influence of bottle storage for four years, through a broader analytical approach was determined, involving chromatic and physicochemical characteristics (alcoholic strength, acidity and total dry extract), phenolic content (total phenolic index), low molecular weight compounds concentrations (HPLC-DAD technique), antioxidant activities and phenolic characterization (HPLC-DAD-ESI-MS/MS technique) of the WSs aged with chestnut wood using TAT and AAT.

2. Results and Discussion

2.1. Effect of the Storage Time in the Bottle on the Antioxidant Activities and Phenolic Content

Phenolic compounds (PCs) present in aged WS are predominantly extracted from the wood during the ageing process, being partly responsible for its aroma, taste, colour and overall quality. In Figure 1 are shown the results concerning the determination of phenolic content (Total Phenolic Index – TPI) and Antioxidant Activity (AA) of the aged WSs obtaining using TAT (B modality) and AAT (O15, O30, O60, N modalities) after storage time in the bottle. During the storage in the bottle, PCs undergo continuous oxidation and condensation reactions mediated by oxygen dissolved into aged WS and also present in the headspace of the bottle, shifting and producing changes in the characteristics and quality of WS [18,20,21]. A gradual evolution of the spirit beverages occurs over storage in the bottle even in an environment with a very low oxygen content. Therefore, the determination of phenolic content throughout storage in the bottle is fundamental to understand the ageing chemistry of WS, determining whether the characteristics imparted by the ageing modality are preserved during its storage. Figure 1(a) shows the effect on TPI content of aged WS after one- and four years' storage in the bottle.

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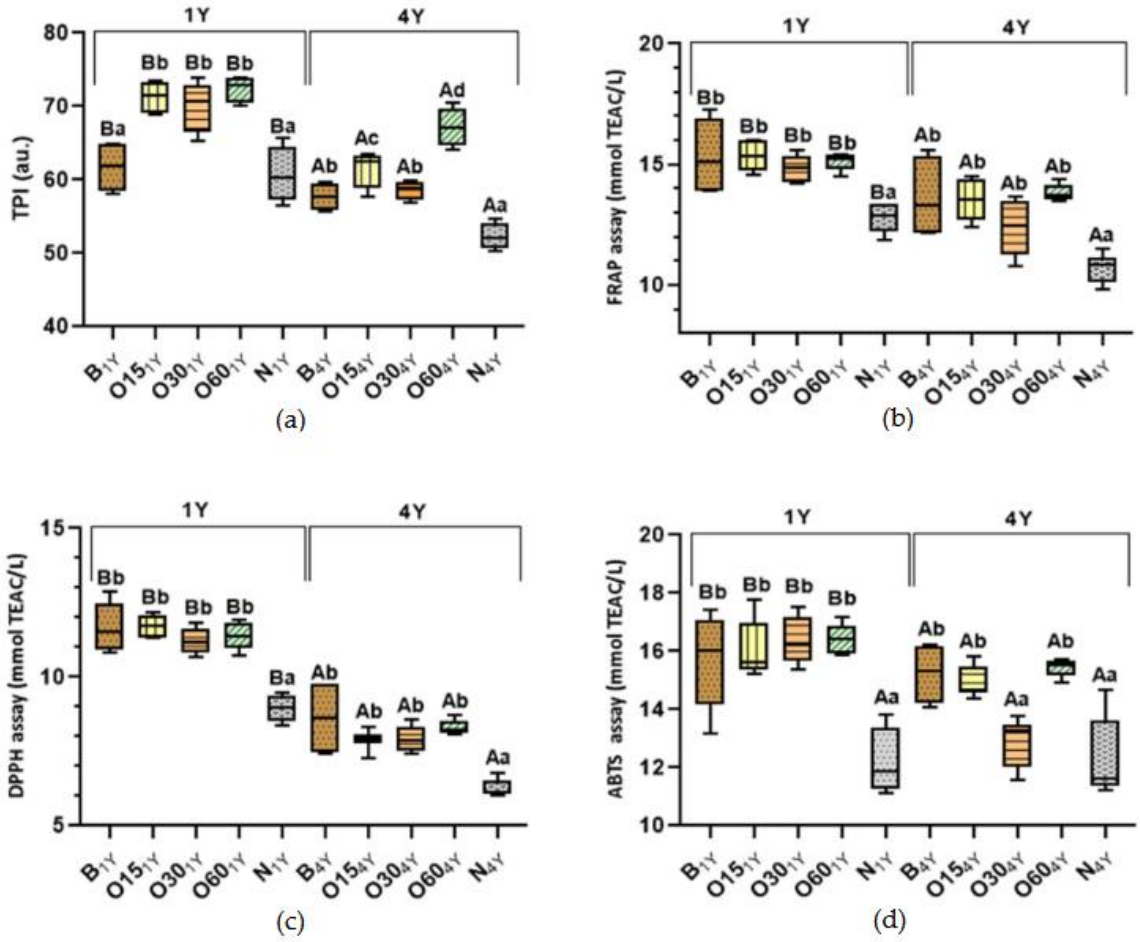

Figure 1. Box-plot diagrams of average values of Total Phenolic Index (TPI) and Antioxidant Activities (AA) of aged wine spirits in each storage time (one year (1Y) and four years (4Y)) according to ageing technologies (Traditional: B modality; Alternative: O15, O30, O60 and N modalities). Results are expressed as mean values ± standard deviation (n = 4). Phenolic determination: (a) TPI. AA determination: (b) FRAP assay; (c) DPPH assay; (d) ABTS assay. For each analytical determination: different uppercase letters (A, B) in the box indicate significant differences between storage times (1Y and 4Y) for each ageing modality by unpaired t-test ($p < 0.05$); and different lowercase letters (a, b, c, d) in box indicate significant differences between ageing modalities in each storage time by Tukey's test ($p < 0.05$).

The statistical analysis demonstrated that TPI values of the alternative ageing technology (O15 (71.29 ± 2.19), O30 (70.11 ± 3.28), O60 (72.40 ± 1.71) were not significantly different between them at bottle storage (1Y), but significantly higher than N modality (60.70 ± 3.51); and traditional technology (B modality (61.70 ± 3.22)). These results agree with those reported by Anjos et al. [23] for WS aged by alternative technology with chestnut staves.

After four years in the bottle (4Y), the results show that TPI values of WS from ageing modality O60 (67.21 ± 2.40) and B (57.66 ± 1.84) exhibited a similar reduction of approximately 7%, while O15 (61.54 ± 2.35), O30 (58.66 ± 1.27) and N (52.28 ± 1.65) displayed a reduction of approximately 14% compared to 1Y. Furthermore, the alternative ageing modalities (O15, O30, O60) had become significantly different between them at bottle storage (4Y). Despite of the reduction of TPI values in all modalities, TPI value of the O60 modality was significantly higher than other modalities (B, O15, O30 and N), retained better the phenolic content. According to previous studies [24–26] the reduction of TPI values indicates reactions evolution that occur in alcoholic beverages during the storage in the bottle. These reactions would presumably lead to changes in the antioxidant capacity of the aged WSs, therefore this study assessed their AA. It is crucial to highlight that there are competing

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mechanisms, such as gallic acid hydrolysis and galloyl-glucose oxidation. Gallic acid consumption shifts the hydrolysis balance, reducing galloyl-glucose ester production [27].

A global profile of the antioxidant capacity of aged WS can be evaluated using methods that assess hydrophilic and lipophilic antioxidant capabilities, which use single-electron transfer mechanisms (SET) [28]. The SET-based assays, such as ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), FRAP(ferric reducing antioxidant power) and DPPH (2,2-diphenyl-1-picrylhydrazyl), measure the capacity of an antioxidant in the reduction of an oxidant [29]. Studies show that ABTS, DPPH and FRAP methods provide comparable values for the antioxidant activity in beverages [18,28]. Thus, ABTS, DPPH and FRAP methods were chosen to evaluate the AA of aged WSs, as a result to their solubility with the samples, straightforward operation, quick execution, and good repeatability and reproducibility.

After one-year storage in the bottle (1Y), the antioxidant activities of the WSs aged by traditional (B modality (11.68; 15.33; 15.62 mmol TEAC/L)) and alternative technologies (O15 (11,71; 15.35; 16.03 mmol TEAC/L), O30 (11.19; 14.85; 16.35 mmol TEAC/L) and O60 (11.38; 15.11; 16.45 mmol TEAC/L) modalities) were not significantly different when measured by DPPH, FRAP and ABTS methods, respectively (Figure 1– b-d). Exception for the control modality (N: 8.96; 12.79; 12.21 mmol TEAC/L) from alternative technology, where a significantly lower AA (DPPH, FRAP and ABTS methods) was observed, while the other modalities behaved similarly. This resulted is expected, since lower phenolic content was obtained in this N modality (Figure 1–a).

After four years storage in the bottle (4Y), N modality (10.72; 6.25; 12.27 mmol TEAC/L), maintained its profile, AA values were significantly lower comparing to other modalities. Results show that, AA values of the ageing modalities (O15 (7.89; 13.56 mmol TEAC/L), O30 (7.93; 12.44 mmol TEAC/L) and O60 (8.31; 13.82 mmol TEAC/L)) were not significantly different when measured by DPPH and FRAP methods, respectively. On the other hand, ABTS method presented the lowest values for O30 (12.87 mmol TEAC/L) in relation the ageing modalities (B (15.25 mmol TEAC/L), O15 (14.91 mmol TEAC/L), O60 (15.44 mmol TEAC/L). According to the ABTS method, the radical $ABTS^{\cdot}$ allows the determination of the antioxidant capacity of both lipophilic and hydrophilic compounds [29], therefore, a reduction of lipophilic compounds, such as fatty acids and aliphatic compounds, due to oxidation reactions in the O30 modality may explain this result.

It is important to emphasize that the average results of FRAP and ABTS values after one year (1Y) were similar, with the exception of DPPH values that were lower. This behaviour can be explained by the reaction mechanisms between antioxidants and radical $DPPH^{\cdot}$, which depend on the structural conformation of the antioxidants [30–32]. Therefore, many antioxidants may react with different kinetics or may not react at all, estimating a lower the antioxidant capacity of the sample. However, according to Pearson's correlation analysis of results, a positive and significant correlation was determined between the values obtained of TPI and AA methods ($r_{\sum DPPH}$ = 0.75; $r_{\sum FRAP}$ =0.77; $r_{\sum ABTS}$ =0.68) of all modalities, indicating the efficiency of these methods for the in vitro evaluation of antioxidant capacity of aged WSs. Studies have demonstrated a positive correlation between phenolic content and AA for cognacs [33], whiskies [34], brandies [35], and aged wine spirits [8,18].

Comparing all results, AA decreased significantly after four years of bottle storage compared to one year for both technologies, TAT and AAT (Figure 1–b-d), displaying an average reduction of 28% in DPPH values, 13% in FRAP values, and 9% in ABTS values. As expected, the O60 modality showed a lower reduction of AA values, being coherent with TPI values (Figure 1–a). The results obtained suggest that longer storage time results in a decrease in phenolic content and, consequently, in a reduction in the antioxidant capacity of WS aged in both technologies. These results are in accordance with those of Nowak et al. [36] who reported a decrease in antioxidant activity during long storage in the bottle (eight years) for fruit alcoholic beverage, due to a decrease in polyphenol content. A previous study [25] showed that herbal liqueur stored after one year in the bottle obtained a reduction of AA (DPPH assay) of 29%, while phenolic content reduction was 7%. Likewise, according to de Beer et al. [37], antioxidant activities of red and white wines decreased after one year of storage in the

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bottles at 15 °C, displaying an average reduction of 17% to 21% (ABTS assay) and 29% to 59% (DPPH assay), respectively.

Several studies [8,35,37,38] confirm that AA from aged WS is dependent on the phenolic composition, which involves its chemical properties, oxidation degree and concentration. In fact, each phenolic compound has an antioxidant capacity profile based on its chemical structure and oxidation potential. Therefore, for our four-years' storage bottle samples, it was crucial to quantify and correlate LMW compounds with AA, in order to understand the contribution/correlation of these compounds to AA values obtained. Table 1 depicts the concentrations of LMW compounds of each storage time (one- and four years) according to the ageing modalities.

Table 1. Evolution of LMW compounds from aged wine spirits after one year and four years of storage in the bottle.

LMW Compounds (mg/L)	**1Y**					**4Y**				
	B_{1Y}	**$O15_{1Y}$**	**$O30_{1Y}$**	**$O60_{1Y}$**	**N_{1Y}**	**B_{4Y}**	**$O15_{4Y}$**	**$O30_{4Y}$**	**$O60_{4Y}$**	**N_{4Y}**
HMF	40.75 ± 1.46Ba	33.38 ± 11.36Aa	30.41 ± 4.68Ba	26.63 ± 1.63Aa	29.72 ± 5.80Ba	31.89 ± 0.73Ab	20.94 ± 6.72Ab	18.91 ± 2.71Aa	20.90 ± 7.48Ab	17.35 ± 4.21Aa
Furf	51.92 ± 1.72Ba	72.81 ± 5.83Ac	67.94 ± 3.73Bc	72.42 ± 3.55Ac	62.95 ± 0.97Bb	46.49 ± 1.80Aa	64.83 ± 5.80Ac	59.39 ± 2.88Ac	67.16 ± 4.19Ac	56.16 ± 0.99Ab
5Mfurf	0.87 ± 0.20Bb	0.82 ± 0.23Bab	0.71 ± 0.09Bab	0.60 ± 0.15Bab	0.48 ± 0.17Ba	0.27 ± 0.11Aa	0.21 ± 0.090Aa	0.21 ± 0.12Aa	0.34 ± 0.04Aa	0.19 ± 0.06Aa
Gall	171.70 ± 12.74Bc	122.50 ± 14.73Bb	102.30 ± 14.41Bab	104.60 ± 1.08Bab	84.14 ± 15.17Ba	137.70 ± 17.51Ab	58.06 ± 7.26Aa	59.36 ± 8.60Aa	67.34 ± 18.52Aa	41.66 ± 8.34Aa
Ellag	17.72 ± 1.03Aa	21.73 ± 2.29Abc	21.83 ± 0.86Bbc	23.44 ± 0.45Ac	19.31 ± 0.90Bab	16.17 ± 0.71Aa	18.68 ± 1.73Aa	19.04 ± 0.89Aa	22.91 ± 2.84Ab	16.72 ± 0.55Aa
Van	12.58 ± 0.40Aa	17.42 ± 5.83Aa	17.18 ± 2.41Aa	15.01 ± 0.14Aa	16.07 ± 2.59Aa	16.62 ± 2.86Ba	16.29 ± 6.25Aa	17.78 ± 3.91Aa	15.05 ± 0.94Aa	15.15 ± 3.48Aa
Syrg	6.02 ± 0.50Aa	13.12 ± 1.49Ab	12.16 ± 0.92Ab	12.97 ± 0.85Ab	11.27 ± 0.72Ab	6.75 ± 0.26Aa	13.51 ± 0.99Ac	13.10 ± 0.76Abc	14.31 ± 1.12Ac	11.48 ± 1.03Ab
Fer	0.26 ± 0.03Aa	0.37 ± 0.11Aab	0.46 ± 0.08Ab	0.43 ± 0.04Ab	0.33 ± 0.02Aab	0.29 ± 0.04Aa	0.55 ± 0.15Ab	0.50 ± 0.13Aab	0.48 ± 0.12Aab	0.37 ± 0.03Aab
Vanil	6.17 ± 0.08Ab	6.53 ± 0.20Abc	6.29 ± 0.27Abc	6.88 ± 0.29Ac	4.94 ± 0.49Aa	6.50 ± 0.35Aab	6.51 ± 0.46Aab	6.32 ± 0.12Aab	7.93 ± 1.99Ab	5.15 ± 0.56Aa
Syrde	13.59 ± 0.19Aa	17.20 ± 0.11Ab	16.01 ± 0.49Ab	17.50 ± 0.38Ab	13.53 ± 1.89Aa	17.59 ± 0.78Ba	20.99 ± 0.91Bab	19.82 ± 0.88Bab	24.30 ± 5.43Bb	16.68 ± 2.30Ba
Cofde	9.17 ± 0.35Ab	5.82 ± 0.23Aa	5.81 ± 0.31Aa	6.09 ± 0.89Aa	5.62 ± 0.27Aa	8.90 ± 0.23Ab	5.57 ± 0.54Aa	5.51 ± 0.37Aa	5.96 ± 0.88Aa	5.36 ± 0.05Aa
Sipde	30.36 ± 1.43Bb	25.45 ± 0.39Bba	23.98 ± 0.85Ba	27.06 ± 2.25Ba	24.96 ± 1.40Ba	21.47 ± 2.29Aa	17.04 ± 2.91Aa	15.95 ± 2.95Aa	20.51 ± 2.87Aa	17.43 ± 1.15Aa
LMW Compounds (mg/L)	**1Y**					**4Y**				
	B_{1Y}	**$O15_{1Y}$**	**$O30_{1Y}$**	**$O60_{1Y}$**	**N_{1Y}**	**B_{4Y}**	**$O15_{4Y}$**	**$O30_{4Y}$**	**$O60_{4Y}$**	**N_{4Y}**

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Total furanic aldehydes	93.54 ± 3.27Ba	107.01 ± 16.91Ba	99.06 ± 1.57Ba	99.65 ± 5.32Ba	93.16 ± 6.25Ba	78.65 ± 2.49Aa	85.97 ± 12.62Aa	78.51 ± 1.39Aa	88.40 ± 11.54Aa	73.69 ± 4.82Aa
Total phenolic acids	208.30 ± 12.83Bc	175.20 ± 5.76Bb	153.90 ± 16.91Bab	156.40 ± 1.13Bab	131.10 ± 17.17Ba	177.50 ± 21.18Ab	107.10 ± 6.02Aa	109.80 ± 12.80Aa	120.10 ± 19.39Aa	85.37 ± 10.61Aa
Total phenolic aldehydes	59.28 ± 1.91Bb	54.99 ± 0.57Ab	52.09 ± 1.71Aab	57.53 ± 3.75Ab	49.04 ± 3.92Aa	54.46 ± 1.68Ab	50.10 ± 3.83Aab	47.60 ± 3.55Aab	58.69 ± 10.81Ab	44.62 ± 2.36Aa
Total LMWC	361.10 ± 12.85Bc	337.10 ± 12.24Bbc	305.10 ± 17.44Bb	313.60 ± 7.91Ab	273.30 ± 20.67Ba	310.60 ± 24.48Ac	243.20 ± 17.76Aab	235.90 ± 14.98Aab	267.20 ± 40.99Abc	203.70 ± 12.64Aa

Results are expressed as mean values ± standard deviation (n = 4) of aged wine spirits in each storage time (one year (1Y) and four years (4Y)) according to ageing technologies (Traditional: B; Alternative: O15, O30, O60 and N). For each analytical determination: different uppercase letters (A, B) in the same row denote significant differences between storage times (one year (1Y) and four years (4Y)) for each ageing modality by unpaired *t*-test ($p < 0.05$); different lowercase letters (a, b, c, d) in the same row denote significant differences between ageing modalities in each storage time by Tukey's test ($p < 0.05$). LMW compounds: Total LMWC —sum of content of individual LMW compounds; Total phenolic acids — sum of content phenolic acids; Total furanic aldehydes — sum of content of furanic aldehydes; Total phenolic aldehydes— sum of content of phenolic aldehydes; Gall—gallic acid; Ellag—ellagic acid; Van—vanillic acid; Syrg—syringic acid; Fer—ferulic acid; Vanil—vanillin; Syrde—syringaldehyde; Cofde—coniferaldehyde; Sipde—sinapaldehyde; Furf— furfural; HMF—5-hydroxymethylfurfural; 5Mfurf—5-methyfurfural.

Based on these results, a positive Pearson's correlation between AA values and Total LMW compounds concentration ($r_{\sum DPPH} = 0.82$; $r_{\sum FRAP} = 0.71$; $r_{\sum ABTS} = 0.61$) also was obtained, confirming that these compounds may play an important role in the antioxidant capacity of aged WS during storage time in the bottle (Table 1). In order to assess if a specific compound has more influence than any other in determining the antioxidant capacity, a correlation analysis was performed between the values of AA for each method and individual LMW compounds content of aged WS. Strong Pearson's correlations (0.99 to 0.50 indicate correlation) were observed only between antioxidant activity and LMW compound content for gallic acid ($r_{\sum DPPH} = 0.71$; $r_{\sum FRAP} = 0.61$; $r_{\sum ABTS} = 0.50$) and sinapaldehyde ($r_{\sum DPPH} = 0.77$; $r_{\sum FRAP} = 0.60$; $r_{\sum ABTS} = 0.41$), indicating that these compounds contribute to the overall antioxidant potential of the aged WS. The other phenolic acids and phenolic aldehydes (ferulic acid, vanillic acid, syringic acid, ellagic acid, vanillin, syringaldehyde and coniferaldehyde) showed weak correlation to the antioxidant capacity. Therefore, the antioxidant activity exhibited by aged WS could be partially explained due to the richness in gallic acid (gall) and sinapaldehyde. These results are according to previous studies, gallic acid and sinapaldehyde act as an antioxidant by scavenging the available free radicals, being able to reduce and inhibit the generation of free radicals [40–42].

Considering the results of the control modality (N) in each storage time in the bottle (1Y; 4Y), lower TPI and AA values were obtained, which is in line with the lower content of Total LMW compounds (Table 1). These outcomes are in line with what was seen throughout the ageing experiment [15,16], which shows how important oxygen (MOX application) is for getting the target compounds out of AAT. On the other hand, the Total LMW phenolic composition of traditional technology was higher than the alternative technology, but not significant for the respective modalities (1Y: O30 and 4Y: O60). According to Table 1, the higher Total LMW phenolic content for traditional technology is directly related to its higher phenolic acid content. Total LMW concentrations for both technologies agree with the findings of previous studies [7,10]. During the ageing process [14,15], the MOX modalities (O15, O30 and O60) were designed to reproduce and accelerate reactions involving wood extractive compounds such as those that occur in wooden barrels (B), but the results showed these reactions were not similar, possibly due to differences in dissolved oxygen (DO) content. The dissolved oxygen content in alternative technology (O15, O30 and O60)

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were higher (approximately 10 mg/L) than those observed in the wooden barrel (approximately 4 mg/L), given that the oxygen dissolved in a wooden barrel is always the result of a balance between the oxygen that passes through the wood and the oxygen that is consumed in the reactions [15,43].

Reduced levels of dissolved oxygen in the B modality compared to MOX modalities (O15, O30 and O60) may suggest increased consumption or a lower oxygen influx through the barrel, implying that this oxygen balance may play a role in attenuating the oxidative reactions of wood extractive compounds, resulting in a higher content of total phenolic acids in the B modality (Table 2). The application of micro-oxygenation in red and white wines has confirmed effects in the reduction of monomer and oligomeric phenolic compounds [44,45]. These results are in concordance with those obtained in previous research works [8,27,46], which reported higher concentrations of phenolic acids (gallic, ellagic, vanillic and syringic acids) in aged WS using traditional technology in comparison with those acquired by the alternative technology (MOX combined with wood staves).

Table 2. Effect of the storage time in a bottle on physicochemical characteristics of wine spirits aged by traditional and alternative technologies.

Analytical parameters	**1Y**					**4Y**				
	B_{1y}	**$O15_{1y}$**	**$O30_{1y}$**	**$O60_{1y}$**	**N_{1y}**	**B_{4y}**	**$O15_{4y}$**	**$O30_{4y}$**	**$O60_{4y}$**	**N_{4y}**
Alcoholic strength by volume (% v/v)	76.38 ± 0.13Aa	77.09 ± 0.11Ab	77.09 ± 0.11Ab	77.16 ± 0.17Ab	76.72 ± 0.26Aab	76.14 ± 0.20Aa	77.05 ± 0.17Ab	77.04 ± 0.21Ab	77.24 ± 0.22Ab	76.37 ± 0.33Aa
Total acidity (g acetic acid/L AE)	0.89 ± 0.13Bd	0.69 ± 0.01Ac	0.66 ± 0.03Ab	0.67 ± 0.01Abc	0.58 ± 0.01Aa	0.82 ± 0.03Ad	0.74 ± 0.01Bc	0.70 ± 0.01Bb	0.71 ± 0.01Bb	0.64 ± 0.02Ba
Fixed acidity (g acetic acid/L AE)	0.44 ± 0.02Bc	0.34 ± 0.01Ab	0.32 ± 0.02Ab	0.33 ± 0.01Ab	0.27 ± 0.02Aa	0.34 ± 0.02Ac	0.33 ± 0.03Abc	0.32 ± 0.02Ab	0.32 ± 0.01Abc	0.27 ± 0.01Aa
Volatile acidity (g acetic acid/L AE)	0.45 ± 0.02Ac	0.35 ± 0.01Ab	0.35 ± 0.02Ab	0.34 ± 0.01Ab	0.31 ± 0.01Aa	0.48 ± 0.03Bc	0.41 ± 0.03Bb	0.39 ± 0.01Bba	0.39 ± 0.01Bba	0.37 ± 0.03Ba
Total dry extract (g/L)	2.37 ± 0.23Ab	2.43 ± 0.08Ab	2.27 ± 0.14Ab	2.37 ± 0.04Ab	2.03 ± 0.06Aa	2.46 ± 0.10Ac	2.44 ± 0.08Abc	2.36 ± 0.01Ab	2.38 ± 0.05Abc	2.07 ± 0.01Aa
pH	4.11 ± 0.05Ba	4.16 ± 0.02Bb	4.14 ± 0.01Bab	4.18 ± 0.01Bb	4.24 ± 0.02Bc	3.97 ± 0.09Aa	4.04 ± 0.04Ab	4.07 ± 0.02Ab	3.95 ± 0.03Aa	4.12 ± 0.04Ac

Results are expressed as mean values ± standard deviation (n = 4) of aged wine spirits in each storage time (one year (1Y) and four years (4Y)) according to ageing technologies (Traditional: B; Alternative: O15, O30, O60 and N). For each analytical determination: different uppercase letters (A, B) in the same row denote significant differences between storage times (one year (1Y) and four years (4Y)) for each ageing modality by unpaired *t*-test ($p < 0.05$); different lowercase letters (a, b, c, d) in the same row denote significant differences between ageing modalities in each storage time by Tukey's test ($p < 0.05$).

Regarding the evolution of the storage in the bottle (4Y), the total LMW compounds reduced significantly in both technologies; however, for the O60 modality, this reduction was not significant (Table 1). It is worth highlighting that the TPI value of the O60 modality at 4Y was significantly higher than other modalities (B, O15, O30, N), which may explain this result, confirming better conservation of LMW compounds during bottle storage. The results obtained for the B modality (1Y) in this study are according to those found by Canas et al. [47], who reported that the total LMW compounds was 397.88 ± 37.07 mg/L. Also, the total LMW compounds were found to be the same using an alternative technology (320.00 ± 77.00 mg/L) [7]. Concerning total furanic aldehydes and total phenolic acids, a significant reduction was demonstrated in both technologies after four years of storage in the bottle (4Y), while for total phenolic aldehydes the reduction occurred only in traditional technology. The alternative technology better preserved their total phenolic aldehydes. Interestingly, phenolic aldehydes contribute positively to the aroma quality of aged WS, being responsible for the aroma

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characteristics, such as cream, vanilla, cinnamon, spicy, and pepper, according to their thresholds [48].

Comparing each furanic aldehydes at 4Y, 5Mfurfural content reduced significantly for both technologies (overall 69%), while furfural and HMF contents have similar behaviour, showing significant reduction for B (11%; 21%), O30 (12%; 37%) and N (11%; 30%) modalities, respectively. Furfural oxidation may have occurred over time and can affect the aroma quality of aged WS, as furanic aldehydes confer positive aroma, such as caramel, dry fruits and toasted-almond-like aromas [49,50]. Furfural oxidation is a simple oxidation reaction, in which a formyl group bonded to a furan ring is converted to carboxylic acid (5-methyl-2-furonic acid; furoic acid) [51]. Furthermore, the furfuryl alcohol (2-furanmethanol) can be formed by degradation of the furfuryl aldehydes during storage in the bottle [52].

Concerning phenolic aldehydes, a significant increase in syringaldehyde concentration was observed over time, while sinapaldehyde decreased significantly. These results can be explained by oxidative cleavage of double C-C bond of the aliphatic chain of these aldehydes, yielding the corresponding benzoic aldehydes; therefore, the oxidation of sinapaldehyde gives rise to syringaldehyde, which may be oxidized to syringic acid [10,53]. Vanillin and coniferaldehyde concentrations did not undergo a significant reduction. Vanillin content, in particular, is one of the most important compounds contributing to a positive sensorial appreciation. This is because vanillin content has a strong relationship with the intensity of vanilla and sweet aromas in wine spirits [48,49,54]. Furthermore, vanillin and syringaldehyde are commonly used as markers of the ageing process [55,56], and their preservation in aged WS during bottle storage is a key factor in ensuring its quality, maintaining its market value.

The phenolic acids, ferulic and syringic acids contents variation were not significant over time for both technologies. However, vanillic acid content increased significantly for traditional technology, while its content for alternative technologies did not change. The hydrolysis and oxidation of lignin-related compounds dissolved in aged WS, such as 5-carboxyvanillic acid and β-hydroxypropiovanillone, contribute to the produce of vanillic acid [57]. This may be one reason why the amount of this phenolic acid rises during storage.

For ellagic acid content at 4Y, no significant changes were found for B, O15 and O60 modalities, while O30 and N modalities reduced to 13%. Ellagic acid is a condensed dimer of the gallic acid [58]. Conversely, gallic acid content decreased for both technologies; the greatest reduction was observed for alternative technology (overall 45%), followed by 20% for traditional technology. Hernanz et al. [59] also evidenced the reduction of 20% of gallic acid content after one year of storage in the bottle in white wine. Burin et al. [60] demonstrated a reduction of 50% of gallic acid content in red wines after 11 months of storage in the bottle. García-Falcón et al. [61] reported for red wines, after one year of bottle storage, an average reduction of 40% in the gallic acid concentration, while vanillic acid and syringic acid concentrations did not change.

The reduction of gallic acid content may have occurred due to oxidation and dimerization reactions. Gallic acid is susceptible to autoxidation in the presence of oxygen [62]. As the three hydroxyl groups that are attached to the gallic acid's aromatic ring are prone to oxidation, which results in the formation of hydrogen peroxide, quinones, and semiquinones [63,64]. Studies show that gallic acid oxidizes and dimerizes, regenerating the hydroquinone form (regenerative polymerization), and then the dimer oxidizes to give its quinone and hydrogen peroxide, consequently producing the oxidized dimer [65,66].

In general, phenolic oxidation occurs more slowly at low pH (characteristic pH of aged WS), reduced oxygen concentration and low temperature, which may explain the preservation of ferulic, vanillic and syringic acid concentrations over time [63,64,67]. Furthermore, the antioxidant properties of phenolic acids are related to their redox potential and, therefore, a deeper knowledge of their oxidation–reduction behaviour is essential for a detailed understanding of the antioxidation process during bottle storage. Galato et al. [69] reported that gallic acid has a lower oxidation potential (Epa), higher redox potential (ΔE), and higher antioxidant capacity than vanillic and ferulic acids. In

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addition, the oxidation potential of the ellagic acid is higher compared to gallic acid [69], which helps explain the relatively lower reduction of its content in aged WS compared to gallic acid over time. Also, some research shows that gallic acid is a better antioxidant than ellagic and protocatechuic acids [70,71].

The free radical scavenging capacity of phenolic compounds is dependent on the number and position of hydroxyl groups, the presence of other functional groups, and mainly the ortho-hydroxyl arrangements in the aromatic ring. The antioxidant activity of a molecule increases with an increase in the number of hydroxyl groups attached to the aromatic ring [64,68]. Rice-Evans et al. [72] described that gallic acid has a total antioxidant capacity of 3.0 mM relative to TEAC (Trolox Equivalent Activity Capacity), corresponding to the three available hydroxyl groups; however, its esterification in the carboxylate group decreases this capacity (2.4 mM). Equally, the substitution of the 3- and 5-hydroxyl with methoxy groups in syringic acid demonstrates an effective diminution in antioxidant activity (1.36 mM TEAC) compared to the trihydroxy derivative. Additionally, analysing these compounds by SET methods, HMF and 5Mfur did not show any antioxidant activity in DPPH and FRAP assays at concentrations below 200 mg/L [73]. Among phenolic aldehydes, their antioxidant capacity decreased in this order: sinapaldehyde > syringic acid > coniferaldehyde [74]. Therefore, this depletion in antioxidant activity during long storage in the bottle is strongly correlated with the reduction of LMW compounds, specifically, sinapaldehyde and gallic acid.

In summary, oxygen concentration in the headspace of the bottle samples, and the previously dissolved oxygen in aged WS had a significant impact on the evolution of the phenolic compounds and antioxidant activity over time. The hydrolysis and oxidation reactions may be responsible for the changes in the phenolic composition of aged WS during storage time in the bottle. Results showed that sinapaldehyde and gallic acid were the individual phenolic compounds that exhibited the most marked reductions after four years of storage in the bottle for both technologies. Among modalities, the O60 modality resulted in the higher preservation of the phenolic content and antioxidant activity of aged WS, ensuring its quality. According to this study, this technological alternative might be the most appropriate for wine spirit quality and ageing sustainability.

2.2. Effect of the Storage Time in the Bottle on the Chromatic Characteristics of Aged WSs

Wooden barrels and staves used for the maturation of spirits are exposed to a heat treatment, as a result, toasted wood can release a greater number of LMW and Maillard reaction compounds (melanoidins, pyrazines and furanic compounds) for distillate, which are partially responsible for the colour and aromas of aged WS [75]. Colour is one of the fundamental attributes of aged beverage's appearance. Colour measurement of aged WS has been used as an indirect measure of quality indicators, such as flavour, phenolic content, and physicochemical properties [16,76]. In fact, the colour is the first quality parameter evaluated by consumers, contributing for impulse purchases, therefore, changes in the aged WS`s colour during long periods of storage in the bottle can impact its acceptance and market share.

The beverage industry has applied the CIELab method to measure and determine colour divergent from a set standard, and evolution during the storage time of the product. The CIELab method is based on the perception of just noticeable colour differences in the cylindrical coordinates of the system (L*, a*, b*). Thus, the determination of the chromatic characteristics L*, a* and b* in CIELab method serve to define the location of any colour in the uniform colour space [76,77]. The coordinate a* takes positive values for reddish colours, whereas b* takes positive values for yellowish colours [76]. In addition, L* is an approximate measurement of luminosity, taking values within the range 0-100 [78]. Chroma (C*) is the quantitative attribute of colorfulness, being used to determine the degree of difference of a hue in comparison to a grey color with the same Lightness. The higher C* values of a sample, higher is the color intensity perceived by human eyes [76,78].

The chromatic characteristics of aged WSs after one and four years of storage in the bottle are shown in Figure 2.

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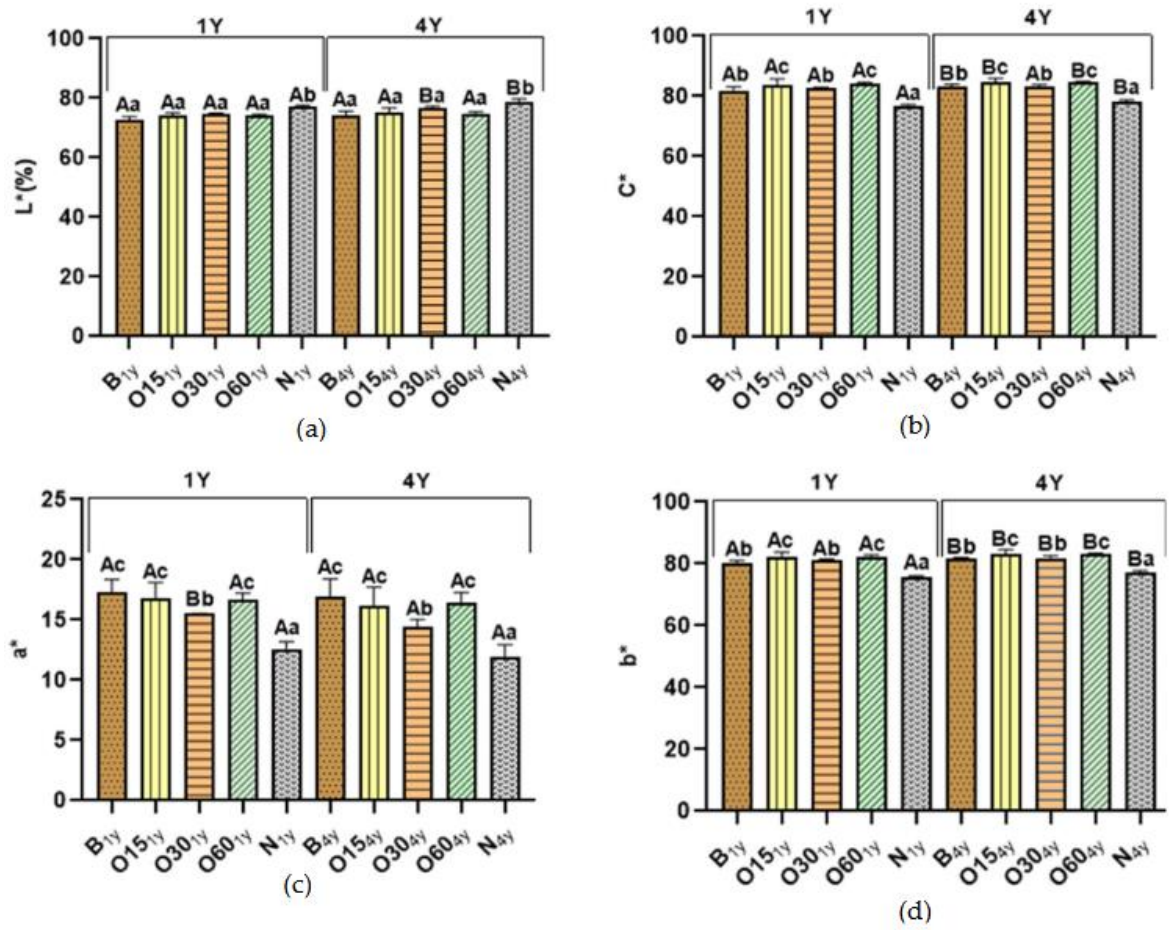

Figure 2. Effect of the storage time in the bottle in the chromatic characteristics of the wine spirits aged by ageing technologies (Traditional: B; Alternative: O15, O30, O60 and N): (a) Lightness (L*); (b) Chroma (C*); (c) chromaticity coordinate a*; (d) chromaticity coordinate b*. For each analytical determination: different uppercase letters (A, B) in the bars indicate significant differences between storage times (one year (1Y) and four years (4Y)) for each ageing modality by unpaired *t*-test ($p < 0.05$); and different lowercase letters (a, b, c, d) in bars indicate significant differences between ageing modalities in each storage time by Tukey's test ($p < 0.05$).

Regarding each storage time (1Y and 4Y) in the bottle, L* values of both technologies show no difference, except for control (N) modality, as expected (Figure 2). Aged WSs from N modality presented significantly higher L* values than aged WSs obtained from other ageing modalities (B (72.85; 74.18), O15 (74.01; 75.38), O30 (74.93; 76.78); O60 (74.18; 74.93)), respectively (Figure 2a). This result is explained by the lower extraction of compounds from chestnut wood during the ageing process, which is confirmed by lower TPI values (Figure 1) and Total LMW compounds content (Table 1).

The statistical analysis demonstrated that after four years´ bottle storage, L* values of O30 (74.93; 76.78) and N (77.11; 78.68) modalities significantly increased, while no changes were observed in B, O15 and O60 modalities. The higher L* values in N and O30 modalities emphasizes the role of oxygen during ageing process, and oxidation of WSs compounds during bottle conservation, as higher reduction of TPI values and total LMW compounds content was observed in these modalities (Figure 1–a and Table 1, respectively).

Concerning C* values (Figure 1–b), a similar evolution/behaviour is observed for all ageing modalities for each storage time (1Y and 4Y) in the bottle, being that O15 (83.85; 84.70) and O60 (84.09; 84.68) modalities obtained higher values compared to the B and O30 modalities, while N modality (76.97; 78.98) showed significant lower values. The modalities, B (81.97; 83.45) and O30 (82.52; 83.12), were not different between them. Interestingly, B, O15, O60, N modalities were significantly higher

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after four years´ bottle storage, while the O30 modality did not show difference. The increase of C* values in all modalities confirms the presence of mechanisms of reactions (as oxidation, polymerization, hydrolysis, among others) occur during storage in the bottle, indicating the chemical evolution of aged WS.

Figure 1c depicts the coordinate a* values of both technologies for each storage time (1Y and 4Y) in the bottle, demonstrating that B (17.33; 16.95), O15 (16.77; 16.20) and O60 (16.65; 16.41) displayed significant higher values compared to the O30 (15,52; 14.47) and N (12.61; 11.94) modalities. In addition, the coordinate b* values (Figure 1d) exhibited the same behaviour of C* for all ageing modalities for each storage time (1Y and 4Y) in the bottle, being that O15 (82.15; 83.38) and O60 (82.42; 83.22) displayed higher values compared B (80.11;81.70) and O30 (81.05; 81.85), while N modality (75.92; 77.16) showed significant lower values. It is important to highlight the significant increase of b* values (yellow hue) for both technologies after four years storage in the bottle, while a* values were not significantly different, despite of a slight downward trend. The increase of b* values contributed to higher values of C*.

In summary, aged WS from alternative technology, specifically O15 and O60 modalities, evidenced a greater colour evolution after four years of storage in the bottle due to the higher values of coordinate b* and C*, without significant changes in values of the coordinate a* and L*, confirming the role of oxygen in the development/evolution of colour. The oxidation of tannins and carboxyl ellagic acid in aged WS responsible for the formation of compounds with yellow colour may explain the increase of coordinate b*, directly reflecting the increase of C* [79]. Furthermore, it is known that the colour evolution during storage in the bottle, of aged WSs, can occur due to oxidation and hydrolysis of phenolic compounds and furfural aldehydes, and other pigment degradations [18,22].

2.3. Effect of the Storage Time in the Bottle on the Physicochemical Characteristics of Aged WSs

The physicochemical characteristics, mainly volatile, fixed and total acidity are important parameters that determine the high-quality spirits. Table 2 shows the effect of the storage time in the bottle on physicochemical characteristics of wine spirits aged by traditional and alternative technologies. It is worth noting that at each storage time (1Y and 4Y), the O15, O30 and O60 modalities presented significantly higher alcoholic strength by volume values than the traditional technology (B modality). Because glass demijohns were used, the ethanol evaporation phenomenon of WS was lower during the ageing process using alternative technology. This is what was expected. After four years of storage in bottle, the alcoholic strength values did not show significant changes in the aged WS for both technologies. The closure and the permeability of the stopper (such as the effective diffusion coefficient) and the transfer of oxygen at the interface between the cork stopper and the glass bottleneck can contribute to the oxidation and evaporation of aged WS during the bottle ageing [80].

The statistical analysis demonstrated that the values of the total acidity, fixed acidity, and volatile acidity were significantly higher in traditional technology than the alternative technology in each storage time (1Y and 4Y), being characteristic of the ageing process in a barrel. A recent study demonstrated higher values of total acidity in WS aged using traditional technology than alternative technology [46]. Among the modalities from alternative technology, the O15 modality showed slightly higher values of total acidity, fixed acidity, and volatile acidity than the O30 and O60 modalities, while the control modality (N) was significantly lower. After four years of storage in the bottle, for the traditional technology there was a reduction of total acidity and fixed acidity, while the volatile acidity was higher. Conversely, the values of total acidity and volatile acidity were significantly higher in alternative technology, while for the fixed acidity no changes were observed.

During storage time in the bottle, volatile acidity was the factor contributing to the total acidity in aged WS, resulting from the hydrolysis of acetyl groups to acetic acid [81,82]. Furthermore, the oxidation of acetaldehyde also may contribute to the acetic acid content of the aged WS [83]. Total acidity of aged WS considers all types of acids, such as inorganic acids, organic acids, amino acids and sulfurous, as well as phenolic acids. The increase of the total acidity can be derived both from

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the release of compounds from the hydrolysis of acetyl groups to acetic acid, and from the oxidation of aldehydes, esters, and alcohols to acids [61,84,85]. In addition, pH values were higher in the control (N) modality in each storage time (1Y and 4Y) compared to other modalities. Interestingly, pH values reduced in both technologies after four years of storage bottle. The pH value reflects the amount and strength of acids, and is primarily dependent upon the total amount of existing acids in aged WS [86]. The formation of acids resulting from the hydrolysis, oxidation and condensation reactions of organic and phenolic compounds contributes to the decrease of pH value over storage bottle. Previous study reported the increase of total acids and decrease of total esters and pH value in Chinese distilled spirit (Fenjiu) after three years of storage in the bottle [87].

Regarding total dry extract, there was no significant change for both technologies during the storage time in the bottle, revealing a good conservation of the compounds resulting from the extraction/oxidation of wood that occurred during the ageing process. The total dry extract includes all matter that is non-volatile. Comparing the technologies, lower values of total dry extract are observed only for the N modality in each storage time (1Y and 4Y), and the B, O15, O30, and O60 modalities did not display differences.

Indeed, the WSs aged from alternative technology, specifically the O15, O30, and O60 modalities, showed similar behaviour in the changes of the physicochemical characteristics over four years of storage in the bottle compared to the B modality, indicating that the different level MOX weakly influences these parameters.

2.4. Multivariate Analysis

Principal Component Analysis (PCA) was used to investigate the effect on the aged WSs' characteristics during the storage in the bottle (Figure 3). At this stage, all data obtained were considered to assess similarities between the ageing modalities (antioxidant activities, TPI, LMW compounds content, physicochemical and chromatic determinations) during storage time in the bottle. The first and second components (PC1 × PC2), explained 56.8% of the total variability of results. PC1 (35.2%) was influenced by the storage time bottle, four years (4Y), phytochemical parameters and chemical composition of the WSs aged using alternative technology with micro-oxygenation (O15, O30, O60 and N modalities) and traditional technology (B modality).

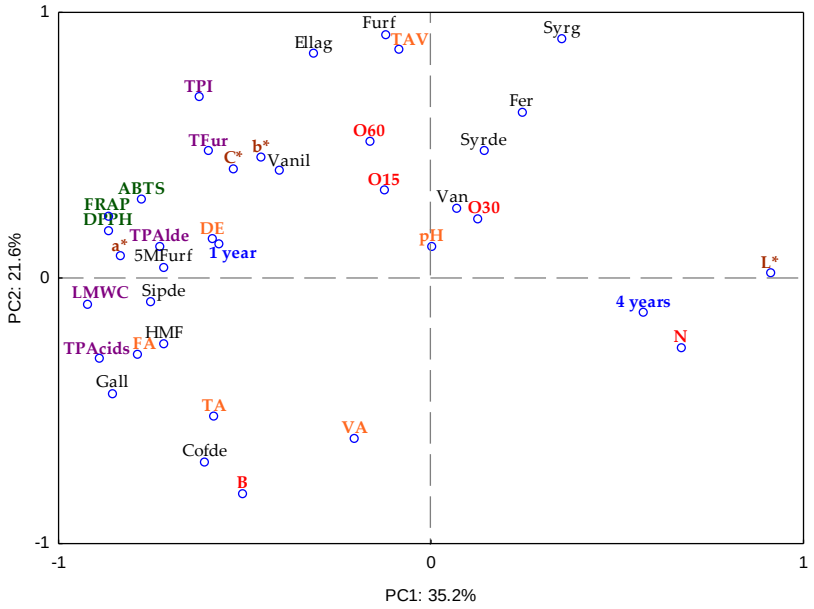

Figure 3. Principal Components Analysis (standardized scores and loadings)) with results of chromatic characteristics (L* —Lightness, C*— Chroma, a*—chromaticity coordinate a*, b*—chromaticity coordinate b*); physicochemical determinations (TAV—alcoholic strength, TA—total acidity, FA—fixed acidity, VA—volatile acidity, DE—total dry extract, pH); antioxidant activity (DPPH, FRAP, ABTS methods); phenolic content (total phenolic index —TPI) and total and individual LMW (Low molecular weight) compounds content from aged

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WS using ageing technologies (Traditional: B; Alternative: O15, O30, O60 and N) over storage time (one year (1Y) and four years (4Y)) in the bottles. LMW compounds: TLMWC—sum of content of individual LMW compounds; TPacids — sum of phenolic acids; TFur — sum of furanic aldehydes; TPalde— sum of phenolic aldehydes; Gall—gallic acid; Ellag—ellagic acid; Van—vanillic acid; Syrg—syringic acid; Fer—ferulic acid; Vanil—vanillin; Syrg—syringaldehyde; Cofde—coniferaldehyde; Sipde—sinapaldehyde; Furf— furfural; HMF—5-hydroxymethylfurfural; 5Mfurf—5-methyfurfural.

In addition, the O30 and N modalities are positively located in relation to CP1, while the O15 and O60 is negatively located. The difference between MOX modalities (O15, O30 and O60) and the control modality (N) was significantly demonstrated at the end of the ageing process, and it was maintained throughout bottle ageing [15,16]. In fact, the N modality presented a higher L* compared to other modalities (Figure 2). It can be noted that the O15 and O60 modalities show a more pronounced differentiation themselves of the O30 modality according to variables analytics. Regarding the variables analysed, there was separation from PC1, where vanillic, syringic and ferulic acids and syringaldehyde were located positively; and negatively in relation to vanillin, ellagic acid, furfural, TPI and antioxidant activity (ABTS, DPPH and FRAP assay), respectively. These data agree with those described in Tables 1 and 2. In addition, PC2 accounted for 21.6% of the variation associated with traditional technology. In detail, the B modality showed correlations with total LMW compounds content and Total phenolic acids.

In summary, these results show that the percentage of variation explained by PC1 and PC2 is low, indicating that there is weak differentiation of the aged WSs' characteristics according to the storage time in bottle (one year and four years). When compared to other alternative technology modalities, the O60 modality resulted in the lower changes and higher retention of its phenolic content and chromatic characteristics, ensuring its overall quality.

2.5. Phenolic Characterisation of the Four Years' Bottle Storage

The LWM compounds from WSs aged by Traditional and Alternative Technology were tentatively identified by LC-DAD-ESI-MS/MS technique using an electrospray ionization source (ESI) in both negative and positive ionization modes. The ionization conditions in the mass spectrometer were optimized to detect the m/z values corresponding to the precursor ions. In Table 3 is presented a list of 36 compounds tentatively identified, including their retention time (RT), UV absorption maximum (λmax), precursor ion, MS/MS product ions, identification of the ageing modality and the bibliographic references to support the identification.

Table 3. Characterization of phenolic compounds in the wine spirits aged using Traditional and Alternative Technologies after four years of storage in bottle by LC-DAD-ESI-MS/MS.

Peak	RT (min.)	λmax (nm)	Precursor Ion (*m*/*z*) $[M-H]^+$	Precursor Ion (*m*/*z*) $[M-H]^-$	Product Ions *m*/*z* (% Base Peak)	Tentative Identification	References
1	6.2			147	103(100)	Citramalic acid	[88,89]
2	7.87			133	115(100), 113(40), 71(20)	Malic acid	[88,90]
3	8.13	273		331	169(100), 125(35)	Galloyl glucose	[27,91,92]
4	8.42	271		331	169(80), 271(20), 211(35), 125(55)	Monogalloyl-glucose	[27,92,93]
5	15.5	271		169	125(100)	Gallic acid	[94–96]
6	20.03	284	127		127(40), 109(100), 81(30)	HMF	[97,98]

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7	22.40	290, 326		153	153(50), 109(100)	Protocatechuic acid	[95,96,99]
8	29.85	281	97		97(100), 69(30)	Furfural	[97,98]
9	33.97	274		483	483 (100), 331(20), 313(30), 271(20), 169(60)	Digalloylglucose 1	[27,91,95]
10	34.48	273		483	483 (100), 331(20), 313(30), 271(20), 169(60)	Digalloylglucose 2	[27,91,95]
11	35.33	279		341	341(100), 169(10), 125(10)	Gallic acid-glucoside	[27,92]
12	36.27	273		321	169(100), 125(10)	Digallate	[27,92]
13	39.95	273		635	635(50), 483(30), 465(20), 313(15), 211(10), 169(10)	Trigalloyl glucose	[27,95]
14	40.89	263, 292		167	167(100), 152(30), 108(23), 123(10)	Vanillic acid	[94,95]
15	41.86	273		197	197(100), 161(30), 182(25), 153(60)	Syringic acid	[94–96]
16	42.92	271		493	493(100), 331(10), 313(10), 271(20), 211(30), 169(10)	Monogalloyl-diglucose	[93,100]
17	44.05	278		289	289(30), 245(100), 203(10), 179(10)	Epicatechin	[101,102]
18	45.17	276		787	787(30), 635(20), 617(20), 465(15), 313(10)	Tetragalloy glucose	[27,95,103]
19	46.35	231, 325		193	193(50), 178(15), 149(20), 134(100),	Ferulic acid	[95,99]
20	46.65	276		939	939(100), 787(50), 769(40), 635(30), 617(10)	Pentagalloy glucose	[27,92]

Peak	**RT (min.)**	**λmax (nm)**	**Precursor Ion (*m*/*z*) $[M-H]^+$**	**Precursor Ion (*m*/*z*) $[M-H]^-$**	**Product Ions *m*/*z* (% Base Peak)**	**Tentative Identification**	**References**
21	46.72	254, 365		301	301(100), 229(10)	Ellagic acid	[92,95,101]
22	47.25	280, 328		151	151(60), 136(100)	Vanillin	[94,95,104]
23	48.17	229, 306		181	181(80), 166(45), 151(20)	Syringaldehyde	[95,96,101]
24	48.69	283, 307		167	167(30), 109(70)	Methyl protocatechuate	[94,98]

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25	49.38	250, 361	585	585(100), 301(30)	Ellagic acid dimer dehydrated	[95,103,104]
26	50.48	320	307	307(100), 261(20), 235(15)	3-Carbethoxymethyl-flavone	[94]
27	50.93	290	361	361(40), 181(50), 137(100)	Homovanillic acid	[94]
28	51.66	271	663	663(20), 331(100), 169(10)	Monogalloyl-glucose dimer	[91,103]
29	52.3	250, 362	433	443(100), 301(50)	Ellagic acid pentoside	[27,91,100]
30	52.85	244, 345	207	207(100), 192(50)	Sinapaldehyde	[95,103,104]
31	53.24	238, 340	177	177(100), 162(90)	Coniferaldehyde	[95,103,104]
33	54.02	274	197	197(100), 169(20), 125(45)	Ethyl gallate	[94,105]
34	55.88	265, 362	447	447(20), 285(100)	Kaempherol-hexoside	[100]
35	57.82	265, 360	285	285(100), 283(40), 193(50), 177(20)	Kaempferol	[96,101]
36	59.28	254, 355	367	367(100), 301(80)	Ellagic acid derivative	[92,95]

The chromatographic profiles at 280 nm (Figure A1 – Appendix A) of aged WSs from all modalities (B, O15, O30, O60 and N) were very similar, confirming that alternative technology can mimic the traditional technology in terms of furanic aldehydes and phenolic profiles. The 36 LMW compounds were tentatively identified in all modalities under study (Table 3): 2 organic acids (peaks 1 and 2), 2 furanic aldehydes (peaks 6 and 8), 13 phenolic acids (peaks 5, 7, 11, 12, 14, 15, 19, 21, 24, 25, 27, 29 and 36), 8 gallotannins (peaks 3, 4, 9, 10, 13, 16, 18, 20 and 28), 4 phenolic aldehydes (peaks 22, 23, 30 and 31), and 4 flavonoids (peaks 17, 25, 34 and 35). According to the literature, these phenolic acids, aldehydes phenolics, flavonoids and hydrolysable tannins have been identified in heartwood extracts from chestnut by HPLC-DAD-ESI-MS/MS technique [95,103,104]. It is noteworthy that chestnut wood has been used in the ageing process of wine and spirit drinks due to its richness in hydroxybenzoic acids and hydrolysable tannins [95,106,107], as the presence of these compounds is confirmed in aged WSs (Table 3).

Organic acids, citramalic ($[M-H]^-$ *m/z* 147) and malic acids ($[M-H]^-$ *m/z* 133), were identified in aged WSs, originating directly from the grape and/or from the fermentation/distillation processes (Figure 4–A). The organic acids have important roles in distilled alcohol beverages since they affect their organoleptic properties, stability and acceptability [108]. Malic acid has been quantified in wine distillates, brandies and whiskeys [108,109].

The precursor ions $[M-H]^-$ at *m/z* 331, 483, 635, 787 and 939 are related to the gallotannin groups (Figure 4), belonging to class of hydrolysable tannins [27]. Fernandes et al. [27] by a targeted metabolomics approach, depicted the loss of one or more galloyls groups (152 u) and/or gallic acid (170 u), from mono, di, tri and tetragalloyl glucopyranose, contributing to an increase in gallic and ellagic acid over time. Besides, isobaric gallotannins were highlighted, specifically digalloylglucose with $[M-H]^-$ at *m/z* 483 (peak 9 and 10). Studies have demonstrated that gallotannins have a wide range of biological activities, including reduced incidence of cardiovascular disease, diabetes,

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cataracts, inflammation, colorectal cancer and inhibition of tumour growth [110,111]. Furthermore, these compounds have astringency and antiradical properties, affecting colour, roundness and mouthfeel of aged WS. In our previous study, we identified gallotannins in WSs aged with chestnut wood and explained their degradation pathway [27].

Noteworthy, the esterification reactions of gallic acid may be confirmed by identification of digallate (peak 12) in aged WSs [27]. The oxidation-reduction reactions of flavonoids, on the other hand, were clearly observed in all modalities by identifying 3-carbethoxymethyl-flavone (peak 26), which showed the typical fragmentation pattern of this compound, with the product ions at *m/z* 261 and 179. The oxidation-reduction reactions of flavones to form methoxy-3,4-flavandiones and methoxy-3-(carbethoxymethylene) flavones has been demonstrated by Smith [112]. Regarding flavonoids, Regalado et al. [94] identified 3-(carbethoxymethyl)-flavone and kaempferol in rum aged in oak barrels.

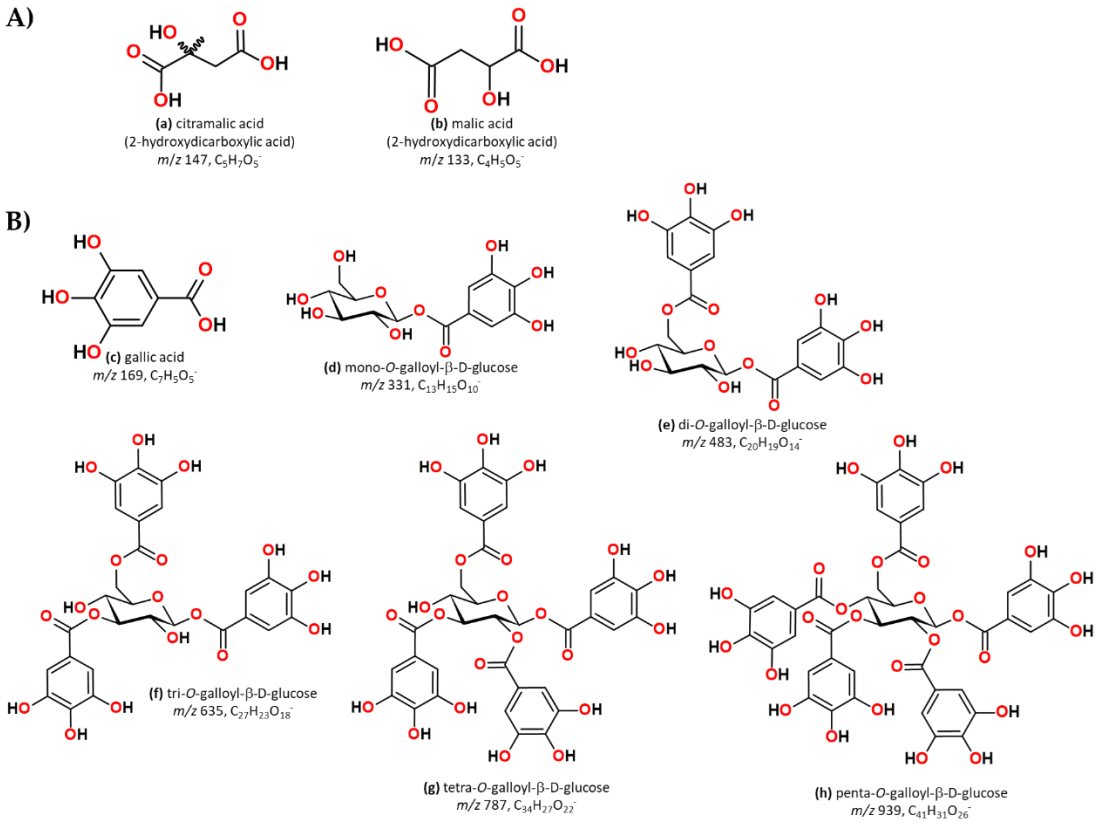

Figure 4. A) Chemical structures of (a) citramalic acid and (b) malic acid; B) Chemical structures of (c) gallic acid and gallotannins (d) mono-*O*-galloyl-β-D-glucose, (e) di-*O*-galloyl-β-D-glucose, (f) tri-*O*-galloyl-β-D-glucose, (g) tetra-*O*-galloyl-β-D-glucose, (h) penta-*O*-galloyl-β-D-glucose.

In general, phytochemical characterisation of aged WSs stored in the bottles for four years was performed, demonstrating that different groups of compounds, such as hydrolysable tannins, dimer phenolic acids and oxidative flavonoids were maintained in a similar manner in both technologies, with these compounds being of crucial importance, because they are responsible for organoleptic characteristics, bioactive activity and consumer' acceptability.

3. Materials and Methods

3.1. Chemical and Reagents

During experiments, the distilled water (conductivity < 6.0 μS/cm) and the ultrapure water (conductivity < 0.055 μS/cm) were obtained from Arium Comfort System (Sartorius, Goettingen, Germany). Acetonitrile (CH_3CN, 99.9% v/v, LC gradient grade), methanol (MeOH, 99.9% v/v, LC

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gradient grade) and formic acid (HCOOH, 98% v/v, analytical grade) were purchased from Merck (Darmstadt, Germany), and ethanol (CH_3CH_2OH, 99.9% v/v, LC gradient grade) was purchased from Carlo Erba (Val de Reuil, France).

Antioxidant activities were conducted using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical obtained from TCI (Tokyo, Japan), and TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), Trolox (6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid), sodium acetate trihydrate purchased from Sigma-Aldrich (Steinheim, Germany). Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) and potassium persulfate ($K_2S_2O_8$) were purchased from Honeywell Fluka (Seelze, Germany).

All the standard phenolic compounds (purity > 98%) were dissolved in ethanol/water (75:25, v/v) and stored in darkness at 7 °C before used. Ellagic acid dehydrate (ellag), vanillin (vanil), vanillic acid (van), syringic acid (syr), ferulic acid (ferul), 5-hydroxymethylfurfural (HMF), furfural (furf) and 5-methylfurfural (5mfurf) were purchased from Fluka (Buchs, Switzerland). Gallic acid (gal), 4-hydroxybenzaldehyd, syringaldehyde (syrde), coniferaldehyde (cofde) and sinapaldehyde (sipde) were bought from Sigma-Aldrich (Steinheim, Germany).

3.2. Experimental Design and Aged WSs Sampling

The experimental design, consisting of two phases, was provided in detail by Canas et al. [15](Figure 4):

1) Ageing trial was carried out on a pilot scale in 50 L glass demijohns, covering five ageing modalities: i) chestnut barrels (B, representing the traditional ageing technology), and (ii) three MOX modalities (O15, O30, O60) and one control modality with nitrogen (N) application (representing the alternative ageing technologies). Portuguese chestnut (*Castanea sativa* Mill.) barrels (250 L) and staves (50 cm length × 5 cm width × 1.8 cm thickness) were manufactured by J. M. Gonçalves cooperage (Palaçoulo, Portugal). The chestnut staves were toasted at a with medium plus toasting level (90 min at an average temperature of 240 °C; 1.8 cm of toasting thickness) in an industrial oven. While, the barrels were heated over a fire of wood offcuts, under certain conditions of temperature to ensure similar level of toasting. The quantity of staves inserted into the demijohns in four modalities (O15, O30, O60, N) was calculated to reproduce the surface area to volume ratio of a 250 L barrel (85 cm^2/L). In this study, two replicates of each ageing modality were carried out. The wine distillate produced by Adega Cooperativa da Lourinhã (Lourinhã, Portugal) through column distillation (alcoholic strength by volume, 78.3 %v/v; total acidity, 0.12 g acetic acid/L of absolute ethanol; volatile acidity, 0.09 g acetic acid/L of absolute ethanol; pH, 5.33) was used to fill the barrels and demijohns.

MOX was applied to the WS during the ageing time, supplying pure oxygen (X50S Food, Gasin, Portugal) through a multiple diffuser micro-oxygenator (VISIO 6, Vivelys, France) with ceramic diffusers, at different flow rates according to the ageing modality (O): i) O15 — 50 L glass demijohns with chestnut staves and micro-oxygenation (flow rate of 2 mL/L/month during the first 15 days followed by 0.6 mL/L/ month until 365 days); ii) O30 — 50 L glass demijohns with chestnut staves and micro-oxygenation (flow rate of 2 mL/L/month during the first 30 days followed by 0.6 mL/L/ month until 365 days); and iii) O60 — 50 L glass demijohns with chestnut staves and micro-oxygenation (flow rate of 2 mL/L/month during the first 60 days followed by 0.6 mL/L/ month until 365 days).

Pure nitrogen (X50S Food, Gasin, Portugal) was applied in the control modality (N) continuously (flow rate of 20 mL/L/month) over the ageing time through a specific device (Gasin, Portugal). The N modality aims to minimize dissolved oxygen in WS, thus acting as a control. During ageing process, the ten experimental units were stored in the cellar of Adega Cooperativa da Lourinhã (Lourinhã, Portugal) in the same environmental conditions.

2) Storage in the bottle: after 365 days of ageing process, the ten aged WS were bottled on the same day in amber glass bottles (750 mL, two bottles from each demijohn), ensuring the same level of WS in each bottle. Regarding the bottle, the headspace was set at 9.8 mL of air in all bottles to ensure that the oxygen ingress into the aged WS was similar, so that the oxidation promoted was

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controlled and the main effects observed were due to the ageing modality and the storage time [80]. The cork stoppers were sealed with parafilm (Parafilm®, Bemis Company, Neenah, WI, EUA) to reduce evaporation. The bottles were transported in the same day and stored in the cellar of INIAV – Dois Portos at 19 °C and 80% relative humidity for 48 months. Sampling was carried out at 12 and 48 months after bottling. A total of 40 samples (5 modalities × 2 replicates × 2 sampling bottles × 3 storage times) of WS were taken and analysed to determine the chromatic and chemical characteristics, total phenolic index, antioxidant activities, low molecular weight composition, phenolic profile and their correlations as well.

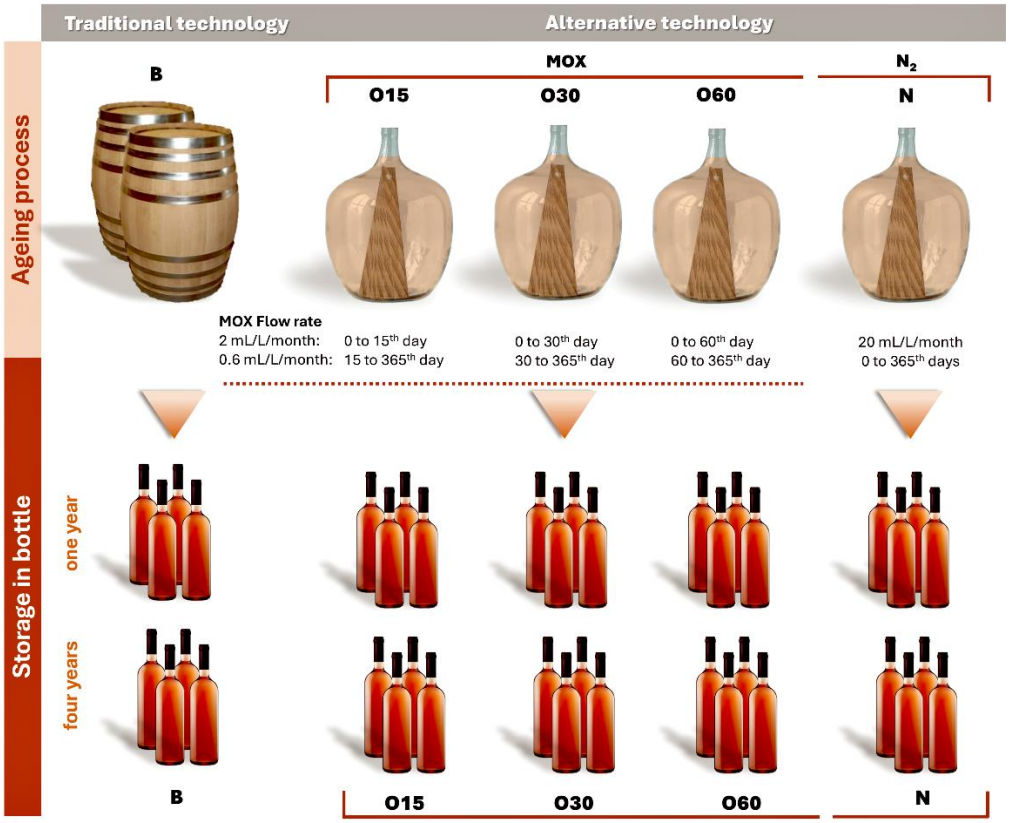

Figure 4. Illustration of the experimental design for the ageing trial of aged wine spirits with Traditional and Alternative Ageing Technologies.

3.3. Chromatic Characteristics

The chromatic characteristics (lightness (L*), chroma (C), chromaticity coordinates (a* and b*) and absorbance at 470 nm) of aged WSs were determined by the CIELab/CIELCh method [76] using a Varian Cary 100 Bio spectrophotometer (Santa Clara, California, USA) and a 10-mm glass cell, as described by Canas et al. [7]. Transmittance measurement was carried out every 10 nm from 380 to 770 nm, using a D65 illuminant and a 10◦ standard observer. The analyses were performed in duplicate.

3.4. Physicochemical Characteristics

The basic chemical characteristics of the aged WSs were determined in duplicate: alcoholic strength by volume, pH, total dry extract, total acidity, fixed acidity and volatile acidity. Alcoholic strength was obtained by distillation and electronic densimetry (DMA 5001, Anton Paar, Graz, Austria) [113]; the corresponding results were expressed as a volumetric percentage of ethanol in the WS. Total dry extract was analysed by gravimetry [35]; the corresponding results were expressed as grams per litre. pH was determined by potentiometry [35] using a potentiometer (micro pH2002, Crison, Barcelona, Spain) with a glass electrode and reference electrolyte (lithium chloride (LiCl) in

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an ethanol medium (1 mol/L) [113]. Total acidity was assessed by colourimetric titration and fixed acidity by colorimetric titration of the water solution of dry extract [81], and the corresponding results were expressed as grams of acetic acid per litre of absolute ethanol. Volatile acidity was obtained by calculation of the total acidity minus fixed acidity [81]. The analyses were performed in triplicate for each bottle. The total phenolic index (TPI) of the aged WSs was determined according to Cetó et al. [114]. Briefly, aged WSs were diluted with distilled water (1:100, v/v) and the absorbance was measured directly at 280 nm using a Varian Cary 100 Bio spectrophotometer (Santa Clara, California, USA) and a 10 mm quartz cuvette. TPI value of each bottle was calculated by multiplying the measured absorbance by the dilution factor. The analyses were performed in triplicate.

3.5. Antioxidant Activity Analyses

3.5.1. ABTS Assay

The ABTS assay was obtained according to the method reported by Rufino et al. [115], with some modifications. Briefly, the $ABTS^{\bullet+}$ radical cations were prepared by reacting 4 mL of a 7 mmol/L ABTS stock solution with 70.4 µL of 140 mmol/L potassium persulfate solution for 16 h, at room temperature and in the dark. Thereafter, the ABTS solution was diluted by adding ethanol (99.5%) to the $ABTS^{\bullet+}$ radical solution until measured absorbance reached 0.700 ± 0.02 at 734 nm using a Varian Cary 100 Bio spectrophotometer (Santa Clara, CA, USA). The aged WSs were diluted with absolute ethanol (1:50 v/v). In tube, 3 mL of ABTS solution was added to 30 µL of sample or 30 µL of standard solution, mixed and placed in the water bath (Selecta Digiterm 3000542, Barcelona, Spain) for 6 min at 30 °C. After reaction, the absorbance was measured at 734 nm at room temperature. The control absorbance (30 µL of absolute ethanol plus 3 mL of ABTS solution) was measured at the beginning and end of the assay. Trolox standard curve (0.08–2.0 mM TEAC) was used as reference antioxidant. Results were expressed as mmol Trolox equivalent antioxidant capacity (TEAC)/L of WS. The analysis was carried out in triplicate.

3.5.2. DPPH Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity was carried out using the method described by Nocera et al. [8]. Briefly, 10 µL of aged WS was added to 3 mL of 8.5 x 10^{-5} M DPPH methanolic solution in a glass tube wrapped with aluminum foil. The tube was vortexed for 10 s and then immediately placed in a water bath (Selecta, Digiterm 3000542, Barcelona, Spain) at 30 ± 1 °C for 60 min; the tube was shaken every 10 min and placed back in the water bath. After cooling to room temperature, the absorbance was measured at a wavelength of 515 nm using a Varian Cary 100 Bio spectrophotometer (Santa Clara, CA, USA). The control absorbance (10 µL de methanol and 3 mL of 8.5 x 10^{-5} M DPPH methanolic solution) was measured at the beginning and end of the assay. Trolox was used as a reference standard curve (1–15 mM TEAC) and the results were expressed as mmol Trolox equivalent antioxidant capacity (TEAC)/L of WS. The analyses were performed in triplicate.

3.5.3. FRAP Assay

The ferric reducing ability was determined by FRAP (Ferric Reducing Antioxidant Power) method, according to Benzie and Strain [116]; and Pulido et al. [117], with modifications. Briefly, FRAP reagent was prepared with 75 mL of a 0.3 M sodium acetate buffer (pH 3.6), 7.5 mL of 10 mmol/L TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) in a 40 mmol/L HCl solution, plus 7.5 mL of 20 mmol/L $FeCl_3 \cdot 6H_2O$ in the dark. The aged WSs were diluted with ethanol (1:50, v/v). Sample or standard solutions, distilled water and FRAP reagent (90 µL sample or standard solution, 270 µL distilled water plus 2.7 mL FRAP reagent) were mixed and kept in a water bath (Selecta, Digiterm 3000542, Barcelona, Spain) for 30 min at 37 °C. After cooling to room temperature, absorbance was measured at 595 nm using a Varian Cary 100 Bio spectrophotometer (Santa Clara, CA, USA). The control absorbance (90 µL ethanol, 270 µL distillated water plus 2.7 mL FRAP reagent) was measured at the beginning of the assay. A Trolox standard curve was also prepared (0.08–1.5 mM TEAC). Results

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were expressed as mmol Trolox equivalent antioxidant capacity (TEAC)/L of WS. The assay was performed in triplicate.

3.6. Analyses of Low Molecular Weight Compounds

3.6.1. HPLC-DAD-ESI-MS/MS Identification

The characterization of the phenolic compounds of aged WS was performed in a Waters Alliance 2695 HPLC system (Waters, Milford, MA, USA) equipped with a quaternary pump, solvent degasser, auto sampler, and column oven, coupled to a diode array detector (Detector Waters 2996, Milford, MA, USA). For the separation of compounds, a reversed phase C18 column (LiCrospher 100 RP-18, 250 × 4 mm; 5 μm) in a thermostatic oven at 35 °C was used. A pre-column (100 RP-18, 5 μm) was also used. The mobile phase consisted of water:formic acid (99.5%:0.5%) as eluent A and acetonitrile:formic acid (99.5%:0.5%) as eluent B at a flow rate of 0.30 mL/min. All solvents were filtered through a 0.22 mm PVDF membrane (Millipore, Billerica, MA, USA) prior to analysis. The system was run with the following gradient elution program: 0–10 min from 99 to 95% A; 10–30 min from 95 to 82% A; 30–44 min from 82 to 64% A; 44–64 min at 64% A; 64–90 min from 64 to 10% A; 90–100 min at 10% A; 100–101 min from 10 to 95% A; 101–120 min at 95% A; and a final step to return to the initial conditions. The injection volume was 20 μL. DAD was used to scan the wavelength absorption from 200 to 650 nm. Tandem mass spectrometry (MS/MS) detection was performed using an electrospray ionization source (ESI) at 120 °C, applying a capillary voltage of 2.5 kV, cone voltage of 30 V and collision energy of 20 eV. The compounds were ionized in the negative and positive mode and spectra were recorded in the range of *m/z* 60–1500. Ultra-high purity argon (Ar) was used as a collision gas. High purity nitrogen (N_2) was used both as a drying gas and nebulizing gas. For data acquisition and processing, MassLynx software (version 4.1, Waters Corporation, Milford, MA, USA) was used to control analytical conditions and to collect the data from HPLC-DAD-MS/MS. For compounds identification purposes, mass and UV spectra were compared with spectra already published in the literature. When standards were commercially available, the identification was based on the comparison of their fragmentation patterns and retention times.

3.6.2. Low Molecular Weight Composition Determination

Low molecular weight (LMW) compounds of aged WSs were quantified according to the method of Canas et al. [118]. Chromatography separation of compounds was performed using a HPLC Lachrom Merck Hitachi system (Merck, Darmstadt, Germany) equipped with a quaternary pump L-7100, a column oven L-7350, a UV-Vis detector L-7400, and an autosampler L-7250, coupled with HSM D-7000 software (Merck, Darmstadt, Germany) for management, acquisition and treatment of data. A LiChrospher RP 18 (5 μm, 250 mm × 4 mm ID) column (Merck, Darmstadt, Germany) was used as a stationary phase. The mobile phase consisted of water/formic acid (98:2 v/v) as eluent A, and methanol/water/formic acid (70:28:2 v/v/v) as eluent B, at a flow rate of 1 mL/min and column temperature of 40 °C. All solvents were filtered through a 0.45 μm PVDF membrane (Cronus filter, Gloucester, UK) prior to analysis. The auto sampler's temperature was set at 18 °C and the injection volume was 20 μL. Samples were spiked with an internal standard (20 mg/L of 4-hydroxybenzaldehyde). The elution program was as follows: 0–3 min at 0% isocratic B; 3–25 min from 0% to 40% B; 25–43 min from 40% to 60% B; 43–55 min at 60% isocratic B; 55–60 min from 60 to 80% A; 60–65 min at 80% isocratic B; 65–75 min from 80 to 0% B, and finally returning to the initial conditions. Detection was made at 280 nm for phenolic acids (gall, van, syrg, fer and ellag acids) and furanic aldehydes (HMF, furf and 5mfurf), and at 320 nm for phenolic aldehydes (vanil, syrde, cofde and sipde). Quantification of these compounds was performed through calibration curves (mg/L).

3.7. Statistical Analysis

Data of antioxidant activities, TPI and low molecular weight composition were expressed as mean ± standard deviation of independent duplicates. One-way analysis of variance (ANOVA) was applied to assess the effects of the ageing modalities on the antioxidant activities, TPI and LMW

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compounds contents of the aged WSs for each storage time in the bottle. Another one-way ANOVA was carried out to assess the significance of the antioxidant activities, TPI and LMW compounds over storage time in the bottle. Tukey's test was made to compare the average values when a significant difference ($p < 0.05$) was found. The correlation between antioxidant activities, TPI and LMW compounds concentrations were determined through the Pearson's correlation coefficient test, considering a confidence level of 95% ($p < 0.05$). Principal component analysis (PCA) of results was used to evaluate the possible grouping of total LWS compounds for all modalities during the storage time in botte. Statistical analysis and PCA were performed using Statistica version 7.0 (StatSoft Inc., Tulsa, OK, EUA). The results of scores and loadings are standardized and present in the same graphic identifying the influence of each factor.

4. Conclusions

The findings presented herein enhance the understanding of the impact on the physicochemical and phytochemical characteristics of WS aged with chestnut wood using traditional and alternative technologies over four years of storage in the bottle. During bottle ageing, the O60 modality enabled more preservation of the phenolic content, demonstrating a higher total phenolic index value and a lower reduction of the phenolic acids and phenolic aldehydes in relation to the other modalities. The antioxidant activity values and phytochemical characterization of aged WSs from traditional and alternative technologies showed comparable profiles. Positive correlations were found for total phenolic content and antioxidant activity, in particular, gallic acid content showing a highest correlation, followed by sinapaldehyde content. The experimental conditions allowed the identification of gallic acid derivatives, such as gallic acid esters and gallotannins, in all modalities. The results demonstrated an increase in b* coordinates and Chroma values in all modalities, evidencing colour evolution through the formation of yellowish compounds. In conclusion, few changes were noted in alternative technology via the O60 modality after four years of storage in the bottle, suggesting that this eco-efficient ageing technique appears to preserve the overall quality of wine spirits. This study supplies new insights into the evolution phenolic of aged wine spirits during storage time in the bottle using alternative technology, potentially providing for spirit industry a cost effective and sustainable solution.

Author Contributions: Conceptualization, S.O-A., Sa.C. and So.C.; methodology, S.O-A., Sa.C. and So.C.; software, S.O-A.; validation, S.O-A., Sa.C., So.C. and T.A.F.; formal analysis, S.O-A., S.L, J.G.-S., A.B.S. and M.R.B.; investigation, S.O-A, T.A.F, Sa.C. and So.C. and.; resources, Sa.C., So.C. and M.R.B.; writing—original draft preparation, S.O.-A., T.A.F and Sa.C.; writing—review and editing, S.O.-A., S.L., J.G-S., A.B.S, M.R.B., T.A.F., Sa.C. and So.C; supervision, Sa.C. and So.C.; project administration, Sa.C. and So.C.; funding acquisition, Sa.C. and So.C. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

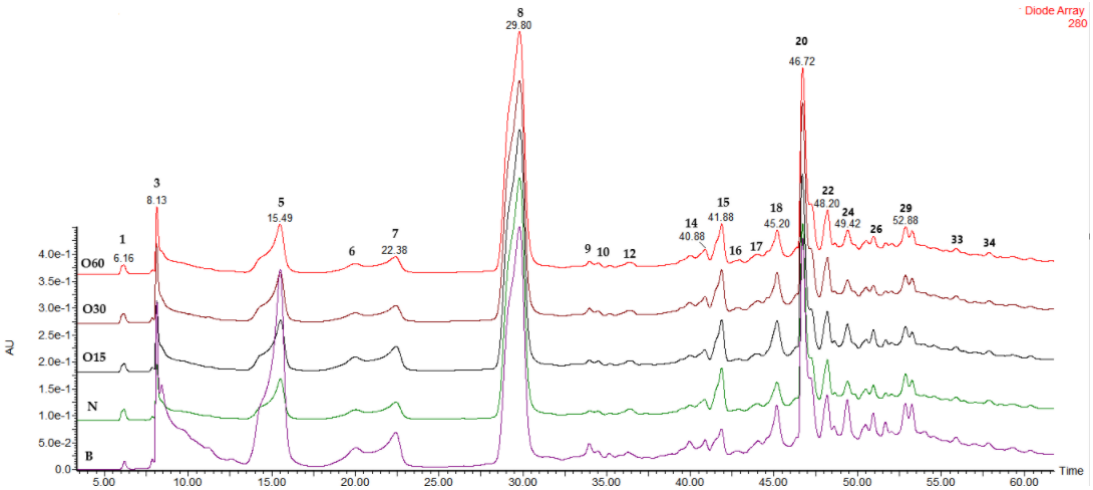

Figure A1. Chromatographic profile at 280 nm of wine spirits aged using ageing technologies (Traditional: B; Alternative: O15, O30, O60 and N) after four years of storage in the bottle.

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