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Characterisation of Refined Marc Distillates with Alternative Oak Products Using Different Analytical Approaches / Petrozziello, M; Rosso, L; Portesi, C; Asproudi, A; Bonello, F; Nardi, T; Rossi, Am; Schiavone, C; Scuppa, S; Cantamessa, S; Pollon, M; Chiarabaglio, Pm. - In: APPLIED SCIENCES. - ISSN 2076-3417. - 12:17(2022), p. 8444. [10.3390/app12178444]

Availability:

This version is available at: 11696/76380 since: 2023-03-05T22:04:39Z

Publisher:

MDPI

Published

DOI:10.3390/app12178444

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Article

Characterisation of Refined Marc Distillates with Alternative Oak Products Using Different Analytical Approaches

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Citation: Petrozziello, M.; Rosso, L.; Portesi, C.; Asproudi, A.; Bonello, F.; Nardi, T.; Rossi, A.M.; Schiavone, C.; Scuppa, S.; Cantamessa, S.; et al. Characterisation of Refined Marc Distillates with Alternative Oak Products Using Different Analytical Approaches. *Appl. Sci.* **2022**, *12*, 8444. <https://doi.org/10.3390/app12178444>

Academic Editor: Alessandro Genovese

Received: 15 July 2022

Accepted: 19 August 2022

Published: 24 August 2022

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Abstract: The use of oak barrel alternatives, including oak chips, oak staves and oak powder, is quite common in the production of spirits obtained from the distillation of vegetal fermented products such as grape pomace. This work explored the use of unconventional wood formats such as peeled and sliced wood. The use of poplar wood was also evaluated to verify its technological uses to produce aged spirits. To this aim, GC-MS analyses were carried out to obtain an aromatic characterisation of experimental distillates treated with these products. Moreover, the same spirits were studied for classification purposes using NMR, NIR and e-nose. A significant change in the original composition of grape pomace distillate due to sorption phenomena was observed; the intensity of this effect was greater for poplar wood. The release of aroma compounds from wood depended both on the toasting level and wood assortment. Higher levels of xylovolatiles, namely, whisky lactone, were measured in samples aged using sliced woods. Both the NIR and NMR analyses highlighted similarities among samples refined with oak tablets, differentiating them from the other wood types. Finally, E-nose seemed to be a promising alternative to spectroscopic methods both for the simplicity of sample preparation and method portability.

Keywords: spirits; VOCs; oak chips; NMR; NIR; GC-MS; e-nose

1. Introduction

The use of wood fragments (chips) for the ageing of wines, distillates and vinegars is a widespread practice within the European Union and complies with specific regulations to defend the Protected Geographical Indication (PGI) from possible fraud. Their use, when authorized, is intended to reduce production time and costs [1].

Different spirits, such as grappa, rum, Armagnac, cognac, brandy, whisky and tequila, are refined in barrels or, if allowed by regulations, using wood chips in their ageing processes [2]. Wood may remain in the distillate from a few days to a few weeks at a dosage of 0.5–2.5 g per L. This process results in a fast change in aromatic complexity and colour intensity [3] due to the extraction of wood volatile compounds from wood, also named xylovolatiles [4], and other extractables, such as polyphenols [5].

Most xylovolatiles arise from the depolymerisation of lignin, cellulose and hemicellulose during both the seasoning and toasting of the wood and include phenolic aldehydes

(vanillin and syringaldehyde) and hydroxycinnamic aldehydes (coniferaldehyde and sinapaldehyde), as well as free phenolic acids such as vanillic, gallic, ellagic, ferulic and syringic acids. Moreover, other compounds can be found in oak-aged distillates. Some of them may derive from the Maillard reaction, which occurs during the wood-toasting phase and leads to the formation of coloured compounds (melanoidins) and several aromas with a high olfactory impact, such as pyrazines and furan derivatives [1,6,7].

Among factors that affect the transfer of xylovolatiles to the distillate the most, the geographic and botanical origin of the wood, the wood grain coarseness, the drying/seasoning methods, the infusion duration, the shape and size of fragments and the surface/volume ratio should be considered [1], but the wood-toasting phase is crucial to the final quality of the distillate. In fact, toasting profoundly changes the structure, chemical composition and physical properties of the wood and is strictly dependent on the applied heat intensity.

Wood chips for wine ageing are exclusively obtained from pedunculate oak (*Quercus robur*), sessile oak (*Quercus petraea*) or white oak (*Quercus alba*). For the refinement of spirits, other types of wood are also available, including chestnut (*Castanea sativa*), cherry (*Prunus avium*), mulberry (*Morus alba*), fir (*Abies alba*) and walnut (*Juglans regia*) [2,7]. The shape and dimensions are the same as those employed in winemaking: fine grains (2 mm), wood chips (2–7 mm), wooden cubes (1 or 2 cm) and small staves.

Sliced wood and peeled wood or untoasted poplar chips used once during fermentation to clarify wines and improve their body could be an alternative to conventional chips or barrels for distillate ageing [1,8]. Sliced wood is obtained by cutting the trunk using a very sharp blade of length at least equal to that of the workpiece (trunk or square portion), with a series of cuts that follow one another from its lateral surface along planes parallel to the fixing base. Wooden sheets with a thickness of a few tenths of a millimetre are obtained (the most common thicknesses are between 0.4 and 0.6 mm).

Peeled wood is obtained from the trunk fixed to spindles that is rotated on its axis against a blade, obtaining a continuous sheet of wood, generally between 1 and 3 mm thick and as wide as it is along the trunk; this wooden strip is then cut transversely to obtain single sheets (possibly discarding the defective portions). A circular section with limited tapering, the regularity of the stem and the absence of ribs is required, and it need to be free of major defects (wounds, knots, etc.) depending on the use, i.e., decorative, structural or intended for packaging [9].

Some vibrational spectroscopy techniques, such as mid- and near-infrared spectroscopy (MIR and NIR), can be used to discriminate wines aged for variable periods in woods obtained from different essences (oak, acacia, chestnut) or wines aged in different ways (barriques, chips, steel) [10]. The same techniques have been recently employed for the analysis of spirits, either for authentication purposes [11,12] or for the discrimination of ageing techniques [13,14]. Furthermore, ^1H NMR analysis is one of the main analytical approaches, able to produce highly reliable and reproducible datasets suitable for non-targeted multivariate statistical analyses aimed to metabolomic studies. NMR has recently and successfully been used for the classification of wines to find an association between their metabolic profile and some environmental, agronomic (in the vineyard) and processing (during wine production) factors. The advantages of high-resolution ^1H -NMR are absolute reproducibility and laboratory-to-laboratory transferability, meaning that it is unmatched by any other method currently used in food analysis. The reproducibility of NMR allows statistical investigations to be conducted, e.g., for the recognition of the variety, geographical origin and adulterations, where minimal changes in many components must be revealed at the same time.

The aim of the paper was twofold: primarily, peeled and sliced oak wood were tested for the first time to evaluate their suitability for use during distillate ageing, and poplar wood was explored as a cheap and available alternative to be used in place of oak. Indeed, a preliminary test on two poplar samples (as a less explored species in the aging field) was performed to evaluate its feasibility and compared with several samples made with different assortments and toasting of oak, in order to evaluate the differences among them.

Secondarily, from a methodological point of view, a multiple analytical approach was pursued; the effects of treatments on the general aromatic profile of the distillates were evaluated using GC-MS [10], while the combined application of more traditional “in-lab” analytical techniques such as GC-MS and NMR was supported by portable and low-cost techniques such as NIR spectroscopy, as an attempt to develop a flexible but robust method to be applied both to the classification of aged distillates and to the control of food fraud. To complete the research study, the samples were also analysed using a portable electronic nose, an analytical approach that can evaluate the overall aromatic quality of agro-food products.

2. Materials and Methods

2.1. Samples and Solutions

The distillate used for testing was obtained from virgin Moscato pomace, fermented and distilled, according to the method employed for grappa production [15]. The raw distillate, with an alcohol content of 70% *v/v*, was diluted to obtain an alcohol content of approximately 40% before chip infusion; a volume of 7.5 L of this solution was divided into 15 aliquots. Each sample had a final volume of 500 mL. Each one, apart from control, contained oak (12 samples) or poplar (2 samples) wood fragments of differing sizes and toasting levels for us to assess their impact on the final aromatic composition of the spirit (Table 1).

Table 1. Experimental plan adopted during the study.

Sample Code	Toasting Level	Format	Ageing Conditions	Wood
1	NT ¹	peeled wood	room temperature	poplar
2	heavy	peeled wood	room temperature	poplar
3	NT	peeled wood	room temperature	oak
4	heavy	peeled wood	room temperature	oak
5	light	peeled wood	room temperature	oak
6	NT	peeled wood	climatic chamber	oak
7	heavy	peeled wood	climatic chamber	oak
8	light	peeled wood	climatic chamber	oak
9	NT	sliced wood	room temperature	oak
10	heavy	sliced wood	room temperature	oak
11	light	sliced wood	room temperature	oak
12	NT	wood tablets	room temperature	oak
13	heavy	wood tablets	room temperature	oak
14	light	wood tablets	room temperature	oak
15	control	control	room temperature	control

¹ NT: not toasted.

To investigate how the wood affected the release of aromatic compounds during infusion, three different formats of alternative products were considered, of 2.5 mm (peeled wood), 0.56 mm (sliced wood) and 18.0 mm (cubes or wood tablets) in thickness, respectively.

The samples of alternative woods were treated ad hoc by simulating the process steps adopted for large-scale production:

- Seasoning of wood: Wood was immersed in deionized water, to reproduce the leaching action achieved by atmospheric precipitations during the open-air seasoning of staves used to produce barriques. The permanence time, submerged in water, was different for veneers and tablets (veneers, 1 h; tablets, 6 h; two washing cycles). Wood fragments were then stored in an oven at 25–30 °C, to simulate the environmental conditions of wood permanence outdoors during “natural seasoning”;
- Toasting phase: Three different levels of heating were considered, non-toasted samples (NT), light toasting (50 °C for 15 min) and heavy toasting (180 °C for 50 min).

A barrel generally has a surface-to-volume ratio ranging between 80–90 cm²/L; this ratio was applied to select the wood quantities to use for each 500 mL sample.

The “peeled wood” samples, subjected to the three different levels of toasting, were replicated twice. A first set of samples (3, 4 and 5 in Table 1) was kept at room temperature during the refining phases, and another set of samples (6, 7 and 8 in Table 1) was stored in a climatic chamber at a controlled temperature and a constant relative humidity of 65%. The following temperature cycle was performed in order to simulate the typical excursion temperature in a refining cellar. The cycle was carried out for about 12 months, and the first set of samples was stored at room temperature for the same period:

- A temperature of 5 °C for 8 days;
- A temperature of 15 °C for 8 days;
- A temperature of 30 °C for 8 days;
- A temperature of 15 °C for 8 days;
- A temperature of 5 °C for 8 days.

Samples were then stored in a temperature-controlled chamber protected from light.

2.2. Volatile Compounds Analysis—GC-MS Methods

All standards were purchased from Merck KgaA (Darmstadt, Germany); methanol and dichloromethane (HPLC grade) were purchased from Carlo Erba Reagents (Rodano, Milan, Italy). Ultrapure water was obtained using a Milli-Q gradient A10 instrument (Millipore Corporation, Billerica, MA, USA). The solid-phase extraction (SPE) cartridges used for sample preparation were polymeric reversed-phase cartridges (Strata X; Phenomenex, Torrance, CA, USA).

The method described by Petrozziello et al. [16] for xylovolatile analysis was used, with the following changes: 1-heptanol (250 µL of 78 mg/L) and 3,4-dimethylphenol (250 µL of 50 mg/L) were added, as internal standards, to 5 mL of distillate; then, 20 mL of water was added to reduce the concentration of alcohol to less than 5%. SPE cartridges were activated with 5 mL of dichloromethane, 5 mL of methanol and then 5 mL of ultrapure water without drying the cartridges between passages.

The sample was passed through the activated cartridge at a maximum flow rate of 2 mL/min on a 24-port SPE vacuum manifold; the cartridge was then washed with 5 mL of ultrapure water and was dried at room temperature. The volatile compounds were extracted with 5 mL of dichloromethane, dehydrated with anhydrous sodium sulphate and then partially concentrated to a volume of 2 mL. Samples were stored at −18 °C until GC analyses. The initial volume was further reduced, immediately before analysis, to approximately 500 µL using a slight stream of nitrogen. The analysis was performed with a GC 7890A system coupled to a 5975 MSD detector (Agilent Technologies, Santa Clara, CA, USA). A volume of 1 µL of extract in dichloromethane was injected in splitless mode. The split/splitless injection port was heated to 250 °C, and the split vent was opened after 2 min. The column used was a 60 m HP-Innowax (Agilent J&W GC Columns, Santa Clara, CA, USA) fused silica capillary column 60 m in length × 0.25 mm in internal diameter × 0.25 µm in polyethylene glycol film thickness. Helium was used as the carrier gas with a linear flux of about 1.1 mL/min.

Some selected key aromatic compounds derived from wood (e.g., acetophenone, acetovanillone, eugenol, isoeugenol, guaiacol, maltol, p-cresol, vanillin, β-methyl-γ-octalactone) were quantified using appropriate calibration curves. Commercial analytical standards were dissolved in a model solution (40% ethanol) to prepare the different levels of concentrations for each compound. Each calibration level was analysed using the same method previously described, and the regression analysis method was applied for quantification. All the other compounds (including varietal and fermentative compounds) for which the calibration curve was not made were reported as equivalents of the internal standard, 1-heptanol. The analysis of the volatile compounds, both the semiquantitative (general aromatic profile) and quantitative evaluation (xylovolatiles) were performed acquiring the chromatogram in Total Ion Current mode (TIC). Mass spectra were recorded across the range of 30–300 *m/z*. As regards the analytes considered in this paper, any coelution phenomenon with other compounds was excluded.

The tentative identification of volatile compounds was performed by comparing the recorded mass spectra with those of the NIST15 and WILEY275 databases. Moreover, the retention index calculated for each compound was compared with those available in the literature [17].

2.3. NMR Analysis

NMR analyses were carried out in the laboratories of Metrological Infrastructure for Food Safety (IMPreSA) in Turin. The instrument used was a 600 MHz NMR AVANCE neo 600 from Bruker (Bruker Biospin GmbH, Rheinstetten, Baden-Württemberg, Germany), equipped with a 5 mm probe, controlled temperature and autosampler.

All reagents and the deuterated solvent were purchased from Merck KgaA (Darmstadt, Germany), and the ultrapure water was obtained with a Milli-Q gradient IQ 7000 instrument (Millipore Corporation, Billerica, MA, USA).

Sample preparation: A volume of 300 μL of the sample was filtered using 0.45 μm PTFE filters, and 300 μL of buffer solution was added. The buffer comprised a solution of 190 μL of D_2O (containing 0.05% wt of TMS), 60 μL of phosphate buffer (pH 7) and 50 μL of pure ethanol, and the preparation was then filtered using 0.45 μm Nylon filters. D_2O was used for the lock on deuterium resonance, and ethanol was used for stabilizing the samples and buffer.

$^1\text{D}^1\text{H}$ -NMR spectra were acquired via ICON-NMR automation (Bruker Biospin GmbH). Lock, tune and shimming were performed automatically.

A modified standard Bruker pulse program was used for the multi-suppression of water and ethanol signals. Spectra were obtained at the ^1H frequency of 600.529 MHz applying a standard zgpr pulse sequence for O1 (frequency of water peak) identification and a standard noesyppps1d pulse sequence for the multi-suppression of water and ethanol peaks. The experimental parameters were as follows: temperature of 298 K, sweep width of 9615.38 Hz, recycle delay (d1) of 4 s and acquisition time of 1.7 s. For peak suppression, the width of narrow, off-resonance suppression was 2.5 Hz, and the width of broad, on-resonance suppression was 25 Hz. The spectra were acquired with 4 prior dummy scans, and 64 scans were recorded. After acquisition, spectra were processed with Topspin 4.1.3 (Bruker Biospin GmbH). Phase correction was performed automatically. The chemical shifts (δ) were referenced to the TMS resonance.

The spectral region from 11 to 5.5 ppm of the ^1H -NMR spectra was chosen as the input data for statistical analyses, thus focusing on the region of the spectra where main structural differences related to important aromatic compounds of the samples should have been visible (phenols, aldehydes, aromatic groups). AssureNMR software was used to segment the NMR spectra into rectangular buckets. The width of the buckets was user-defined and equal to 0.05 ppm for ^1H -NMR data [18]. Integration was achieved using the “sum of total intensities” mode, and the spectra were scaled to the peak of TMS, using the region between 0.07 ppm and -0.07 ppm as a reference region. The datasets were scaled with the Pareto scaling method [19] and used for principal component analysis (PCA).

2.4. NIR Analysis

NIR spectra were recorded as described by Nardi et al. [10] in transmittance mode on an MPA Bruker near-infrared spectrometer (Bruker Optik GmbH, Ettlingen, Germany) equipped with a TE-InGaAs detector; the range was $11,500\text{--}4000\text{ cm}^{-1}$ at a temperature of 30°C , using 1 mL volume and 6 mm internal pathlength clear glass vials, sealed with polyethylene snap caps. For each sample, 32 scans were recorded with a spectral resolution of 4 cm^{-1} and then averaged. A preliminary analysis of spectra using instrumental software (OPUS 6.5; Bruker Optik GmbH, Ettlingen, Germany) allowed us to identify the ranges that were useful for processing with further chemometrics analyses. The ranges of $6900\text{--}6800\text{ cm}^{-1}$ and $5500\text{--}4000\text{ cm}^{-1}$ were chosen as suitable for spirit characterisation, according to recent literature findings [14], and slightly adapted by taking into account the full-spectrum wavelength loading contribution to global variance in our dataset.

2.5. Analysis with Electronic Nose PEN-2

A commercial portable electronic nose, PEN2 (WMA Airsense Analysetechnik GmbH, Schwerin, Germany), device was used to differentiate and monitor the changes in the profile of volatile compound contents during the ageing period.

PEN2 consisted of a sampling apparatus, a detector unit containing the array of sensors and pattern recognition software for data recording. The core component of the electronic-nose system is the sensor array, which is composed of 10 different metal oxide semiconductors (MOS-type chemical sensors; Table 2). Each sensor generates a specific response to a corresponding aroma substance in the sample headspace, with the purpose of simulating the human nose.

Table 2. Description of sensors in the PEN2 electronic nose (WMA Airsense, Schwerin, Germany).

Sensor Name	Description
W1C-aromatic	Aromatic compound.
W5S-broadrange	Broad range sensitivity reacts to nitrogen oxides and ozone.
W3C-aromatic	Ammonia, used as a sensor for aromatic compounds.
W6S-hydrogen	Mainly hydrogen, selectively (breath gases).
W5C-arom-aliph	Alkanes, aromatic compounds, less polar compounds.
W1S-broad-methane	Sensitive to methane (environment), ca. 10 mg/kg.
W1W-sulphur-organic	Reacts to sulphur compounds (0.1 mg/kg H ₂ S) and terpenes.
W2S-broad-alcohol	Detects alcohol, partially aromatic compounds; broad range.
W2W-sulph-chlor	Aromatic compounds, sulphur organic compounds.
W3S-methane-aliph	Reacts to high levels >100 ppm; sometimes very selective.

From each sample, 3 mL of distillate was taken and left in special vials of 35 mL for 1 h at 30 °C to facilitate the diffusion of volatile compounds in the vial headspace. The sensor array was positioned in a small chamber with a volume of 1.8 mL. The measurement phase lasted 140 s, and data were recorded using interface unit PC software (Winmuster v.1.6 software).

During the measurement process, the headspace gas of a sample was pumped into the sensor chamber at a constant rate of 100 mL/min via Teflon tubing connected to a needle. When the gas accumulated in the vial headspace, it was pumped into the sensor chamber, and the ratio of conductance of each sensor changed. The sensor response was expressed as the ratio of conductance (G/G₀) (G and G₀, conductivity of the sensors when the sample gas or zero gas blows over).

The sample interval was 1 s. Finally, when a measurement was completed, a stand-by phase was activated (60 s) to clean the aspiration circuit and return sensors to their baseline values. Ambient air filtered through activated charcoal was used as the reference gas to clean the circuit.

2.6. Statistical Analyses

Statistical treatments were carried out using XLSTAT 19.4 biomed version software (Addinsoft, New York, NY, USA; 2016). With regards to GC-MS, the results were statistically analysed using univariate (ANOVA) and PCAs. Some analyses and related graphic representations were performed with the statistical freeware PAST 3.26 [20] program.

The statistical analyses of the NMR spectra were preliminary performed using the AssureNMR program from Bruker. Variations in the data were explored using PCAs, which were used for unsupervised pattern recognition, allowing the observation of trends and similarities between samples to be conducted. Statistical treatments of NIR data were performed with SIMCA 15.0.2 software (Umetrics–Sartorius, Sweden).

Data obtained using PEN2 E-nose were analysed using PCAs with the ggbiplot package in R-3.4.4.

3. Results and Discussion

3.1. GC-MS

3.1.1. General Aromatic Composition of the Raw Distillate

In Table 3, the main volatile compounds present in the samples derived from the distillation of fermented grape pomace are listed. As expected, the raw distillate is characterized by an abundant concentration of monoterpenols, namely, linalool, geraniol, citronellol and nerol, and their respective acetates (namely, geranyl acetate). Significant concentrations of other terpenoids were also detected, e.g., α -terpineol, p-menth-1-en-9-ol and terpinen-4-ol, and linalool oxides such as *cis*-linalool oxide and *trans*-linalool oxide. These latter compounds could originate via several transformations of monoterpenoids both during the storage of grape pomace and due to the action of yeasts starting from the monoterpenes present in the pomace during the fermentation processes and their conservation in silos before distillation and during distillation via hydrolytic reactions promoted by the high ethanolic content and high temperature [21]. Some megastigman norisoprenoids, such as β -damascenone and β -ionone, were detected in trace amounts. Other varietal compounds retrieved in Moscato grappa in previous works [22], such as sesquiterpenes, were not identified.

Table 3. Volatile compounds identified in distillates via mass spectrometry coupled to gas chromatography.

Name	IUPAC Name	Gr ¹	RT ²	LRI Lit ³	LRI Cal. ⁴	MQ% ⁵
hexyl acetate	hexyl acetate	AE	16.68	1265–1280	1273	90
benzyl acetate	benzyl acetate	AE	34.83	1697–1742	1719	93
β -phenylethyl acetate	2-phenylethyl acetate	AE	37.97	1797–1827	1810	83
isoamyl alcohol (mg/L)	3-methylbutan-1-ol	A	14.70	1198–1217	1210	90
1-pentanol	pentan-1-ol	A	15.78	1238–1256	1249	90
3-buten-1-ol-3-methyl	3-methyl-3-buten-1-ol	A	15.85	1263 [§]	1258	96
1-pentanol-4-methyl	4-methylpentan-1-ol	A	18.15	1301 [§]	1307	72
2-heptanol	heptan-2-ol	A	18.34	1304–1326	1312	83
2-penten-1-ol	pent-2-en-1-ol	A	18.47	1310–1324	1315	87
1-pentanol-3-methyl	3-methylpentan-1-ol	A	18.68	1325 [§]	1321	64
1-hexanol (mg/L)	hexan-1-ol	A	20.02	1344–1360	1341	90
<i>trans</i> -3-hexen-1-ol	(E)-hex-3-en-1-ol	A	20.30	1364–1385	1364	96
<i>cis</i> -3-hexen-1-ol	(Z)-hex-3-en-1-ol	A	21.12	1370–1390	1381	96
<i>trans</i> -2-hexen-1-ol	(E)-hex-2-en-1-ol	A	21.92	1389–1410	1401	91
<i>trans</i> -4-hexen-1-ol	(E)-hex-4-en-1-ol	A	22.10	1408 [§]	1406	74
<i>trans</i> -2-hexen-1-ol	(Z)-hex-2-en-1-ol	A	22.30	1416 [§]	1410	43
1-octen-3-ol	oct-1-en-3-ol	A	23.64	1437–1454	1443	90
<i>trans</i> -hept-4-enol	(E)-Hept-4-en-1-ol	A	25.43	1502 [§]	1488	91
<i>cis</i> -4-hepten-1-ol	(Z)-hept-4-en-1-ol	A	25.83	1527 [§]	1498	94
1-octanol	octan-1-ol	A	27.94	1544–1562	1552	91
<i>trans</i> -2-octen-1-ol	(E)-oct-2-en-1-ol	A	30.12	1620 [§]	1608	91
<i>cis</i> -2-octen-1-ol	(Z)-oct-2-en-1-ol	A	30.25	1552 [§]	1611	93
1-nonanol	nonan-1-ol	A	31.90	1649–1665	1655	91
<i>trans</i> -3-nonen-1-ol	(E)-non-3-en-1-ol	A	32.90	1688 [§]	1678	96
alpha-Cyclogeraniol	(2,6,6-trimethylcyclohex-2-en-1-yl)methanol	A	33.66	Nf	1697	98
<i>cis</i> -6-nonen-1-ol	(Z)-non-6-en-1-ol	A	34.06	1711 [§]	1709	89
decanol	decan-1-ol	A	35.71	1744–1766	1755	91
Benzyl alcohol	phenylmethanol	A	40.20	1852–1881	1870	97
2-phenylethanol	2-phenylethanol	A	41.40	1890–1920	1908	94
2,4-decadien-1-ol	deca-2,4-dien-1-ol	A	43.92	Nf	1980	72
nonanal	nonanal	AL	21.52	1385–1400	1395	96
2,4-heptadienal	hepta-2,4-dienal	AL	25.70	1497 [§]	1495	93
benzaldehyde	benzaldehyde	AL	27.07	1504–1533	1529	94
<i>trans</i> -2-decenal	(E)-dec-2-enal	AL	31.45	1630–1655	1643	97
ethyl hexanoate	ethyl hexanoate	EE	15.25	1224–1241	1234	98
ethyl lactate	ethyl 2-hydroxypropanoate	EE	19.59	1353 [§]	1350	78
ethyl 2-hydroxyisovalerate	ethyl 2-hydroxy-3-methylbutanoate	EE	22.89	1426 [§]	1422	72
ethyl octanoate	ethyl octanoate	EE	23.10	1428–1441	1436	98
ethyl decanoate	ethyl decanoate	EE	31.09	1626–1644	1636	99
ethyl benzoate	ethyl benzoate	EE	32.50	1650–1677	1664	94

Table 3. Cont.

Name	IUPAC Name	Gr ¹	RT ²	LRI Lit ³	LRI Cal. ⁴	MQ% ⁵
diethylsuccinate	diethyl butanedioate	EE	32.69	1687 [§]	1670	97
ethyl phenylacetate	ethyl 2-phenylacetate	EE	36.88	1784 [§]	1780	70
ethyl laurate	ethyl dodecanoate	EE	38.60	1826–1850	1841	99
ethyl hydrocinnamate	ethyl 3-phenylpropanoate	EE	40.39	1907	1879	96
Diethyladipate	diethyl hexanedioate	EE	40.60	1858	1887	90
ethyl isopentyl succinate	1-O-ethyl 4-O-(3-methylbutyl) butanedioate	EE	40.75	1928	1980	91
ethyl myristate	Ethyl tetradecanoate	EE	45.5	2044	2043	99
ethyl palmitate	Ethyl Hexadecanoate	EE	51.91	2250	2250	99
ethyl-9-hexadecenoate	ethyl hexadec-9-enoate	EE	52.68	2278	2274	98
linoleate	ethyl octadeca-9,12-dienoate	EE	59.65	2538	2471	94
6-methyl-5-hepten-2-one	6-methylhept-5-en-2-one	K	19.33	1329–1346	1335	96
6-methyl-3,5-heptadien-2-one	(3E)-6-methylhepta-3,5-dien-2-one	K	29.56	1602 [§]	1592	95
3-tert-butylcyclohexan-1-one	3-tert-butylcyclohexan-1-one	K	31.30	1645 [§]	1640	91
decanoic acid methyl ester	methyl decanoate	ME	29.41	1590 [§]	1590	97
methyl salicylate	methyl 2-hydroxybenzoate	ME	36.68	1745–1794	1774	94
trans- β -ocimene	(3E)-3,7-dimethylocta-1,3,6-triene	T	16.03	1244–1257	1255	95
Linalool oxide A	2-(5-ethenyl-5-methyloxolan-2-yl)propan-2-ol	T	23.53	1427–1465	1441	91
Linalool oxide B	2-(5-ethenyl-5-methyloxolan-2-yl)propan-2-ol	T	24.68	1446–1464	1469	94
linalool	3,7-dimethylocta-1,6-dien-3-ol	T	27.61	1537–1553	1541	97
terpinen-4-ol	4-methyl-1-(propan-2-yl)cyclohex-3-en-1-ol	T	29.86	1592–1611	1597	42
α -terpineol	2-[4-methylcyclohex-3-en-1-yl]propan-2-ol	T	33.51	1682–1706	1692	87
linalool oxide C	(3R,6S)-6-ethenyl-2,2,6-trimethyloxan-3-ol	T	35.04	1725–1750	1732	86
geranyl acetate	[(2E)-3,7-dimethylocta-2,6-dienyl] acetate	T	35.56	1743–1764	1749	91
citronellol	3,7-dimethyloct-6-en-1-ol	T	35.90	1756–1774	1759	98
nerol	(2Z)-3,7-dimethylocta-2,6-dien-1-ol	T	37.24	1782–1808	1794	96
geraniol	(2E)-3,7-dimethylocta-2,6-dien-1-ol	T	38.88	1830–1857	1839	94
p-menth-1-en-9-ol	2-(4-methylcyclohex-3-en-1-yl)propan-1-ol	T	42.03	1946	1935	83
3,7-dimethyl-1,5-octadien-3,7-diol	(3E)-2,6-dimethylocta-3,7-diene-2,6-diol	T	42.19	1936	1928	64
3,7-dimethyloct-1-en-3,7-diol	2,6-Dimethyl-7-octene-2,6-diol	T	43.21	1996	1949	91

¹ Gr. Chemical group: AE, acetate esters; A, alcohols; AL, aldehydes; EE, ethyl esters; K, ketones; ME, methyl esters; T, terpenes; ² RT, retention time expressed in min; ³ Linear Retention Index values retrieved from [23] (50% confidence interval of RI literature data values) or from [24] where indicated with [§]. Nf, Not found in the literature; ⁴ Linear Retention Index calculated comparing retention times of a homologous series of n-alkanes and analytes, separated with the same GC method; ⁵ Match Quality values obtained comparing mass spectra with reference mass spectra of commercial libraries.

Fermentative compounds are the main group of compounds in the distillate. Among them, higher alcohols, isoamyl alcohol and 1-hexanol and some medium-chain fatty acid esters, such as ethyl hexanoate, ethyl octanoate and ethyl decanoate, are the most abundant. Ethyl esters have low perception thresholds and pleasant fruity and floral notes that positively characterize the final product from an olfactory point of view.

3.1.2. Effect of Wood on the General Aromatic Composition of the Distillate

The statistical analysis (ANOVA) of the results obtained via GC-MS, related to the composition of varietal and fermentative compounds of the distillate, highlights how both the toasting factor (Table 4) and the format (Table 5) of the wood have a direct influence on the concentration of the main compounds present in the marc distillate.

Table 4. Effect of toasting level on the general aromatic composition of the distillate. All data are expressed in µg/L except where specifically indicated. Different letters within each row denote a significant difference between the wines at $p < 0.05$ (Tukey's post hoc test). *, **, *** and ns: differences significant at $p < 0.05$, 0.01, 0.001 and not significant, respectively. The main groups of compounds analyzed are highlighted in bold.

Name	Control	Heavy	Light	NT	Sig.
Acetated esters					
hexyl acetate	1006 a	331 b	255 b	285 b	***
benzyl acetate	228	178	151	178	ns
β-phenylethyl acetate	901 a	733 a	504 b	598 ab	*
Alcohols					
isoamyl alcohol (mg/L)	72.41	77.90	78.25	75.51	ns
1-pentanol	1761	1779	1755	1588	ns
3-buten-1-ol-3-methyl	430	575	1054	626	ns
1-pentanol-4-methyl	117	125	128	117	ns
2-heptanol	173	283	386	263	ns
2-penten-1-ol	587	678	852	743	ns
1-pentanol-3-methyl	138	78	116	102	ns
1-hexanol (mg/L)	26.00	28.34	27.41	26.51	ns
trans-3-hexen-1-ol	923	1111	1017	1015	ns
cis-3-hexen-1-ol	1721	1799	1596	1698	ns
trans-2-hexen-1-ol	1032	1053	1037	965	ns
trans-4-hexen-1-ol	135	148	152	145	ns
trans-2-hexen-1-ol	502	490	448	440	ns
1-octen-3-ol	772	913	815	1559	ns
trans-hept-4-enol	145	154	145	145	ns
cis-4-hepten-1-ol	251	250	241	234	ns
1-octanol	986	1078	997	957	ns
trans-2-octen-1-ol	97	99	91	86	ns
cis-2-octen-1-ol	327	347	324	309	ns
1-nonanol	1281 ab	1388 a	1160 b	1135 b	*
trans-3-nonen-1-ol	185	177	160	157	ns
2-cyclohexene-1-methanol, 2,6,6-trimethyl	650	683	635	613	ns
cis-6-nonen-1-ol	105 ab	126 a	104 ab	99 b	*
decanol	753 ab	830 a	658 b	635 b	**
Benzyl alcohol	259	312	326	290	ns
2-phenylethanol	2382	2949	2966	2748	ns
2,4-decadien-1-ol	46 ab	48 a	24 b	30 b	**
Aldehydes					
nonanal	656 a	74 b	124 b	129 b	**
2,4-heptadienal	176 ab	177 a	129 b	125 b	***
benzaldehyde	3583 a	2475 b	2022 b	2168 b	***
trans-2-decenal	91 a	43 b	32 b	32 b	***
Ethyl esters					
ethyl hexanoate	3212 a	1197 b	992 b	1035 b	***
ethyl lactate	3457	5453	5184	6000	ns
ethyl 2-hydroxyisovalerate	502	549	501	541	ns
ethyl octanoate	4864 a	2184 b	1474 b	1582 b	**
ethyl decanoate	7334 a	3904 ab	2206 b	2248 b	**
ethyl benzoate	147	109	97	93	ns
diethylsuccinate	3654	3916	3458	3684	ns
ethyl phenylacetate	528	514	396	442	ns
ethyl laurate	5278 a	2103 b	1150 b	989 b	***
ethyl hydrocinnamate	138 a	117 a	90 b	95 b	**
diethyladipate	47	17	55	70	ns
ethyl isopentyl succinate	190	179	154	166	ns
ethyl myristate	2747 a	1134 b	655 b	592 b	***
ethyl palmitate	7363 a	3952 b	1886 c	2245 c	***
ethyl-9-hexadecenoate	836	611	347	372	ns
linoleate	1318 a	652 ab	324 b	377 b	**
Ketones					
6-methyl-5-hepten-2-one	285	230	249	196	ns
6-methyl-3,5-heptadien-2-one	150	164	159	134	ns
cyclohexanone, 4-(1,1-dimethylethyl)	177	197	168	170	ns
Methyl esters					
decanoic acid methyl ester	175 a	56 b	64 b	45 b	**
methyl salicylate	678 a	545 ab	441 b	445 b	**

Table 4. Cont.

Name	Control	Heavy	Light	NT	Sig.
Terpenes					
<i>trans</i> - β -ocimene	3	104	97	200	ns
<i>cis</i> -linalool oxide (furanoid)	6922	7711	6226	7033	ns
<i>trans</i> -linalool oxide (furanoid)	4677	5625	5433	5411	ns
linalool	9134 ab	9424 a	8219 b	8653 b	*
terpinen-4-ol	492 a	384 ab	297 b	310 ab	*
α -terpineol	4078	4778	5075	4581	ns
linalool oxide (pyranoid) trans	422	478	506	492	ns
geranyl acetate	253 a	163 ab	112 b	115 b	**
citronellol	2151	2353	2125	2110	ns
nerol	3226 ab	3420 a	2435 b	2937 ab	***
geraniol	2549 c	4237 a	3331 bc	3644 b	***
p-menth-1-en-9-ol	53	48	41	38	ns
3,7-dimethyl-1,5-octadien-3,7-diol	105 ab	144 a	100 b	93 b	***
3,7-dimethyloct-1-en-3,7-diol	120	121	462	146	ns

Table 5. Effect of wood format on the general aromatic composition of the distillate. All data are expressed in $\mu\text{g/L}$ except where expressly indicated. Different letters within each row denote significant difference between the wines at $p < 0.05$ (Tukey's post hoc test). *, **, *** and ns: differences significant at $p < 0.05$, 0.01, 0.001 and not significant, respectively. The main groups of compounds analyzed are highlighted in bold.

Name	Control	Peeled	Sliced	Tablets	Sig.
Acetated esters					
hexyl acetate	1006 a	201 c	496 b	334 bc	***
benzyl acetate	228	174	182	149	ns
β -phenylethyl acetate	902 a	679 a	678 a	402 b	***
Alcohols					
isoamyl alcohol (mg/L)	72.41	75.06	78.36	81.48	ns
1-pentanol	1761	1758	1681	1582	ns
3-buten-1-ol-3-methyl	430	878	503	562	ns
1-pentanol-4-methyl	116	122	122	127	ns
2-heptanol	173	316	280	302	ns
2-penten-1-ol	587 ab	915 a	583 b	481 b	***
1-pentanol-3-methyl	138	92	113	95	ns
1-hexanol (mg/L)	26,045	27,306	26,871	28,264	ns
trans-3-hexen-1-ol	924	1114	964	964	ns
cis-3-hexen-1-ol	1722	1824	1664	1428	ns
trans-2-hexen-1-ol	1033	1073	964	918	ns
trans-4-hexen-1-ol	135	156	140	135	ns
trans-2-hexen-1-ol	502	476	435	442	ns
1-octen-3-ol	772	839	2117	852	ns
trans-hept-4-enol	146	154	143	137	ns
cis-4-hepten-1-ol	251 ab	257 a	229 ab	216 b	*
1-octanol	986	1033	983	983	ns
trans-2-octen-1-ol	97	94	93	85	ns
cis-2-octen-1-ol	328	343	313	297	ns
1-nonanol	1281	1221	1259	1235	ns
trans-3-nonen-1-ol	186 a	177 a	162 ab	136 b	***
2-cyclohexene-1-methanol, 2,6,6-trimethyl	650	660	628	621	ns
cis-6-nonen-1-ol	105	110	114	105	ns
decanol	753	692	739	735	ns
Benzyl alcohol	260	335	269	276	ns
2-phenylethanol	2382	3095	2488	2709	ns
2,4-decadien-1-ol	47	37	37	27	ns

Table 5. Cont.

Name	Control	Peeled	Sliced	Tablets	Sig.
Aldehydes					
nonanal	656 a	99 b	85 b	157 b	**
2,4-heptadienal	176	140	166	135	ns
benzaldehyde	3584 a	2046 c	2279 c	2702 b	***
trans-2-decenal	91 a	29 b	46 b	44 b	***
Ethyl esters					
ethyl hexanoate	3212 a	622 c	1636 b	1750 b	***
ethyl lactate	3457	6276	5309	3959	**
ethyl 2-hydroxyisovalerate	502 ab	582 a	496 b	437 b	***
ethyl octanoate	4864 a	1090 c	2644 b	2689 b	***
ethyl decanoate	7335 a	1693 c	4344 b	4335 b	***
ethyl benzoate	148 a	89 b	107 ab	123 a	**
diethylsuccinate	3655 ab	4116 a	3421b	2882 b	***
ethyl phenylacetate	529	475	439	417	ns
ethyl laurate	5278 a	796 c	2315 b	2251 b	***
ethyl hydrocinnamate	138 a	106 ab	103 ab	89 b	*
Diethyladipate	47 ab	21 b	115 a	47 ab	*
ethyl isopentyl succinate	190 a	188 a	147 ab	133 b	***
ethyl myristate	2747 a	465 c	1233 b	1278 b	***
ethyl palmitate	7364 a	1971 c	3585 bc	4002 b	***
ethyl-9-hexadecenoate	836	251	754	677	**
linoleate	1318 a	283 b	672 a	720 a	***
Ketones					
6-methyl-5-hepten-2-one	285	201	231	275	ns
6-methyl-3,5-heptadien-2-one	150	155	159	136	ns
cyclohexanone, 4-(1,1-dimethylethyl)	177	183	183	165	ns
Methyl esters					
decanoic acid methyl ester	175 a	38 b	85 b	67 b	***
methyl salicilate	679 a	449 b	542 ab	499 b	*
Terpenes					
trans-b-ocimene	169	161	105	102	ns
cis-linalool oxide (furanoid)	6922 ab	8117 a	5989 ab	5239 b	*
trans-linalool oxide (furanoid)	4677	5733	5054	5294	ns
linalool	9134	8846	8955	8542	ns
terpinen-4-ol	493	278	390	422	***
a-terpineol	4078	5010	4241	4764	ns
linalool oxide (pyranoid) trans	422	526	439	449	ns
geranyl acetate	253 a	128 b	172 ab	101 b	*
citronellol	2151	2300	2053	2088	ns
nerol	3226	3067	3012	2650	ns
geraniol	2549 b	3908 a	3680 ab	3474 ab	*
p-menth-1-en-9-ol	53	42	41	48	ns
3,7-dimethyl-1,5-octadien-3,7-diol	105	114	115	111	ns
3,7-dimethyloct-1-en-3,7-diol	121	262	88	276	ns

Lightly toasted or untoasted wood significantly reduced the concentration of esters, both the ethyl esters of medium-chain fatty acids and the acetate of higher alcohols. Namely, in the case of ethyl hexanoate, the difference between the control thesis and the aged sample with lightly toasted wood was about −69%. Similar variations were also noted for ethyl octanoate (−69%) and ethyl decanoate (−43%) as well as long-chain fatty acid esters (ethyl myristate and ethyl palmitate) (Table 4). The content of acetate esters of higher alcohols underwent an even greater variation when comparing the control thesis and the theses treated with untoasted wood, up to 75% in the case of hexyl acetate. The concentrations of 2,4-heptadienal, benzaldehyde, trans-2-decenal, cis-6-nonen-1-ol, decanol and some terpenes, including linalool and nerol, exhibited a similar, even if not as pronounced, behaviour.

It is interesting to note that the control sample showed some similarities with the samples aged using highly toasted woods. These similarities mainly regarded the fermentative

compounds. In other words, the sorption effect was weaker for heavily toasted wood. Some studies carried out on the effects that wood may have on the aromatic component of wine have highlighted how lignin and hemicellulose are involved in the sorption of aromatic compounds through various types of interactions, both hydrophobic [25] and of the acid–base type [26]. During toasting, the degradation of lignin occurs with the release sinapaldehyde, syringaldehyde, coniferaldehyde and vanillin; these reactions are proportional to the intensity of the applied heat [3]. Consequently, it is plausible that toasting may change the sorbent capacity of the wood, making the sorption of organic volatiles less intense for more toasted products [26].

In Figure 1, the results of a principal component analysis of the samples based on the sum of the fermentative and varietal compounds derived from the marcs are reported (see Table 3).

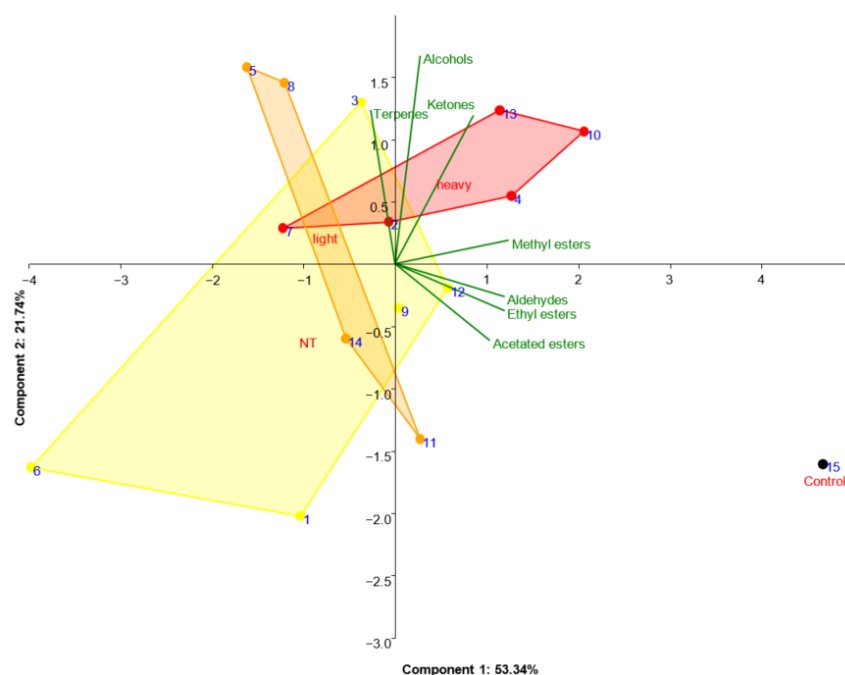


Figure 1. PCA biplot representing spirits aged with different alternative products. The graph highlights the effect of toasting on the spirit composition on volatile compounds grouped by chemical structure. Yellow, NT, non-toasted wood; red, heavy toasting; orange, light toasting.

The first component (PC1) explained 52.93% of the variance, and it was strongly positive correlated (Pearson's $R \geq 0.7$) with methyl esters, benzenoids, ethyl esters, aldehydes and acetated esters. The second component explained 18.72% (PC2) of the variance, and it was strongly positive correlated with alcohols. No strong negative correlations (Pearson's $R \leq -0.7$) were observed between the first two principal components and the original variables.

From this PCA, we could recognize some differences between the test and the treated samples. The test sample was the richest in aldehydes, acetated esters, ethyl esters, benzenoids and methyl esters and poor in alcohols.

Samples 13, 10, 4, 2 (heavy toasting level) and 12 (not toasted) showed a medium–high concentration in compounds strongly correlated with PC1 together with a medium–high concentration in alcohols.

Samples 5, 3, 8 (light or not toasted) and 7 presented a medium–low presence in compounds highly correlated with PC1 with a medium–high presence of alcohols.

Samples 14, 6, 9 and 11 (all light or not toasted) displayed a medium–low concentration of compounds related to the PC1 with a medium–low concentration of alcohols.

Sample 1 (control) had the lowest concentrations in alcohols, in contrast with all the samples treated with wood pieces that were high-toasted, which showed a greater concentration of alcohols.

From these results, it is possible to hypothesize an absorbing effect of wood on some important fermentative compounds belonging to the family of methyl esters, benzenoids, ethyl esters, aldehydes and acetated esters. No clear differentiations were observed for poplar and oak, nor among the different formats of woods.

3.1.3. Analysis of Xilovolatiles with a High Olfactory Impact

Considering the quantitative analysis of the main wood-derived compounds present in the distillate, several statistically significant differences were highlighted among the samples. In this case, only samples treated with oak wood were considered.

The most relevant results are shown in Tables 6 and 7. As previously reported, the heating of wood causes the thermodegradation of lignin and the formation of numerous aromatic compounds, including aromatic hydrocarbons, phenols and aromatic aldehydes [27]. Generally, low/medium levels of heating during cooperage lead to the formation of cinnamic and benzoic aldehydes, such as vanillin and syringaldehyde [16]. In our case, very high concentrations of vanillin, ethyl vanillate and syringaldehyde were obtained using the highest temperatures (Table 6). Is worth to note that the effect of toasting on the level of whisky lactone in spirits was weak, even if a greater concentration of this compound was noted in the untoasted theses.

Table 6. The values represent the means measured for each toasting level \pm standard error (SE). All data are expressed in $\mu\text{g/L}$ except where expressly indicated. Different letters within each row denote significant difference between the wines at $p < 0.05$ (Tukey's post hoc test). *, **, *** and ns: differences significant at $p < 0.05$, 0.01, 0.001 and not significant, respectively. The results refer only to the test samples treated with oak wood.

Compound	NT	Light	Heavy	Sig.
o-Guaiacol	24 \pm 2 a	25 \pm 1 a	28 \pm 1 a	ns
Methylguaiacol	2 \pm 0 b	2 \pm 0 b	33 \pm 16 a	***
Vinylguaiacol	24 \pm 2 b	22 \pm 1 b	34 \pm 2 a	***
Eugenol	102 \pm 89 a	96 \pm 57 ab	72 \pm 16 b	*
Vanillin	660 \pm 118 b	1140 \pm 276 b	7534 \pm 1686 a	***
Syringaldehyde	839 \pm 169 b	2640 \pm 947 b	8624 \pm 2091 a	***
Metoxyeugenol	11 \pm 2	16 \pm 2	15 \pm 3	ns
Phenol	26 \pm 3	23 \pm 1	22 \pm 1	ns
o-Cresol	14 \pm 1 b	14 \pm 0 b	16 \pm 1 a	**
p-Cresol	6 \pm 1	5 \pm 1	5 \pm 1	ns
cis-whisky lactone	97 \pm 63 a	56 \pm 34 b	64 \pm 41 ab	*
trans-whisky lactone	37 \pm 23 a	22 \pm 13 b	27 \pm 16 ab	*
Ethyl vanillate	50 \pm 5 b	68 \pm 11 b	384 \pm 55 a	***
Acetovanillone	23 \pm 7 b	30 \pm 7 b	179 \pm 40 a	***
Propiovanillone	63 \pm 19	60 \pm 15	84 \pm 12	ns

The use of different wood formats influenced the accumulation of xilovolatiles. With regards to the differences among wood assortments, the distillates refined with tablets were characterized by a higher concentration of benzoic aldehydes and, in particular, vanillin. Moreover, Table 7 shows how theses refined with sliced wood had a high content of both whisky lactone (boisé, coconuts) and eugenol (spiced, cloves), two compounds with a primary role in defining the aromatic profile of distillates.

Table 7. The values represent the means measured for each wood format \pm standard error (SE). All data are expressed in $\mu\text{g/L}$ except where expressly indicated. Different letters within each row denote significant difference between the wines at $p < 0.05$ (Tukey's post hoc test). *, **, *** and ns: differences significant at $p < 0.05$, 0.01, 0.001 and not significant, respectively. The results refer only to the test samples treated with oak wood.

Compound	Peeled Wood	Sliced Wood	Tablets	Sig.
o-Guaiacol	26 \pm 1	27 \pm 1	25 \pm 1	ns
Methylguaiacol	3 \pm 0 b	8 \pm 3 b	36 \pm 19 a	***
Vinylguaiacol	28 \pm 3	26 \pm 2	25 \pm 1	ns
Eugenol	57 \pm 3 b	173 \pm 26 a	72 \pm 3 b	***
Vanillin	2012 \pm 395 c	3467 \pm 1484 b	4956 \pm 2527 a	***
Syringaldehyde	3204 \pm 636 b	3778 \pm 1533 ab	5953 \pm 3100 a	*
Metoxyeugenol	12 \pm 2	14 \pm 1	17 \pm 3	ns
Phenol	26 \pm 2	21 \pm 1	21 \pm 1	ns
o-Cresol	15 \pm 1	14 \pm 1	14 \pm 0	ns
p-Cresol	6 \pm 1	4 \pm 1	6 \pm 1	ns
cis-whisky lactone	3 \pm 0 b	281 \pm 33 a	3 \pm 0 b	***
trans-whisky lactone	3 \pm 0 b	108 \pm 11 a	2 \pm 0 b	***
Ethyl vanillate	130 \pm 29 b	192 \pm 76 ab	217 \pm 96 a	**
Acetovanillone	51 \pm 9 b	93 \pm 38 a	114 \pm 58 a	***
Propiovanillone	70 \pm 7 b	112 \pm 16 a	21 \pm 9 c	***

Subsequently, a PCA considering only xylovolatile compounds was carried out with the aim of highlighting compositive differences among the samples depending on the treatments. The analysis was performed on the whole dataset. Cumulatively, the first two main components explain about 61.6% of the dataset variance. In Figure 2, it is possible to identify two main groups of samples. All the distillates aged with heavily toasted woods are grouped on the right, due to their high concentrations of vanillin and related benzoic aldehydes. On the other hand, distillates with a low toasting level or aged with untoasted woods are placed very close on the left side of the score plot. These samples were characterized by the low concentration of aromatic compounds deriving from the degradation of the wood biopolymers that occurred during the toasting phases. This limited compositional complexity explained their proximity to the control sample, as highlighted in Figure 2.

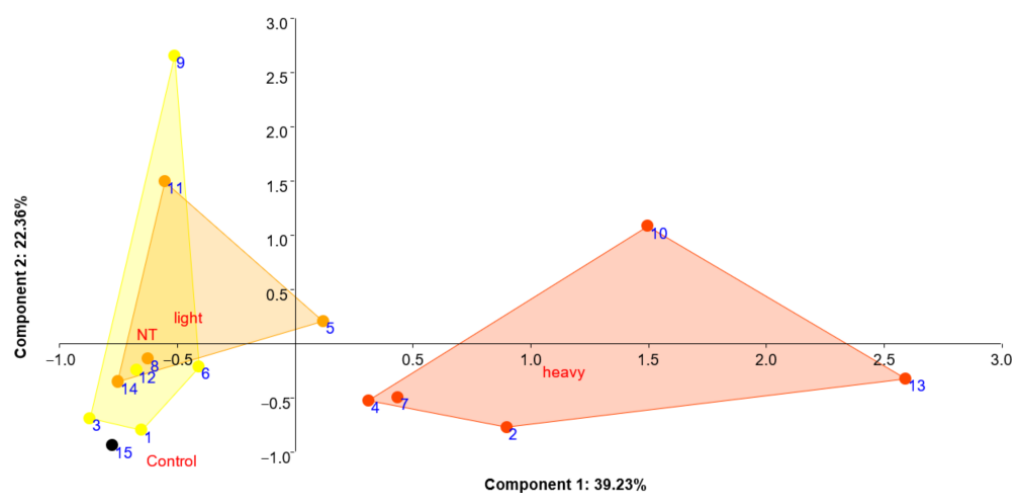


Figure 2. PCA score plot representing spirits aged with different alternative products. Results of GC-MS analysis for xylovolatile compounds. The graph highlights the effect of toasting on spirit composition. Yellow, NT, non-toasted wood; red, heavy toasting; orange, light toasting.

Moreover, PC2 discriminated among wood assortments. The samples refined with sliced wood (samples 9, 10 and 11) were clearly separated along Component 2. This distinction could be easily explained by their high content of whisky lactone (see Table 7).

Finally, from a compositional point of view, there were no statistically significant differences between these stored at room temperature and those stored in a climatic chamber.

3.1.4. Comparing Oak and Poplar

To assess the effect of the botanical origin of wood on the aromatic profile of the distillate, four samples were compared: two refined with peeled poplar wood and two obtained by ageing with peeled oak wood. Each pair of samples was tested at two toasting levels (not toasted and heavy toasting). The data processed using the ANOVA highlighted clear differences between distillates refined with poplar and those refined with oak due to the sorption effect. These differences mainly concerned the concentration of the ethyl esters of the fatty acids contained in the distillate. It is notable how the concentrations of hexyl acetate ($p < 0.01$), ethyl octanoate ($p < 0.001$), ethyldecanoate ($p < 0.001$), ethyl laurate ($p < 0.001$), ethyl myristate ($p < 0.001$), terpinene and acid ethyl ester ($p < 0.05$) were significantly higher in the distillates refined with oak wood. Furthermore, some compounds with an isoprenoidic structure, such as *t*- β -ocimene and 3,7-dimethyl-1,5-octadien-3,7-diol and terpinene-4-ol, were also more concentrated in the same samples. As reported above, the intensity of the sorption effect depended on the level of toasting. The interactions between toasting and the botanical origin of the wood showed how this effect was also strongly conditioned by the type of wood. In the case of oak, toasting strongly limited the intensity of the absorption phenomenon, while in the case of poplar, this effect was not significant (data not reported).

3.2. NMR and NIR Analysis

The same 15 samples analysed via GC-MS were analysed using NMR and NIR spectroscopy. The ^1H -NMR spectra of marc distillates are shown in Figure 3. Each spectrum could be split into three regions mostly containing signals of aromatic compounds (region 1), carbohydrates (region 2) and higher alcohols (region 3).

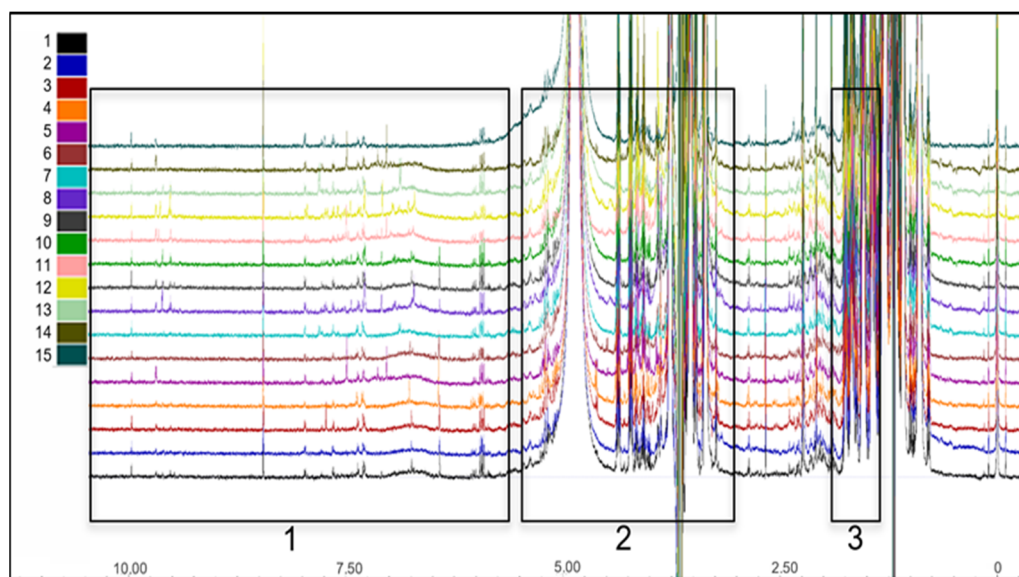


Figure 3. ^1H -NMR stacked spectra of all samples. In box 1, signals related to aromatics; in box 2, signals related to carbohydrates; in box 3, signals related to higher alcohols. For the numbering of the spectra presented in the figure, refer to Table 1.

The region where the main differences were expected, as a function of the different toasting temperatures and wood processing, was found in the aromatic-compounds section,

between 12 ppm and 5.5 ppm. Given that in regions (2) and (3), the signals were partially modified via solvent suppression, the chemometric analysis of the spectra was performed on region 1 of the spectra.

In Figure 3, the spirits refined with non-toasted wood and strongly toasted wood appeared to have similar aromatic NMR fingerprints. Moreover, distillates refined with lightly toasted wood seemed to have quite different aromatic NMR fingerprints from both the control sample and other samples. According to the quantile plot, the main differences among the different spectra were due to the signals at around 9.6 ppm, 7.5 ppm and 6.5 ppm. The assignment of these peaks to specific compounds was beyond the purpose of the study; however, the loading plots emphasized that in spirits refined with lightly toasted woods, aldehyde compounds were present that were not detected in the control samples and other samples refined with highly toasted woods. In the aromatic region, around 8 ppm to 5.5 ppm, the profiles of the samples showed different signals that were not present in the control sample.

Figure 4 shows the results of PCA analysis. The first two principal components together explained 60.61% of the total variance. The score plot showed a good separation of the samples on PC1 based on the toasting temperature. Moreover, the control sample was well distinguished from the other groups. It is noteworthy that samples refined with strongly toasted woods were well separated from lightly toasted ones. With regards to the different wood formats, the PCA analysis highlighted an only partial separation among groups, in particular, between the majority of samples refined with peeled wood and those refined with sliced wood (samples 9, 10 and 11).

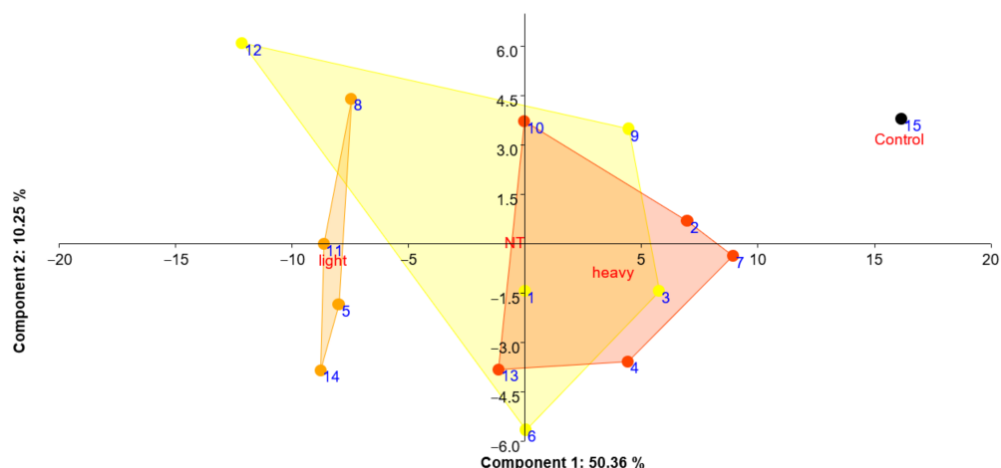


Figure 4. PCA score plot for spirits aged with different alternative products. Results of NMR analyses. The graph highlights the effect of toasting on spirit composition. Yellow, NT, non-toasted wood; red, heavy toasting; orange, light toasting.

NIR spectroscopy was also employed in this study to explore its potential to characterize the wood alternatives. Indeed, the promising performance of this technique has been recently verified for analysing both wines [10] and spirits [14] aged with oak alternatives. The results of the PCA based on the NIR spectra of 15 distillate samples showed that a certain variance was present (summarized by the two main components representing more than 50.60%) and confirmed the interest of previously selected spectral zones for application in aged spirits. Figure 5 highlights that some specific assortments of wood alternatives (e.g., wood tablets for shape) were also grouped using the NIR analysis. The method failed, however, in further characterizing the dataset, due to the low number of available samples, which was certainly sub-optimal for a chemometric approach such as NIR application. Therefore, further trials are needed to extend the potential of the technique in wider datasets, eventually taking advantage of the dedicated application of Functional Data Analysis, which has recently been shown to be useful in discriminating wine and spirit ageing technologies in wide datasets [13].

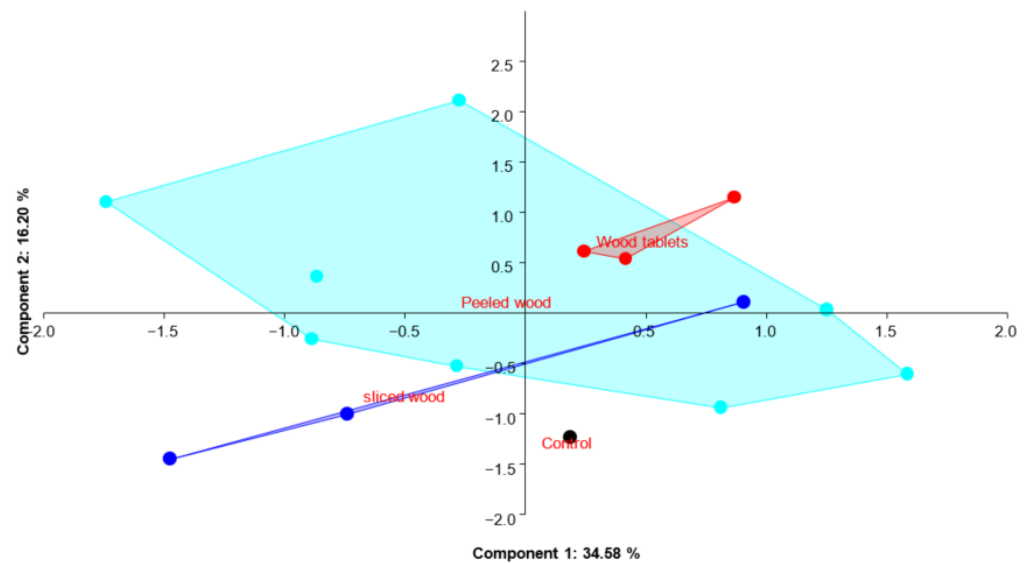


Figure 5. PCA score plot representing spirits aged with different alternative products. Results of NIR data. The graph highlights the effect of the shape of wood fragments on spirit composition. Black, control sample; light blue, peeled wood; red, wood tablets; dark blue, sliced wood.

3.3. PEN2 E-Nose Analysis

In order to highlight possible differences among all samples analysed using PEN2, a PCA was performed. Figure 6 shows the PCA biplot after 180 days of ageing.

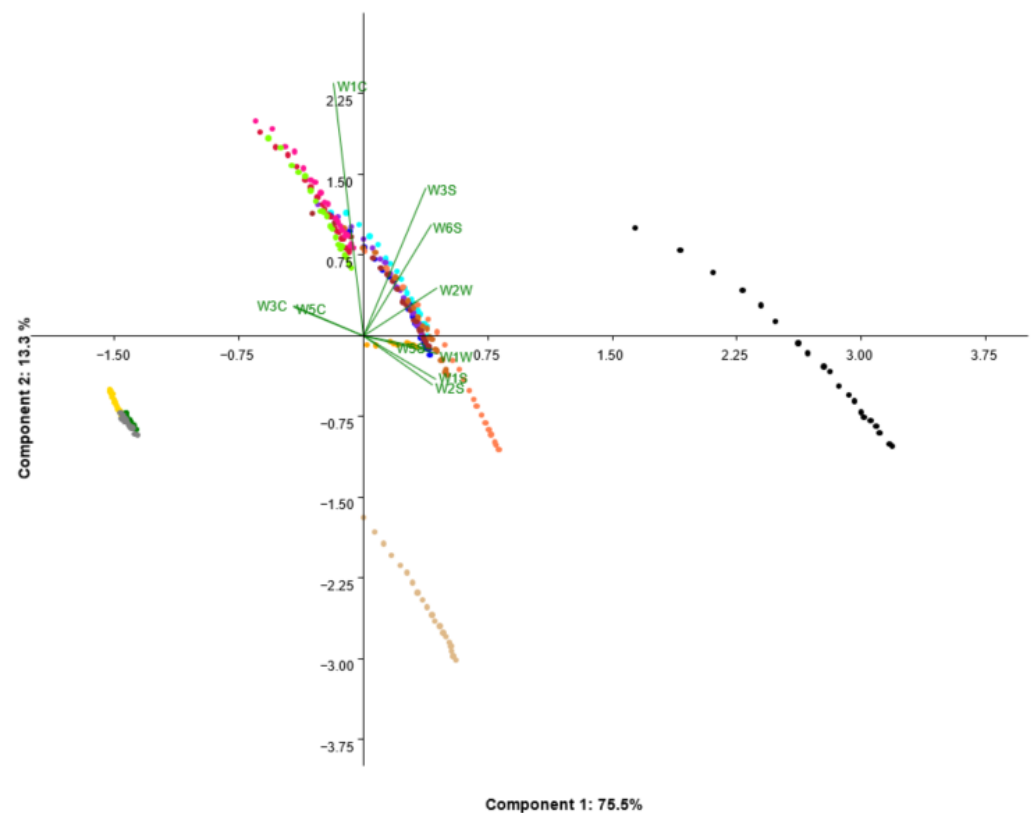


Figure 6. Biplot of PCA based on electronic-nose data for the 15 samples reported in Table 1. Loading plot represents the responses of e-nose sensors as described in Table 2. Colour samples are: 1, orange; 2, aqua; 3, blue; 4, blue-violet; 5, brown; 6, burlywood; 7, chocolate; 8, coral; 9, crimson; 10, deep pink; 11, chartreuse; 12, gold; 13, green; 14, grey; 15, black.

As shown in Figure 6, the first two principal components explained 88.8% of the total variance (75.5% for PC1 and 13.3% for PC2). It is notable that the control (black dots in Figure 6) was well distinguished from the other samples, which must have thus undergone an evident wood effect. Two other groups could be identified: the most numerous, which included all the samples (except for sample 6), and samples 12, 13 and 14 (gold, green and grey dots, respectively), which formed an isolated and very tight group instead. This latter cluster only included samples that had been refined with wood tablets (Figure 7); moreover, the level of toasting did not seem to strongly affect the instrumental response of the PEN2 sensors (Figure 8). It is noteworthy that the control sample was characterized by the highest response to electronic-nose sensors. This result was consistent with the GC-MS analyses, which highlighted the highest concentration of aroma compounds in this sample.

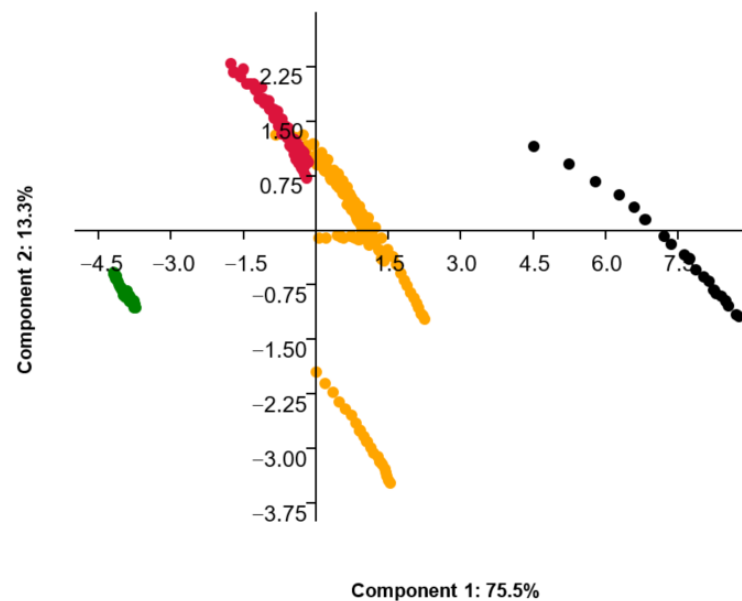


Figure 7. PCA based on electronic-nose data, clustered by wood assortments. Black dots, control sample; orange dots, peeled wood; red dots, sliced wood; green dots, wood tablets.

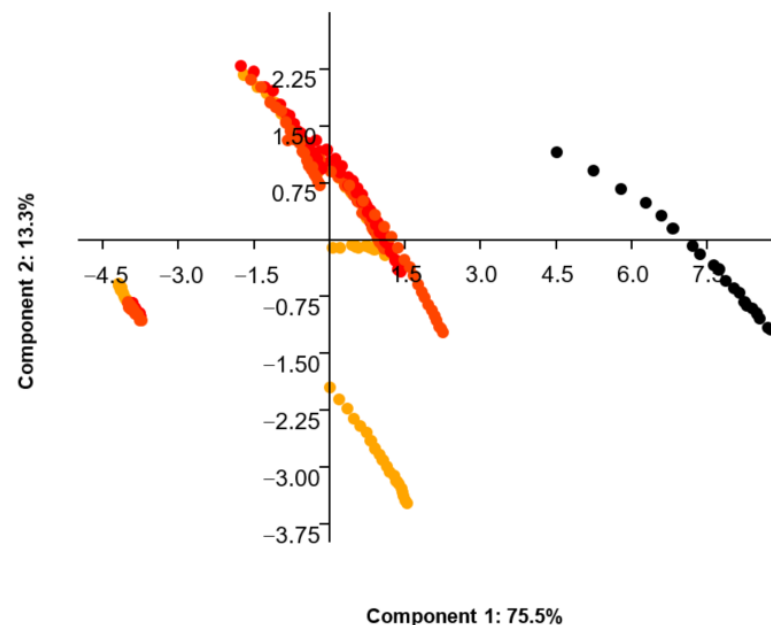


Figure 8. Biplot of PCA based on electronic-nose data clustered by toasting. Black dots, Control sample; red dots, heavy toasting; orange-red dots, light toasting; orange dots, not toasted.

4. Conclusions

These preliminary results indicated, overall, that peeled and sliced oak wood could be an interesting solution for future use in the distillate industry due to their low cost and excellent ability to release desirable aromatic compounds.

Samples aged with peeled and sliced toasted woods showed a significant increase in vanillin content, regardless of the wood assortment used. Moreover, the wood format considerably affected the concentration of *cis*-whisky lactone. Among the trials, the sliced woods, irrespective of the degree of toasting, released abundant quantities of this compound, which is found naturally in oak heartwood both in its free, non-cyclized form (3-methyl-4-hydroxyoctanoic acid) and as precursors.

Moreover, ageing distillates with alternatives to barrique products clearly showed a reduced content of esters, especially ethyl esters of medium and short fatty acids. This phenomenon seemed to be linked to the hydrophobic interactions between the distillate and wood. A greater sorbent effect was noticed using poplar wood. The intensity of these phenomena appeared to be weaker for the more toasted woods, but in the case of poplar, this interaction was negligible.

The ability of some analytical methods to discriminate the distillates refined with different woods was also highlighted in this preliminary study.

The NMR analysis seemed to be a promising tool in order to classify spirit samples based on toasting level. Even if, in the case of the wood assortment, a partial separation among groups was achieved, further research needs to be carried out to improve the results obtained.

The NIR analysis appeared to highlight similarities among the samples refined with wood tablets, distinguishing them from the other wood assortments, although its potential application needs to be further confirmed with a bigger dataset, as this technique requires a wide range of calibrations.

For the objectives defined by this work, the first preliminary results for the use of the E-nose seems encouraging both for the simplicity of sample preparation and for the portability of the method. The best performance was obtained by evaluating the differences among the various assortments of woods used.

More research aimed at integrating the use of these methods could make possible a clear differentiation of distillates refined with different technologies. This aspect could be of great importance both for product traceability and fraud control.

Author Contributions: Conceptualisation, M.P. (Maurizio Petrozziello), L.R., C.P. and P.M.C.; Data curation, M.P. (Maurizio Petrozziello); Formal analysis, M.P. (Maurizio Petrozziello), A.A., F.B., T.N., C.S., S.S., S.C. and M.P. (Matteo Pollon); Investigation, M.P. (Maurizio Petrozziello), L.R., C.P., A.A., F.B., T.N., C.S., S.S., S.C. and P.M.C.; Methodology, C.P., T.N. and M.P. (Matteo Pollon); Resources, A.M.R.; Supervision, P.M.C.; Writing—original draft, M.P. (Maurizio Petrozziello), L.R., C.P., F.B., T.N. and M.P. (Matteo Pollon); Writing—review and editing, L.R., A.A., F.B., A.M.R. and P.M.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are openly available in “figshare” at <https://doi.org/10.6084/m9.figshare.20480292.v1>.

Acknowledgments: This work was born from an idea of our colleague Gaetano Castro, who dedicated with passion his working life to wood technology and its applications. Our thanks go to him not only for the skills he shared with us but also for the passion and enthusiasm he transmitted to us.

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

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