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Influence of the early bacterial biofilms developed on vats made with seven wood types on PDO Vastedda della valle del Belìce cheese characteristics

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ABSTRACT

Early vat bacterial biofilms developed spontaneously through contact with whey have been characterized on seven wood types (*Castanea sativa* Miller, *Cedrus libani*, A. Rich., *Prunus avium* L., *Fraxinus ornus* L., *Juglans regia* L., *Pinus nigra* J.F. Arnold and *Populus nigra* L.). The present study aimed to evaluate the influence of these biofilms on the microbiological, chemical, physical and sensory characteristics of PDO Vastedda della valle del Belìce (VdB) cheese, processed traditionally from raw ewe's milk using wooden tools. To this purpose, the experimental cheeses after 15 d of refrigerated storage were examined. Lactic acid bacteria (LAB) populations dominated the microbial community of all samples. The species more frequently identified were *Lactococcus lactis* among starter LAB and *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, *Lactobacillus fermentum* and *Pediococcus pentosaceus* among non starter LAB. Culture-independent analysis of microbiota diversity was performed by MiSeq Illumina that identified *Streptococcus* as major group followed by members of *Enterobacteriaceae* family, *Lactococcus* and *Lactobacillus*. Generally, the seven tree species did not negatively affect the physico-chemical composition of VdB cheeses. Chestnut (both Sicilian and Calabrian) vats produced cheeses with significant lower hue angle (a*/b*) than other wood types. Among chemical parameters, significant variations were registered for a_w, primary and secondary lipid oxidation state (significantly lower for the VdB cheeses produced with poplar wood), and volatile organic compounds (VOCs). The significant differences detected among the VOCs emitted from cheeses were not perceived by the panelists who recognized all cheeses from the different trials as similar. This study confirmed the suitability of cedar, cherry, ash, walnut, black pine and poplar as alternative woods to chestnut for the production of the wooden vats employed in cheese making for the Sicilian traditional dairy productions.

1. Introduction

The recent re-discovery of typical products (Settanni and Moschetti, 2014) has determined an increase in the demand of traditional Sicilian cheeses (Gaglio et al., 2014a). Among these cheeses, Ragusano, Pecorino Siciliano, Piacentinu Ennese, Provola dei Nebrodi, and Vastedda della valle del Belìce (VdB) enjoy a PDO status. The traditional production protocols for the majority of cheeses produced in Sicily share the use of raw milk of indigenous breeds, the addition of artisan animal rennet and the transformation in wooden equipment (Scatassa et al., 2015). This way of processing ensures the presence of microorganisms from different sources, but the wooden vats, used for centuries to collect and transform milk by farmers and cheesemakers, represent the main reservoir of desirable dairy LAB (Cruciata et al., 2018; Di Grigoli et al., 2015; Scatassa et al., 2015).

LAB are found associated with the wooden vats because they adhere to each other and to the vat surfaces thanks the their self-produced matrix of extracellular polymeric substances (EPS) forming an aggregate of microorganisms referred to as "biofilm" (Vert et al., 2012). In order to investigate the microbial ecology of the vat biofilms, several works were carried out by Italian and French groups to study the bacterial biofilms associated with the wooden vats used in cheese making (Didienne et al., 2012; Gaglio et al., 2016; Licitra et al., 2007; Lortal et al., 2009; Scatassa et al., 2015). These investigations showed the persistence, and the dominance, of certain LAB species; in particular, the common starter LAB (SLAB) such as *Lactobacillus helveticus*, *Lactococcus lactis* and *Leuconostoc mesenteroides* that are responsible for the curd acidification, and several non-starter LAB (NSLAB) such as

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Lactobacillus plantarum and *Lactobacillus casei* that play defining roles during ripening (Settanni and Moschetti, 2010). The specific investigation of pathogenic bacteria never revealed their presence on these vats; this could be due to the ability of biofilmogenic LAB to produce antimicrobial compounds such as bacteriocins in combination with the inhibitory action exerted by the organic acids produced during fermentation (Lortal et al., 2009; Mariani et al., 2011).

Large-scale cheese productions are generally obtained using pasteurized milk transformed in stainless steel equipment (Johnson, 2017). In these conditions, the vats used for milk clotting do not host LAB and it becomes necessary to inoculate commercial starter cultures to allow and drive the acidification of curd (Goerges et al., 2008). The addition of commercial starters influences the features of the final cheeses, since LAB biodiversity associated with raw milk and wooden equipment is considered a key factor for the organoleptic features of artisanal cheeses (Gaglio et al., 2016; Scatassa et al., 2015). The studies conducted by Settanni et al. (2012) and Di Grigoli et al. (2015) on the microbiological characterization of both traditional and standard technologies applied to obtain Caciocavallo Palermitano cheese showed that applying the traditional protocol of production a clear dominance of the *Streptococcus thermophilus* strains and members of the NSLAB population of vat origin ensured cheese typicality.

A recent study carried out to valorize the Sicilian forestry resources showed the ability of LAB to adhere and survive on several wood typologies including those not traditionally employed in cheese making, indicating the suitability of local woods in traditional dairy processes (Cruciata et al., 2018). Following the previous study, this work was performed to evaluate the influence of the early vat bacterial biofilms developed on seven wood types on the final characteristics of PDO Vastedda della valle del Belìce cheese, in order to legitimate the use of local tree species for cheese production as alternative to the common chestnut wood.

2. Materials and methods

2.1. Cheese production and sample collection

Eight experimental wooden vats (15 L) were used in this study. The vats were made from *Castanea sativa* Miller grown in Calabria region (W1) and seven tree species grown in Sicily: *C. sativa* Miller (W2); *Cedrus libani*, A. Rich. (W3); *Prunus avium* L. (W4); *Fraxinus ornus* L. (W5); *Juglans regia* L. (W6); *Pinus nigra* J.F. Arnold (W7) and *Populus nigra* L. (W8). Calabrian chestnut is the most common wood species used for the production of traditional dairy equipment used in western Sicily; for this reason, W1 vat was used as control. All eight vats were used for PDO VdB cheese making, after biofilm formation as reported by Cruciata et al. (2018). VdB cheese productions were performed according to the EU Regulation n. 971 (OJC no. C 42/16 19.2.2010) from raw ewes' milk processed in wooden vats without the addition of starter cultures and curdled with animal rennet paste (Scatassa et al., 2015).

Two cheese making trials were carried out at an artisanal dairy farm ("Ovini e Natura" Società Agricola di Firpo F. & C. s.a.s., Santa Margherita Belice, Italy) belonging to the consortium for the production of PDO VdB cheese. Experimental cheeses (Ch) were obtained from 12 l of raw milk of the autochthonous breed sheep Valle del Belice with 2.6 g of lamb rennet paste (Calza Clemente s.r.l., Acquanegra Cremonese, Italy). Cheese productions were carried out in duplicate and then repeated after seven days, obtaining four cheeses per trial (two cheeses from each vat from the two distinct productions), forming a total of 32 cheeses.

Samples of bulk milk (BM), acidified curd (AC) before stretching, cheese just after stretching (V_0) and cheese after 15 d of refrigerated storage at 5 °C (V_{15}) were collected from each cheese making trial. Cheeses were sampled as follows: each cheese was cut along the vertical axis to obtain two symmetrical halves and cuneiform pieces of approximately 30 g were then cut from the core section until the rind to represent a pool of cheese regions.

2.2. Microbiological analysis and isolation LAB

The main microbial groups useful in dairy technology (Rossetti et al., 2009) and those investigated for quality, hygiene, and safety aspects were analyzed in all dairy samples collected during VdB cheese productions using the vats made from seven woods. Bulk milks were subjected to decimal serial dilutions in Ringer's solution (Oxoid), while 15 g of each solid sample (AC, ChV_0 and ChV_{15}) were first subjected to homogenization in a stomacher (BagMixer® 400, Interscience, Saint Nom, France) for 2 min at the highest speed in sodium citrate (2% w/v) solution and then serially diluted in Ringer's solution. The microbial suspensions were plated and incubated as follows: total mesophilic microorganisms (TMM) on plate count agar (PCA) supplemented with 1 g/l skimmed milk (SkM), incubated aerobically at 30 °C for 72 h; total psychrotrophic counts (TPC) on PCA-SkM, incubated aerobically at 7 °C for 7 d; mesophilic and thermophilic rod LAB on MRS agar, acidified at pH 5.4 with lactic acid (5 mol/l), incubated anaerobically for 48 h at 30 and 44 °C, respectively; mesophilic and thermophilic coccus LAB on M17 agar, incubated anaerobically for 48 h at 30 and 44 °C, respectively; enterococci on kanamycin azide aesculin agar (KAA) incubated aerobically for 24 h at 37 °C; members of the *Enterobacteriaceae* family on Violet Red Bile Glucose Agar (VRBGA) incubated aerobically for 24 h at 37 °C; coagulase-positive staphylococci (CPS), *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* were analyzed as reported by Cruciata et al. (2018). Microbiological counts were carried out in duplicate for all samples. Anaerobic conditions were obtained with the Anaeroben AN25 (Oxoid) system in hermetically sealed jars. All media were purchased from Oxoid.

LAB from refrigerated cheeses (ChV_{15}) were isolated (about five colonies per morphology) from the highest dilutions of cell suspensions plated on MRS and M17 media. All different morphologies were considered in order to evaluate the total LAB diversity. The isolates were streaked by successive subculturing, and their purity was verified by means of an optical microscope. All Gram-positive [Gregersen KOH method (Gregersen, 1978)] and catalase-negative [determined by addition of 5% (v/v) H_2O_2 to fresh colonies] bacterial cultures were considered presumptive LAB and were stored in glycerol stocks at −80 °C until further investigation.

2.3. Phenotyping grouping, strain differentiation and identification of cheese LAB

The cultures isolated from cheeses were subjected to a phenotypic characterization performed by microscopic inspection, growth at 15 and 45 °C, hydrolysis of arginine and esculin, acid production from hexose and pentose carbohydrates (arabinose, ribose, xylose, fructose, galactose, lactose and sucrose) and glycerol, and CO_2 production from glucose tested with Durham's tubes. In order to separate enterococci from other dairy LAB cocci, all cultures characterized by a coccus cell shape were also evaluate for their capacity to grow at pH 9.2 and in the presence of 6.5 g/l NaCl (Gaglio et al., 2014a).

In order to reduce the number of isolates to be processed for identification, the presumptive LAB were first differentiated at strain level. To this purpose, DNAs from broth cultures, developed overnight at the optimal temperatures in the media used for isolation, were extracted by the InstaGene Matrix kits (Bio-Rad, Hercules, CA) following the manufacturer's instructions and then used for PCR. The strain typing was performed random amplification of polymorphic DNA (RAPD)-PCR using the single primers M13, AB111 and AB106 as reported by Gaglio et al. (2017). PCR products and the GeneRuler 100 bp Plus DNA ladder (M Medical Srl, Milan, Italy) were separated by electrophoresis on 1.5% (w/v) agarose gel (Gibco BRL, Cergy Pontoise, France) and visualized by UV transillumination after staining with the SYBR® safe DNA gel stain (Molecular Probes, Eugene, OR). The comparison of RAPD

patterns was performed with GelCompar II software, version 6.5 (Applied Maths, Sint-Marten-Latem, Belgium), and the isolates with different RAPD profiles were considered to represent different strains.

The genotypic identification of the different LAB strains was carried out by amplification and sequencing of the 16S rRNA gene. PCRs were performed as described by Weisburg et al. (1991) while DNA sequencing reactions were performed as described by Cruciata et al. (2018). The identities of the sequences were determined by a blast search against the NBCI non-redundant sequence database and by comparison with the sequences of the sole type strains within the EZTaxon database (http://eztaxon-e.ezbiocloud.net/taxonomy) (Chun et al., 2007).

2.4. V3-V4 amplification and sequencing strategy

To maximize the effective length of the MiSeq's 300PE sequencing reads, the region encompassing the V3 and V4 hypervariable regions of the 16S rRNA gene (approximately 469 bp) was targeted for sequencing. Genomic DNA was extracted from cheese samples using QIAamp DNA Mini Kit and diluted to 5 ng/μl in 10 mM Tris pH 8.5 as requested by Illumina protocol 16S Metagenomic Sequencing Library Preparation, 15,044,223 Rev. B. Briefly, to amplify and sequence the V3-V4 hypervariable region of the 16S rRNA gene, primers were designed that contained overhang adapter sequences that must be appended to the primer pair sequences for compatibility with Illumina (Illumina, San Diego, CA, USA) index and sequencing adapters. Amplification of fragment was obtained following PCR condition suggested by the above mentioned Illumina protocol and the expected fragment's size was ~550 bp. In the following step, adapters and dual-index barcodes were added to the amplicon target obtaining a fragment of ~630 bp. After PCR Clean-Up step, the obtained libraries (~630 bp in length) were quantified with Agilent Bioanalyzer 2100 and QuBit 2.0 Fluorometer (Invitrogen), then normalized to 4 nM and, finally, pooled. PhiX Control library (v3) (Illumina) was combined with the amplicon library (expected at 5%). The libraries were sequenced with MiSeq Reagent Kit v3, 600 Cycles sequencing kit (MS-102-3003) on MiSeq System (Illumina).

2.5. Illumina data analysis and sequence identification by QIIME2

Sequences obtained from Illumina Sequencing were processed using QIIME2 software package version 2018.4 (Caporaso et al., 2010). Briefly, reads were assigned to each sample according to the unique index; pairs of reads from the original DNA fragments were firstly merged using an import tool implemented in QIIME2. Quality check and trimming were performed in order to trim sequences where quality score is < 20 using the DADA2 software package (Callahan et al., 2016) wrapped in QIIME2. Moreover, to remove chimeras from Illumina sequenced fastq files the "consensus" method implemented in DADA2 was used. For taxa comparisons, we used the QIIME2 q2-feature-classifier plugin and the Naïve Bayes classifier that was trained on the Greengenes13.8 99% Operational Taxonomic Units (OTUs) full-length sequences. QIIME2 taxa barplot command was used for visualization of the taxonomic composition of the samples. Alpha diversity analysis was performed with the q2-diversity plugin in QIIME2. In particular, Chao1 metric (Chao and Bunge, 2002) that is a nonparametric abundance-based estimator of species richness and observed OTUs were used to study diversity within each sample.

2.6. Chemical composition of final cheeses

Cheese samples were analyzed for dry matter (DM), fat, protein (TN × 6.38), N-soluble and ash content according to IDF standards 4A (IDF, 1982), 5B (IDF, 1986), 25 (IDF, 1964a) and 27 (IDF, 1964b), respectively. Salt content was determined by Volhard method (AOAC, 2000). Measurements of pH were performed electrometrically by the pH-meter DocuMeter Sartorius (Data Weighing Systems, Inc., Elk Grove, IL, USA). Water activity (a_w) was determined according to the ISO 21807 (2004) using the HygroPalm water activity indicator (Rotronic, Bassersdrof, Germany).

Fatty acids (FA) were determined in lyophilized cheese samples (100 mg) which were directly methylated with 2 ml of 0.5 M $NaOCH_3$ at 50 °C for 15 min, followed by 1 ml of 5% HCl in methanol at 50 °C for 15 min (Lee and Tweed, 2008). Fatty acid methyl esters (FAME) were recovered in hexane (1.5 ml). One microliter of each sample was injected by auto-sampler into an HP 6890 gas chromatography system equipped with a flame-ionization detector (Agilent Technologies Inc., Santa Clara, CA). Fatty acid methyl esters from all samples were separated using a 100-m length, 0.25-mm i.d., 0.25-μm capillary column (cp-sil 88; Chrompack, Middelburg, the Netherlands). The injector temperature was kept at 255 °C and the detector temperature at 250 °C. The carrier gas was at a flow rate of 0.7 ml/min (linear velocity of 14 cm/s). Fatty acid methyl ester hexane mix solution (Nu-Chek Prep Inc., Elysian, MN, USA) was used to identify each FA. The identification of the conjugated linoleic acid (CLA) isomers was performed using a commercial mixture of cis-9 and trans-11 and trans-10 and cis-12 octadecadienoic acid methyl esters (Sigma-Aldrich, Milano, Italy) and published isomeric profiles (Kramer et al., 2004; Luna et al., 2005).

The oxidation status of cheese fat was evaluated based on the assumption that wood antioxidants could be transferred to milk and cheeses. This parameter was determined on freeze-dried samples by determination of peroxide value (POV, mEq O_2/kg fat), as index of primary lipid oxidation (IDF, 1991). In addition, thiobarbituric acid-reactive substances (TBARs), expressed as μg malonylaldehyde (MDA)/kg DM, used as a measure of the secondary lipid oxidation products, was determined according to the method proposed by Tarladgis et al. (1960) and modified by Mele et al. (2011). Cheese extracts were prepared, according to the method of Rashidinejad et al. (2013) with slight modifications, to measure cheese antioxidant status by the determination of total phenolic compound content, measured using the Folin–Ciocalteau colorimetric method, as described by López-Andrés et al. (2014) and trolox equivalent antioxidant capacity (TEAC assay), both detected on three replicates per sample.

Volatile organic compounds (VOC) emitted from VdB cheeses were determined using the headspace solid-phase microextraction (SPME) method coupled with gas chromatography with mass spectrometric detection. The SUPELCO SPME (Bellefonte, PA) fiber holder and fiber used were coated with divinylbenzene/polydimethylsiloxane (DV/PDMS), 65 mm. Cheese samples were kept at −20 °C until analysis. Before analysis, each sample (10 g) was grated, transferred into a 35 ml vial, added with 10 ml of H_2O, 200 μl of internal standard solution (1-Heptanol, 35 mg/l in 20% ethanol aqueous solution) and 1 g of NaCl, the latter added to increase extraction rate of VOCs. Extraction temperature of head-space and time were 60 °C and 30 min, respectively. The samples were gently vortexed during extraction using a magnetic stirrer. Fiber exposition was prolonged for 30 min at 60 °C (Gaglio et al., 2014b). Thermal desorption was performed in the injector at 250 °C for 2 min into a Finnegan Trace MS for GC/MS (Agilent 6890 Series GC system, Agilent 5973 Net Work Mass Selective Detector; Milan, Italy) equipped with a DB-WAX capillary column (Agilent Technologies; 30 m. 0.250 mm i.d. film thickness 0.25 μm). The GC–MS system and chromatographic conditions described by Corona (2010) and Sannino et al. (2013) were used for analysis. Mass spectra were recorded by electronic impact at 70 eV using the ion source temperature of 200 °C. All compounds of *m/z* 33–495 atomic mass units (amu) were detected with this scan mode. Individual peaks were identified by comparing their retention indices and their mass spectra to those within the NIST/EPA/NIH Mass Spectral Library database (Version 2.0d. build 2005). Volatile compounds were expressed as μg/kg. All solvents and reagents were purchased from WWR International (Milan, Italy). All analyses were performed in triplicate.

Table 1
Microbial evolution[a] during experimental VdB cheese production carried out in vats made of seven woods.

Samples	Bacterial counts									
	TMM	TPC	*Enterobacteriaceae*	CPS	*E. coli*	Mesophilic rod LAB	Thermophilic rod LAB	Mesophilic coccus LAB	Thermophilic coccus LAB	Enterococci
BM	6.99 ± 1.15	4.57 ± 0.00	4.52 ± 0.58	3.25 ± 0.24	3.18 ± 1.27	6.10 ± 0.79	6.16 ± 0.06	5.52 ± 0.04	3.84 ± 0.08	4.68 ± 0.71
W1 AC	7.76 ± 0.31 A	4.59 ± 0.26 A	5.98 ± 0.09 B	4.92 ± 0.13 AB	5.70 ± 0.16 A	8.85 ± 0.42 A	7.35 ± 0.21 A	9.06 ± 0.27 A	8.33 ± 0.63 A	6.07 ± 0.20 A
W2 AC	7.65 ± 0.30 AB	3.62 ± 0.31 A	5.94 ± 0.14 B	5.20 ± 0.18 A	5.65 ± 0.31 A	8.97 ± 0.25 A	7.96 ± 0.27 A	7.89 ± 0.26 B	8.28 ± 0.69 A	5.86 ± 0.19 A
W3 AC	7.44 ± 0.14 AB	4.06 ± 0.08 A	6.83 ± 0.14 A	5.03 ± 0.12 AB	5.71 ± 0.26 A	8.79 ± 0.40 A	7.85 ± 0.08 A	8.98 ± 0.35 A	8.42 ± 0.66 A	6.51 ± 0.30 A
W4 AC	7.67 ± 0.07 AB	3.46 ± 0.48 A	5.98 ± 0.14 B	4.61 ± 0.19 AB	5.78 ± 0.17 A	7.66 ± 0.51 A	8.30 ± 0.43 A	9.16 ± 0.23 A	8.31 ± 0.54 A	5.97 ± 0.36 A
W5 AC	7.22 ± 0.05 B	3.53 ± 0.24 A	6.15 ± 0.08 B	4.09 ± 0.17 B	5.78 ± 0.23 A	8.43 ± 0.59 A	8.41 ± 0.42 A	8.98 ± 0.24 A	8.33 ± 0.65 A	6.72 ± 0.28 A
W6 AC	7.89 ± 0.03 A	3.86 ± 1.06 A	6.15 ± 0.11 B	4.86 ± 0.76 AB	4.70 ± 0.16 B	8.76 ± 0.40 A	8.09 ± 0.51 A	8.86 ± 0.20 AB	8.18 ± 0.85 A	6.31 ± 0.65 A
W7 AC	7.87 ± 0.18 A	3.80 ± 1.26 A	6.10 ± 0.02 B	4.44 ± 0.38 AB	4.81 ± 0.20 AB	8.85 ± 0.72 A	8.13 ± 0.55 A	9.01 ± 0.71 A	8.16 ± 1.16 A	6.24 ± 0.49 A
W8 AC	7.86 ± 0.12 A	4.65 ± 0.18 A	6.24 ± 0.29 AB	4.66 ± 0.28 AB	4.51 ± 0.05 B	8.81 ± 0.39 A	8.55 ± 0.08 A	8.84 ± 0.23 AB	7.56 ± 1.75 A	6.28 ± 0.24 A
SEM	0.08	0.16	0.23	0.13	0.19	0.15	0.13	0.14	0.10	0.10
Wooden vat	*	ns	***	*	***	ns	ns	**	ns	ns
Cheese making	**	ns	**	**	*	ns	ns	*	ns	ns
W1 ChV_0	7.06 ± 0.77 A	2.45 ± 0.63 B	2.38 ± 0.43 A	1.31 ± 0.85 A	2.07 ± 0.67 AB	8.25 ± 1.26 AB	7.28 ± 0.11 A	8.67 ± 0.83 A	8.43 ± 0.49 A	5.49 ± 0.30 A
W2 ChV_0	6.66 ± 0.33 A	2.11 ± 0.05 B	2.92 ± 0.40 A	1.98 ± 0.39 A	2.15 ± 1.20 AB	7.74 ± 0.21 AB	8.13 ± 0.74 A	8.65 ± 0.49 A	8.56 ± 0.39 A	5.61 ± 0.30 A
W3 ChV_0	6.73 ± 0.09 A	2.32 ± 0.66 B	2.42 ± 0.44 A	1.30 ± 0.84 A	2.08 ± 0.34 AB	7.78 ± 0.31 AB	8.23 ± 0.65 A	8.57 ± 0.38 A	8.49 ± 0.37 A	5.69 ± 0.03 A
W4 ChV_0	7.04 ± 0.51 A	2.28 ± 0.11 B	3.00 ± 0.05 A	2.49 ± 0.37 A	2.87 ± 0.04 A	6.77 ± 0.06 B	8.33 ± 0.50 A	8.71 ± 0.26 A	8.57 ± 0.44 A	6.24 ± 0.51 A
W5 ChV_0	7.15 ± 0.11 A	2.89 ± 0.16 AB	2.61 ± 0.23 A	2.06 ± 0.31 A	2.39 ± 0.20 AB	7.81 ± 0.16 A	8.23 ± 0.63 A	8.60 ± 0.33 A	8.55 ± 0.53 A	6.15 ± 0.49 A
W6 ChV_0	6.82 ± 0.16 A	3.53 ± 0.58 A	1.79 ± 1.12 A	2.85 ± 0.91 A	1.06 ± 0.49 B	7.97 ± 0.05 A	8.26 ± 0.63 A	8.64 ± 0.40 A	8.61 ± 0.41 A	5.73 ± 0.17 A
W7 ChV_0	7.38 ± 0.65 A	3.03 ± 0.41 AB	1.84 ± 1.18 A	2.09 ± 0.12 A	1.24 ± 0.75 AB	7.89 ± 0.08 A	8.34 ± 0.99 A	8.21 ± 1.03 A	8.59 ± 0.47 A	5.71 ± 0.29 A
W8 ChV_0	6.78 ± 0.13 A	3.28 ± 0.47 AB	2.71 ± 0.20 A	2.67 ± 0.09 A	2.36 ± 0.35 AB	7.64 ± 0.21 AB	8.32 ± 0.66 A	8.70 ± 0.48 A	8.67 ± 0.46 A	5.35 ± 0.14 A
SEM	0.09	0.18	0.15	0.20	0.21	0.15	0.13	0.06	0.03	0.11
Wooden vat	ns	*	ns	ns	*	**	ns	ns	ns	ns
Cheese making	ns	**	ns	*	**	*	ns	ns	ns	ns
W1 ChV_{15}	6.82 ± 0.35 A	2.64 ± 0.48 A	1.42 ± 0.60 A	< 1 B	< 1 B	7.66 ± 0.39 B	8.22 ± 0.57 A	8.60 ± 0.56 A	8.63 ± 0.20 A	5.66 ± 0.01 A
W2 ChV_{15}	6.66 ± 0.48 A	2.49 ± 0.34 A	1.62 ± 0.87 A	1.23 ± 0.74 A	1.04 ± 0.47 A	8.23 ± 0.60 AB	8.52 ± 0.74 A	8.86 ± 0.19 A	8.88 ± 0.29 A	5.63 ± 0.22 A
W3 ChV_{15}	7.15 ± 0.00 A	1.97 ± 1.37 A	2.62 ± 0.54 A	1.00 ± 0.41 A	2.56 ± 0.54 A	8.94 ± 0.54 A	8.58 ± 0.57 A	8.98 ± 0.03 A	8.91 ± 0.12 A	6.04 ± 0.33 A
W4 ChV_{15}	7.24 ± 0.29 A	3.24 ± 0.87 A	2.96 ± 0.12 A	1.28 ± 0.81 A	2.72 ± 0.35 AB	8.70 ± 0.60 AB	8.63 ± 0.53 A	9.00 ± 0.20 A	8.85 ± 0.37 A	6.14 ± 0.87 A
W5 ChV_{15}	6.54 ± 0.33 A	3.47 ± 0.86 A	2.34 ± 0.19 A	< 1 B	1.07 ± 0.52 AB	8.20 ± 0.39 AB	8.55 ± 0.57 A	8.88 ± 0.53 A	8.77 ± 0.29 A	6.27 ± 0.76 A
W6 ChV_{15}	6.83 ± 0.20 A	4.20 ± 1.49 A	1.72 ± 1.02 A	1.17 ± 0.66 A	1.07 ± 0.52 AB	8.42 ± 0.39 AB	8.49 ± 0.27 A	8.81 ± 0.56 A	8.81 ± 0.25 A	5.27 ± 0.10 A
W7 ChV_{15}	7.00 ± 0.11 A	3.30 ± 0.44 A	1.75 ± 1.06 A	< 1 B	1.12 ± 0.58 AB	8.07 ± 0.19 AB	8.61 ± 0.13 A	9.23 ± 0.02 A	8.81 ± 0.05 A	5.32 ± 0.34 A
W8 ChV_{15}	6.79 ± 0.06 A	3.83 ± 1.18 A	1.64 ± 0.90 A	< 1 B	< 1 B	8.25 ± 0.35 AB	8.30 ± 0.93 A	8.81 ± 0.25 A	8.82 ± 0.36 A	5.35 ± 0.20 A
SEM	0.08	0.26	0.20	0.22	0.36	0.14	0.05	0.06	0.03	0.14
Wooden vat	ns	ns	ns	*	*	***	ns	ns	ns	ns
Cheese making	ns	ns	ns	ns	**	*	ns	ns	ns	ns

Abbreviation: TMM, total mesophilic microorganisms; TPC, total psychrotrophic count; CPS, coagulase-positive staphylococci; BM, Bulk milk; W1 AC, acid curd produced in Calabrian Chestnut wooden vat; W1 ChV_0, Vastedda cheese produced in Calabrian Chestnut wooden vat; W1 ChV_{15}, Vastedda cheese produced in Calabrian Chestnut wooden vat after 15 days of refrigerate storage; W2 AC, acid curd produced in Sicilian Chestnut wooden vat; W2 ChV_0, Vastedda cheese produced in Sicilian Chestnut wooden vat; W2 ChV_{15}, Vastedda cheese produced in Sicilian Chestnut wooden vat after 15 days of refrigerate storage; W3 AC, acid curd produced in Cedar wooden vat; W3 ChV_0, Vastedda cheese produced in Cedar wooden vat; W3 ChV_{15}, Vastedda cheese produced in Cedar wooden vat after 15 days of refrigerate storage; W4 AC, acid curd produced in Cherry wooden vat; W4 ChV_0, Vastedda cheese produced in Cherry wooden vat; W4 ChV_{15}, Vastedda cheese produced in Cherry wooden vat after 15 days of refrigerate storage; W5 AC, acid curd produced in Ash wooden vat; W5 ChV_0, Vastedda cheese produced in Ash wooden vat; W5 ChV_{15}, Vastedda cheese produced in Ash wooden vat after 15 days of refrigerate storage, W6 AC, acid curd produced in Walnut wooden vat; W6 ChV_0, Vastedda cheese produced in Walnut wooden vat; W6 ChV_{15}, Vastedda cheese produced in Walnut wooden vat after 15 days of refrigerate storage; W7 AC, acid curd produced in Black pine wooden vat; W7 ChV_0, Vastedda cheese produced in Black pine wooden vat; W7 ChV_{15}, Vastedda cheese produced in Black pine wooden vat after 15 days of refrigerate storage; W8 AC, acid curd produced in Poplar wooden vat; W8 ChV_0, Vastedda cheese produced in Poplar wooden vat; W8 ChV_{15}, Vastedda cheese produced in Poplar wooden vat after 15 days of refrigerate storage.
SEM, standard error of means; ns, not significant.
Results indicate mean values ± standard deviation (SD) of four plate counts (carried out in duplicate for two independent productions).
[a] Units are log CFU/ml for liquid sample and log CFU/g for solid sample.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Table 2
Phenotypic grouping of the LAB isolated from VdB cheeses.

Characters	Clusters				
	I (n = 103)	II (n = 98)	III (n = 84)	IV (n = 139)	V (n = 41)
Morphology[a]	R	R	R	C	C
Cell disposition[b]	sc	sc	sc	sc	t
Growth					
15 °C	−	+	+	+	+
45 °C	+	+	+	−	+
pH 9.6	n.d.	n.d.	n.d.	+	+
6.5% NaCl	n.d.	n.d.	n.d.	−	+
Resistance to 60 °C	+	+	+	+	−
Hydrolysis of					
Arginine	+	+	+	+	+
Aesculin	+	+	+	+	+
Acid production from					
Arabinose	+	+	−	−	+
Ribose	+	+	+	+	+
Xylose	+	+	−	−	+/−
Fructose	+	+	+	+	+
Galactose	+	+	+	+	+
Lactose	+	+	+	+	+
Sucrose	+	+	+	+	−
Glycerol	+	+	+	+	+/−
CO_2 from glucose	+	−	−	−	−

Abbreviation: n.d., not determined.
[a] R, rod; C, coccus.
[b] sc, short chain; lc long chain; t, tetrads.

2.7. Cheese color determination

VdB cheese color was analyzed on the top surface by a Minolta tristimulus Chromometer CR-300 (Minolta, Osaka, Japan) using CIELAB L*a*b* values (Hunter, 1975). The measure of lightness (L* values, range 0–100) represents black to white, the redness measurement (a* values) describes green to red, and the yellowness measurement (b* values) represents blue to yellow. Beside these attributes, a* and b* values were also used to determines the parameters hue angle and chroma: hue angle (a*/b*) gives the predominant wavelength composing the color; chroma or saturation [$\sqrt{(a^2 + b^2)}$] accounts for the vividness or the color purity. The chromometer was standardized using a white standard plate. The results reported are averages of five measurements on the same cheese slice.

2.8. Sensory analysis

After 15 days of refrigerate storage (5 °C) under vacuum, VdB cheeses were also evaluated for their sensory characteristics through a panel test carried out following the ISO 13299 (2003) indications. The analysis included a total of 16 cheeses, two cheeses from the second production week from each of the eight trials. The effect of wood type on the sensory characteristics of the cheeses was evaluated by 12 trained judges (six men and six woman, from 22 to 53 years old). Cheese samples were cut into cubes (3 × 3 × 3 cm) and acclimated for at ambient temperature (about 20 °C) 1 h before being administered to the judges. For each cheese, the judges evaluated several parameters regarding the aspect (color and uniformity of structure), the smell (strength of odor, milk, butter and unpleasant smell), the taste (salty, sweet, acid, spicy and bitter taste), the consistency (soft/hard, solubility and grittiness following mastication) and the overall acceptability.

2.9. Statistical analysis

Microbiological, physico-chemical parameters analyses and volatile organic compounds were analyzed with repeated-measures linear analyses of variance (GLM procedure, SAS 9.1.2 software), which included the fixed effect Wood type. Comparisons among least-square-means was performed by *t*-test; differences were considered significant at $P < 0.05$.

The association between microbial OTUs and VOCs was measured by the Spearman's rank correlation coefficient obtained with the function “psych” and plotted through the “corrplot” package of R.

3. Results

3.1. Microbiological analysis by culture-dependent approach

The levels of the different microbial groups investigated in this study are reported in Table 1. The results for *Salmonella* spp. and *L. monocytogenes* were not reported in Table 1 because these pathogens were not detected in any sample analyzed. The levels of TMM and TPC of bulk milk were 6.99 and 4.57 log CFU/ml, respectively. During all steps of cheese making, TMM were higher than TPC. The levels of TMM in the acidified curds were in the range 7.22–7.89 log CFU/g and decreased slightly in the cheeses just after salting and after 15 d of refrigerated storage. Members of the *Enterobacteriaceae* family in bulk milk were at 4.52 log CFU/mL and increased in AC until 6.83 log CFU/g (W3 AC); lower levels were detected in cheeses (1.42–2.96 log CFU/g). An increasing trend from bulk milk to acidified curds followed by a decrease in cheeses was also observed for the levels of CPS and *E. coli*. In particular, both bacterial groups were undetectable in cheeses produced with the vats W1 and W8 after 15 d of refrigerate storage, while only CPS were below the detection limit in those processed with the vats W5 and W7.

The levels of mesophilic and thermophilic rod LAB in raw milk were comparable at about 6 log CFU/ml, while mesophilic and thermophilic cocci LAB showed lower levels. LAB populations dominated all acidified curds and reached values between 8 and 9 log CFU/g. In particular, thermophilic cocci increased more (almost 5 log cycles on average) than the other LAB groups during acidification. These levels remained almost constant in V_0 and V_{15} cheeses. A slight reduction of mesophilic rod LAB numbers was registered just after salting, but except W1 ChV_{15} all other cheeses were characterized by levels above 8 log CFU/g. Enterococci were registered at approximately 5 log CFU/ml in raw milk and increased of about 1 log cycle during cheese production. In the final

Table 3
Distribution of LAB species within VdB cheeses.

Species	W1 ChV_{15}	W2 ChV_{15}	W3 ChV_{15}	W4 ChV_{15}	W5 ChV_{15}	W6 ChV_{15}	W7 ChV_{15}	W8 ChV_{15}
Lactococcus lactis	■	■	■	■	■	■	■	■
Lactobacillus paracasaei	■	■	■	■	■	■	■	■
Lactobacillus rhamnosus		■	■	■	■	■	■	■
Lactobacillus fermentum			■	■	■	■		
Pediococcus pentosaceus	■	■						

Abbreviation: W1 ChV_{15}, Vastedda cheese produced in Calabrian Chestnut wooden vat after 15 days of refrigerate storage; W2 ChV_{15}, Vastedda cheese produced in Sicilian Chestnut wooden vat after 15 days of refrigerate storage; W3 ChV_{15}, Vastedda cheese produced in Cedar wooden vat after 15 days of refrigerate storage; W4 ChV_{15}, Vastedda cheese produced in Cherry wooden vat after 15 days of refrigerate storage; W5 ChV_{15}, Vastedda cheese produced in Ash wooden vat after 15 days of refrigerate storage, W6 ChV_{15}, Vastedda cheese produced in Walnut wooden vat after 15 days of refrigerate storage; W7 ChV_{15}, Vastedda cheese produced in Black pine wooden vat after 15 days of refrigerate storage; W8 ChV_{15}, Vastedda cheese produced in Poplar wooden vat after 15 days of refrigerate storage.

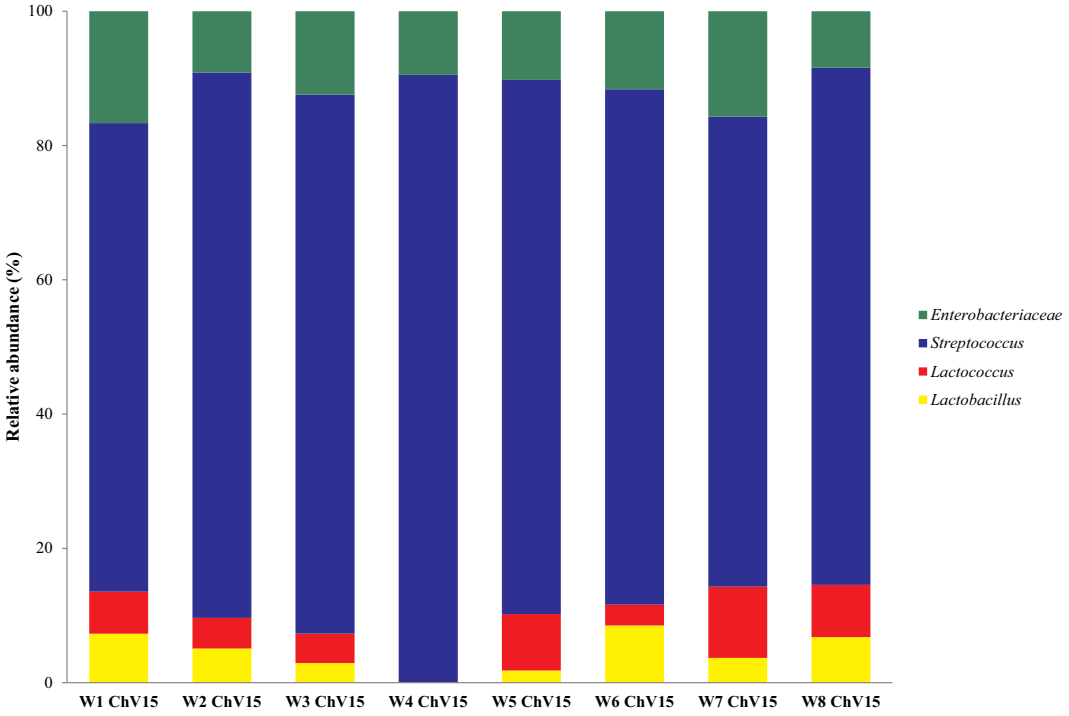

Fig. 1. Relative abundances (%) of bacterial genera identified by MySeq Illumina in VdB cheeses after 15 d of refrigerated storage. Only taxonomic groups with at least two representative sequences per taxonomic unit were retained. Abbreviation: W1 ChV_{15}, Vastedda cheese produced in Calabrian Chestnut wooden vat after 15 d of refrigerated storage; W2 ChV_{15}, Vastedda cheese produced in Sicilian Chestnut wooden vat after 15 d of refrigerated storage; W3 ChV_{15}, Vastedda cheese produced in Cedar wooden vat after 15 d of refrigerated storage; W4 ChV_{15}, Vastedda cheese produced in Cherry wooden vat after 15 d of refrigerated storage; W5 ChV_{15}, Vastedda cheese produced in Ash wooden vat after 15 d of refrigerated storage, W6 ChV_{15}, Vastedda cheese produced in Walnut wooden vat after 15 d of refrigerated storage; W7 ChV_{15}, Vastedda cheese produced in Black pine wooden vat after 15 d of refrigerated storage; W8 ChV_{15}, Vastedda cheese produced in Poplar wooden vat after 15 d of refrigerated storage.

cheeses their levels were above 6 log CFU/g for the cheeses W3–W5.

A total of 465 presumptive LAB colonies were isolated from VdB cheeses obtained through vats made with the seven wood types. All cultures, after purification and microscopic analysis, were separated into 298 cocci and 167 rods. Gram and catalase tests indicated that 271 cocci and 153 rods could be considered presumptive LAB cultures. From the combination of the different phenotypic features, the cultures were separated into five groups (Table 2). About 30% of the total cultures collected, representative for the different VdB cheeses analyzed, were subjected to RAPD analysis. The genotyping differentiation revealed the presence of 19 distinct RAPD profiles (data not shown). The sequencing of the 16S rRNA gene and the sequence comparison within two distinct databases (BLAST and Ez-Taxon) identified 5 main dominating species: *Lc. lactis*, *Lactobacillus fermentum*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus* and *Pediococcus pentosaceus* (Table S1). Table 3 shows the distribution of the dominating species among the cheese samples. *Lc. lactis* and *Lb. paracasei* were isolated from all cheeses, *Lb. rhamnosus* was not found among the dominating LAB community of cheese W1, while *Lb. fermentum* was isolated at high levels from the cheeses W3–W6 and *P. pentosaceus* only from the cheeses processed in chestnut vats (W1 and W2).

3.2. Characterization of cheese microbiota by Illumina analysis

After processing of the demultiplexed fastq files with the DADA2 package, 625,026 reads were obtained with a mean value of 78,128 per samples. The relative abundancies (%) of the bacterial genera identified in VdB cheese after 15 d of refrigerated storage are reported in Fig. 1. Only taxonomic groups with at least two representative sequences per taxonomic unit were retained. LAB genera were represented by the three genera *Streptococcus*, *Lactococcus* and *Lactobacillus* in all samples with the exception of W4, where all LAB community was constituted of streptococci. *Streptococcus* were the main LAB of all samples accounting for 69.79–90.51% of OTUs. The minor part of LAB OTUs belonged to

Table 4
Physicochemical parameters of experimental VdB cheeses.

Cheese samples	pH	Dry matter[a]	Fat[a]	Protein[a]	N soluble[a]	Ash[a]	a_w	Salt[a]
W1 ChV_{15}	5.44	54.47	44.71	45.30	0.63	5.03	0.97b	0.82
W2 ChV_{15}	5.43	54.75	45.28	45.04	0.59	4.95	0.99a	0.76
W3 ChV_{15}	5.43	55.83	47.35	44.47	0.65	4.99	0.99a	0.79
W4 ChV_{15}	5.37	55.84	43.73	45.43	0.61	5.07	0.99a	0.73
W5 ChV_{15}	5.40	55.51	45.18	44.05	0.62	4.86	0.99a	0.53
W6 ChV_{15}	5.44	56.33	46.33	45.17	0.57	5.11	0.97b	0.79
W7 ChV_{15}	5.45	55.69	45.76	44.40	0.60	4.98	0.98ab	0.76
W8 ChV_{15}	5,45	55,71	45,81	45,04	0,60	5,00	0.99a	0,79
SEM	0.03	0.63	0.92	0.90	0.03	0.05	0.006	0.13
Wooden vat	ns	ns	ns	ns	ns	ns	*	ns
Cheese making	ns	ns	*	ns	***	**	*	*

Abbreviation: a_w, water activity; W1 ChV_{15}, Vastedda cheese produced in Calabrian Chestnut wooden vat after 15 days of refrigerate storage; W2 ChV_{15}, Vastedda cheese produced in Sicilian Chestnut wooden vat after 15 days of refrigerate storage; W3 ChV_{15}, Vastedda cheese produced in Cedar wooden vat after 15 days of refrigerate storage; W4 ChV_{15}, Vastedda cheese produced in Cherry wooden vat after 15 days of refrigerate storage; W5 ChV_{15}, Vastedda cheese produced in Ash wooden vat after 15 days of refrigerate storage, W6 ChV_{15}, Vastedda cheese produced in Walnut wooden vat after 15 days of refrigerate storage; W7 ChV_{15}, Vastedda cheese produced in Black pine wooden vat after 15 days of refrigerate storage; W8 ChV_{15}, Vastedda cheese produced in Poplar wooden vat after 15 days of refrigerate storage.
Results indicate mean values of four cheese per trial (carried out in duplicate for two independent productions).
SEM, standard error of means; ns, not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; on the column different letter are significant for $P < 0.05$.
[a] Units are %.

Table 5
Colorimetric characteristic of experimental VdB cheeses.

Cheese samples	Lightness, L*	Redness, a*	Yellowness, b*	Croma[1]	Hue angle[2]
W1 ChV_{15}	83.46	−3.96	14.40	14.94	−0.27 ABb
W2 ChV_{15}	82.10	−3.97	15.02	15.53	−0.26 Bb
W3 ChV_{15}	83.61	−4.04	14.01	14.58	−0.29 Aa
W4 ChV_{15}	83.17	−4.37	14.95	15.58	−0.29 Aa
W5 ChV_{15}	84.47	−4.03	13.80	14.38	−0.29 Aa
W6 ChV_{15}	82.10	−4.34	14.54	15.18	−0.30 Aa
W7 ChV_{15}	82.65	−3.88	13.23	13.78	−0.29 Aa
W8 ChV_{15}	83.38	−4.26	13.96	14.60	−0.30 Aa
SEM	0.59	0.16	0.51	0.52	0.006
Wooden vat	ns	ns	ns	ns	***
Cheese making	ns	***	***	***	*

Abbreviation: W1 ChV_{15}, Vastedda cheese produced in Calabrian Chestnut wooden vat after 15 days of refrigerate storage; W2 ChV_{15}, Vastedda cheese produced in Sicilian Chestnut wooden vat after 15 days of refrigerate storage; W3 ChV_{15}, Vastedda cheese produced in Cedar wooden vat after 15 days of refrigerate storage; W4 ChV_{15}, Vastedda cheese produced in Cherry wooden vat after 15 days of refrigerate storage; W5 ChV_{15}, Vastedda cheese produced in Ash wooden vat after 15 days of refrigerate storage, W6 ChV_{15}, Vastedda cheese produced in Walnut wooden vat after 15 days of refrigerate storage; W7 ChV_{15}, Vastedda cheese produced in Black pine wooden vat after 15 days of refrigerate storage; W8 ChV_{15}, Vastedda cheese produced in Poplar wooden vat after 15 days of refrigerate storage.
Results indicate mean values of four cheese per trial (carried out in duplicate for two independent productions).
SEM, standard error of means; ns, not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; on the column different letter are significant for $P < 0.05$.
[1] Croma = $\sqrt{(a^2 + b^2)}$; [2]Hue angle = a/b.

Lactococcus (3.10–10.60%) and *Lactobacillus* (1.82–8.53%). Members of *Enterobacteriaceae* family were found in all samples at a percentage ranging between 8.41 and 16.67.

In order to retrieve information at species level, the OTUs belonging to the three LAB genera and to the *Enterobacteriaceae* family were manually blasted against the NCBI database. All the *Lactobacillus* OTUs were identified as belonging to *Lb. casei/paracasei/rhamnosus*. *Lactococcus* OTUs were identified as *Lc. lactis*. A higher diversity was revealed among *Streptococcus* genus, represented by *S. plurianimalium*, *S. pseudoporcinus* and *S. thermophilus*. The two OTUs recovered for *Enterobacteriaceae* family belonged to *Enterobacter cloacae* and *Enterobacter tabaci*. No significant differences were found between observed and predicted (Chao1estimator) OTUs. Therefore, the majority of OTUs present in each sample were captured.

3.3. Physicochemical parameters of cheese samples

The physicochemical parameters of the experimental VdB cheeses after 15 days of refrigerated storage are reported in Table 4. The different wooden vats did not determine significant variations of the main physicochemical parameters except for a_w, while cheese making factor showed significant differences for fat, N-soluble, ash and salt percentages and for a_w. VdB cheeses produced in Calabrian chestnut and walnut vat (W1 ChV_{15}; W6 ChV_{15}) were characterized by the lowest a_w value (0.97).

Color parameters are reported in Table 5. Lightness (L*), redness (a*) and yellowness (b*) were not statistically influenced by the eight wooden vats and showed typical color parameters of VdB cheeses (Todaro et al., 2017). However, chestnut wood showed significant lower hue angle parameters than other wooden vats. Cheese making factor significantly influenced all color parameters except Lightness.

3.4. Fatty acid composition, oxidation state and polyphenol levels of cheeses

The effect of the wood type on cheese fatty acid composition is reported in Table 6. Overall, the cheese fatty acidic composition was

Table 6
VdB cheese fatty acid composition (g/100 g FAME).

Fatty acids	W1 ChV_{15}	W2 ChV_{15}	W3 ChV_{15}	W4 ChV_{15}	W5 ChV_{15}	W6 ChV_{15}	W7 ChV_{15}	W8 ChV_{15}	SEM	P-value Wooden vat	P-value Cheese-making
C4:0	2.6	2.7	2.7	3.0	2.6	2.8	2.6	2.7	0.12	ns	***
C6:0	2.2	2.3	2.3	2.5	2.2	2.3	2.2	2.3	0.11	ns	***
C8:0	1.9	2.0	2.1	2.3	2.1	2.1	2.0	2.0	0.09	ns	***
C9:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	ns	***
C10:0	5.3	5.4	5.4	6.0	5.4	5.5	5.4	5.4	0.22	ns	***
C11:0	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.01	ns	ns
C12:0	3.2	3.3	3.2	3.5	3.2	3.3	3.2	3.3	0.11	ns	***
C13:0	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.00	ns	***
C14:0 *iso*	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.00	ns	***
C14:0	9.6	9.8	9.4	10.3	9.6	9.6	9.6	9.6	0.27	ns	***
C15:0 *iso*	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.01	ns	***
C15:0 *anteiso*	0.4	0.5	0.4	0.5	0.4	0.4	0.5	0.5	0.01	ns	**
C14:1 *c9*	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.01	ns	***
C15:0	1.3	1.3	1.2	1.3	1.3	1.2	1.2	1.3	0.03	ns	***
C16:0 *iso*	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.01	ns	**
C16:0	21.3	21.3	20.4	22.1	21.1	20.1	21.1	21.1	0.53	ns	ns
C17:0 *iso*	0.7	0.6	0.6	0.7	0.6	0.6	0.7	0.6	0.03	ns	***
C17:0 *anteiso*	0.4a	0.4a	0.4a	0.4a	0.4a	0.3b	0.4a	0.4a	0.02	*	***
C16:1 *c9*	1.3	1.3	1.2	1.3	1.3	1.2	1.2	1.3	0.03	ns	***
C17:0	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.02	ns	***
C18:0	9.4	9.3	8.9	9.6	9.3	9.0	9.3	9.2	0.21	ns	***
C18:1 *t11*, VA[1]	3.2	3.3	3.0	3.3	3.2	3.1	3.1	3.2	0.09	ns	***
C18:1 *c9*	16.1	16.1	15.5	16.5	16.1	15.6	16.1	15.4	0.42	ns	***
C18:2 n-6 *c9 c12* LA[2]	2.3	2.3	2.2	2.4	2.3	2.3	2.4	2.3	0.06	ns	***
C18:3 n-3 ALA[3]	1.8	1.8	1.7	1.9	1.7	1.7	1.8	1.7	0.05	ns	***
CLA[4] C18:2 *c9 t11*, RA[5]	1.2	1.2	1.2	1.3	1.2	1.2	1.2	1.2	0.03	ns	ns
CLA isomers	0.4	0.3	0.3	0.3	0.4	0.3	0.4	0.4	0.03	ns	*
C20:5 n-3, EPA[6]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.01	ns	*
C22:5 n-3, DPA[7]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.01	ns	*
Saturated FA	61.1	61.8	60.0	65.3	61.2	60.7	61.4	61.3	1.62	ns	***
Monounsaturated FA	25.9	26.1	24.9	26.7	25.9	25.0	25.8	25.7	0.64	ns	***
Polyunsaturated FA	8.4	8.5	8.0	8.8	8.5	8.2	8.7	8.4	0.27	ns	***
Unsaturated FA	34.2	34.5	32.9	35.4	34.4	33.2	34.5	34.2	0.90	ns	ns
Total FA	95.4	96.3	92.9	99.9	95.5	93.9	95.9	95.4	2.44	ns	**
Unsaturated/Saturated	0.56	0.56	0.55	0.55	0.56	0.55	0.56	0.56	0.00	ns	***
Σ omega-6	4.6	4.8	4.5	4.9	4.8	4.6	5.0	4.7	0.18	ns	***
Σ omega-3	2.2	2.2	2.1	2.3	2.2	2.1	2.2	2.1	0.06	ns	***
Omega-6/omega-3	2.2	2.3	2.2	2.2	2.3	2.3	2.4	2.3	0.10	ns	***
BCFA[8]	2.2a	2.1a	2.1a	2.2a	2.1a	2.0b	2.2a	2.1a	0.04	*	***

Abbreviation: W1 ChV_{15}, Vastedda cheese produced in Calabrian Chestnut wooden vat after 15 days of refrigerate storage; W2 ChV_{15}, Vastedda cheese produced in Sicilian Chestnut wooden vat after 15 days of refrigerate storage; W3 ChV_{15}, Vastedda cheese produced in Cedar wooden vat after 15 days of refrigerate storage; W4 ChV_{15}, Vastedda cheese produced in Cherry wooden vat after 15 days of refrigerate storage; W5 ChV_{15}, Vastedda cheese produced in Ash wooden vat after 15 days of refrigerate storage, W6 ChV_{15}, Vastedda cheese produced in Walnut wooden vat after 15 days of refrigerate storage; W7 ChV_{15}, Vastedda cheese produced in Black pine wooden vat after 15 days of refrigerate storage; W8 ChV_{15}, Vastedda cheese produced in Poplar wooden vat after 15 days of refrigerate storage.
a, b, means within a row with different superscripts differ ($P \leq 0.05$), [1]vaccenic acid; [2]linoleic acid; [3]α-linolenic acid; [4]conjugated linoleic acid; [5]rumenic acid; [6]eicosapentaenoic acid; [7]docosapentaenoic acid; [8]branched chain fatty acids.
Results indicate mean values of four cheese per trial (carried out in duplicate for two independent productions).
SEM, standard error of means; ns, not significant;* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; on the column different letter are significant for $P < 0.05$.

not significantly influenced by the type of wood in the vats. Only the walnut vat produced a VdB cheeses with a significant reduction of branched FA (BCFA) due to lower level of C 17:0 *anteiso*.

Table 7 reports the oxidation state and polyphenols levels of experimental VdB cheeses. The primary (POV) and secondary (TBARs) lipid oxidation were statistically influenced by wooden type; in particular, poplar wood produced VdB cheeses with the lowest peroxide value and TBARs, while no significant effect was found on polyphenols and TEAC. Regarding cheese making factor, it significantly influenced POV, TBARs and total phenolic compound content.

3.5. Volatile organic compound composition of cheeses

The volatile organic compounds emitted from VdB cheeses are reported in Table 8. Twenty-five volatile compounds were identified in the headspace of the cheeses: 9 acids, 6 alcohols, 4 esters, 3 ketones, 2 aldehydes and 1 aromatic hydrocarbons. Some differences were revealed among the VOCs of the different samples. In particular, the samples W4 ChV_{15}, W7 ChV_{15} and W8 ChV_{15} showed the highest concentration of volatile compounds, while sample W2 ChV_{15} the lowest. In particular, acids (from C4 to C16) were registered at high concentrations in the samples W8 ChV_{15}, W7 ChV_{15} and W4 ChV_{15}. Hexanoic, heptanoic, octanoic and decanoic acids were the compounds highly concentrated in all samples (on average 1691, 23, 1506 and 928 μg/kg, respectively). Alcohols were present at the highest concentration in W4 ChV_{15}, followed by the samples W7 ChV_{15} and W8 ChV_{15}. High level of isoamyl alcohol were registered in all samples (between 965 and 434 μg/kg) except W2 ChV_{15} (142 μg/kg). 2,3-Butanediol was mostly present in W7 ChV_{15} followed by W4 ChV_{15} and W8 ChV_{15}, with 381, 323 and 293 μg/kg, respectively. Aldehydes were represented only by benzaldehyde detected at high concentrations in W7 ChV_{15}, W8 ChV_{15} and W5 ChV_{15}. Ketons were mostly present in W3 ChV_{15}, W4 ChV_{15} and W5 ChV_{15}. Aromatic hydrocarbons were detected at high concentrations in W5 ChV_{15} and W4 ChV_{15}. Aromatic ethers were detected at high concentrations in W4 ChV_{15}, W3 ChV_{15}, W7 ChV_{15}, W8 ChV_{15}. All volatile compounds were significantly influenced by both factors considered: wooden vat typology and cheese making process.

Table 7
Oxidation state of experimental VdB cheeses.

Cheese samples	Peroxide value (POV), mEq O2/kg fat	TBARs µg MDA/kg DM	Total phenolic compounds, g GAE/Kg DM	TEAC, mmol trolox eq/kg DM
W1 ChV_{15}	3.56 a	4.4 c	7.89	26.87
W2 ChV_{15}	2.86 ab	4.5 c	5.25	20.61
W3 ChV_{15}	2.55 b	4.8 ab	7.00	24.46
W4 ChV_{15}	2.94 ab	5.5 a	4.87	16.54
W5 ChV_{15}	2.58 b	5.3 ab	5.02	24.15
W6 ChV_{15}	2.72 b	4.7 bc	5.11	26.83
W7 ChV_{15}	3.12 ab	4.8 bc	9.25	27.94
W8 ChV_{15}	1.68 c	4.2 c	4.81	21.78
SEM	0.28	0.2	2.09	4.06
Wooden vat	***	*	ns	ns
Cheese making	***	***	*	ns

Abbreviation: TBARs, Thiobarbituric Acid Reactive Substances Test; MAD, malonylaldehyde; GAE, gallic acid equivalent; TEAC = trolox equivalent antioxidant capacity W1 ChV_{15}, Vastedda cheese produced in Calabrian Chestnut wooden vat after 15 days of refrigerate storage; W2 ChV_{15}, Vastedda cheese produced in Sicilian Chestnut wooden vat after 15 days of refrigerate storage; W3 ChV_{15}, Vastedda cheese produced in Cedar wooden vat after 15 days of refrigerate storage; W4 ChV_{15}, Vastedda cheese produced in Cherry wooden vat after 15 days of refrigerate storage; W5 ChV_{15}, Vastedda cheese produced in Ash wooden vat after 15 days of refrigerate storage, W6 ChV_{15}, Vastedda cheese produced in Walnut wooden vat after 15 days of refrigerate storage; W7 ChV_{15}, Vastedda cheese produced in Black pine wooden vat after 15 days of refrigerate storage; W8 ChV_{15}, Vastedda cheese produced in Poplar wooden vat after 15 days of refrigerate storage.
Results indicate mean values of four cheese per trial (carried out in duplicate for two independent productions).
SEM, standard error of means; ns, not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; on the column different letter are significant for $P < 0.05$.

3.6. Correlation between cheese microbiota and volatilomes

The correlation between VOCs and the main bacterial OTUs are shown in Fig. 2. The only significant ($P < 0.05$) associations found were those between the genus *Lactococcus* and the volatile compounds hexanoic acid and butyric acid. In particular, both compounds were positively correlated with this LAB group.

3.7. Sensory analysis

Fig. 3 reports the spider graphic representation of the sensory characteristics evaluated on the VdB cheeses made with the eight wooden vats. Generally, the judges did not score the sensory attributes of the cheeses differently, although cheese W2 ChV_{15} was characterized by the highest overall satisfaction and cheese W8 ChV_{15} by the highest butter odor. The sensory analysis indicated that the seven wood types did not affect consistently the final characteristics of VdB cheeses.

4. Discussion

The final characteristics of traditional Sicilian cheeses made according to PDO protocols (OJC no. C 42/16 19.2.2010) depend on several factors, including raw materials such as milk (Franciosi et al., 2008) and artisan rennet [coagulant enzyme preparation extracted from the abomasum of ruminants that is minced and added with salt (Salvadori del Prato, 1998)] (Cruciata et al., 2014), wooden equipment (Di Grigoli et al., 2015; Licitra et al., 2007; Lortal et al., 2009; Scatassa et al., 2015), dairy environments and dairy technologies (Settanni and Moschetti, 2014). In recent years, several studies have highlighted the scientific value of the microbial biofilm on the acidification of the curd and ripening of traditional cheeses (Di Grigoli et al., 2015; Didienne et al., 2012; Gaglio et al., 2016; Licitra et al., 2007; Lortal et al., 2009; Scatassa et al., 2015). Furthermore, Aviat et al. (2016) stated that the wood in contact with foods has not been found responsible for any foodborne outbreak. Thus, the conditions of production of typical Sicilian cheeses do not compromise the safety of the production chain.

In this study, wooden vats made with seven Sicilian tree species were used in comparison to the control vat made with Calabrian chestnut. All vats were used to perform dairy productions applying PDO VdB cheese technology, in order to evaluate the influence of the seven woods on the final products kept refrigerated for 15 d that corresponds to the average time generally past before consumption. No sample hosted pathogenic *Salmonella* spp. and *L. monocytogenes*, while *E. coli* and CPS, initially present in milk, decreased their levels from stretching step onward. The decrease registered could be a consequence of the high temperature applied during stretching (85–95 °C) (Cruciata et al., in press) and directly related to the dominance of the bacteria showing dairy properties (Mariani et al., 2011). The evolution of LAB populations from acidification of the curds until the end of refrigerated storage were comparable for all trials, including control production. These microorganisms dominated during all phases of the production process and the highest levels were registered for coccus LAB (9.23 log CFU/g). These findings were superimposable on the results reported by Gaglio et al. (2016). Enterococci followed the same trend registered for other LAB groups; however, their levels were lower by about 3 log cycles. Similar data were previously reported by Mucchetti et al. (2008). Despite being indicators of low hygienic quality (Giraffa, 2003), the presence of enterococci is directly linked with cheese typicality (Foulquié Moreno et al., 2006).

LAB from final cheeses were isolated and purified in order to be investigated at strain and species level. Five main phenotypic groups were detected and examined by RAPD-PCR. This method allowed the recognition of 19 strains. The species more frequently identified were *Lc. lactis* among SLAB and *Lb. paracasei*, *Lb. rhamnosus*, *Lb. fermentum* and *P. pentosaceus* among NSLAB. In terms of cell densities, the dominant species were *Lc. lactis* followed by *Lb. paracasaei*. The direct comparison of the polymorphic profiles of the strains isolated from the final cheeses to those of wooden vat surface origin carried out by Cruciata et al. (2018) showed that *Lc. lactis* and *Lb. fermentum* strains found in the cheeses derived from the wooden vats. Among these strains, *Lc. lactis* were characterized by a very fast acidification and a rapid autolysis, whereas *Lb. fermentum* showed antimicrobial activity, a parameter technologically relevant in cheese manufacture because it contributes to the inhibition of pathogenic bacteria Cruciata et al. (2018).

In the last decade, sequencing technologies have changed the approach scientists take to the study of food microbial communities (De Filippis et al., 2018). Nowadays, this approach is routinely applied to investigate on the microbiota of cheeses (Gobbetti et al., 2018), dairy raw materials (Cruciata et al., 2014), as well as the microbiotas associated with the dairy plants (Stellato et al., 2015). The culture-dependent methodologies alone do not allow to detect all bacteria present in a given food matrix, due to the limits of cultivation. For this reason, in this study, the cheeses at 15 d of refrigeration were processed by MiSeq

Table 8
Analysis of volatile organic compounds emitted from experimental VdB cheeses.

	Chemical compounds	W1 ChV_{15}	W2 ChV_{15}	W3 ChV_{15}	W4 ChV_{15}	W5 ChV_{15}	W6 ChV_{15}	W7 ChV_{15}	W8 ChV_{15}	SEM	P-value Wooden vat	P-value Cheese-making
Acids	Acetic acid	194.40 ± 3.14 C	160.39 ± 4.76 D	166.23 ± 3.63 D	305.81 ± 9.50 B	292.20 ± 5.58 B	144.10 ± 3.51 D	405.48 ± 13.52 A	410.87 ± 9.83 A	38.60	***	***
	Butyric acid	551.63 ± 12.34 A	230.89 ± 5.21 D	260.07 ± 9.56 D	373.78 ± 8.84 C	355.50 ± 8.74 C	193.06 ± 9.20 E	500.48 ± 13.69 B	517.75 ± 11.67 B	49.02	***	***
	Hexanoic acid	1835.49 ± 66.31 B	1073.49 ± 44.05 D	1455.52 ± 45.66 C	2143.78 ± 84.13 A	1995.38 ± 78.31 AB ABABAB	1175.46 ± 46.23 D	1864.05 ± 41.69 B	1988.47 ± 51.68 AB AAAAAABABAB	142.57	***	***
	Heptanoic acid	12.51 ± 0.26 C	9.23 ± 0.35 D	5.96 ± 0.21 E	21.56 ± 0.47 B	19.79 ± 0.89 B	13.19 ± 0.67 C	23.46 ± 0.64 A	23.78 ± 0.57 A	2.42	***	***
	Octanoic acid	988.55 ± 22.29 D	941.42 ± 35.25 D	1288.61 ± 52.88 C	2325.12 ± 57.19 A	1714.02 ± 64.19 B	1266.94 ± 63.29 C	1722.61 ± 28.17 B	1799.40 ± 62.24 B	165.64	***	***
	Nonanoic acid	4.64 ± 0.11 DE	6.66 ± 0.25 D	8.14 ± 0.14 D	14.20 ± 0.53 C	4.00 ± 0.14 E	18.09 ± 0.94 C	68.73 ± 1.43 B	186.54 ± 4.33 A	22.39	***	***
	Decanoic acid	386.53 ± 8.99 F	522.09 ± 12.92 E	835.26 ± 20.54 D	1344.89 ± 40.94 A	914.85 ± 21.88 CD	949.24 ± 49.50 C	1282.91 ± 23.78 AB AAB	1191.68 ± 24.77 B	122.15	***	***
	Undecanoic acid	39.02 ± 0.76 G	51.09 ± 2.29 G	106.66 ± 3.51 E	130.98 ± 3.13 D	76.05 ± 2.84 F	143.35 ± 6.55 C	265.66 ± 4.41 A	169.00 ± 2.80 B	25.91	***	***
	Hexadecanoic acid	14.64 ± 0.38 F	18.75 ± 0.56 F	125.05 ± 3.16 D	56.26 ± 2.14 E	41.44 ± 1.05 E	182.09 ± 7.38 C	388.25 ± 11.55 B	644.57 ± 10.76 A	79.01	***	***
Alcohols	2,3-Butanediol	250.07 ± 4.19 D	151.41 ± 5.57 F	163.32 ± 2.85 F	323.43 ± 7.91 B	183.73 ± 4.41 E	70.32 ± 2.48 G	380.62 ± 8.59 A	293.07 ± 5.02 C	36.33	***	***
	Isoamyl alcohol	433.53 ± 7.09 D	141.91 ± 4.58 E	624.52 ± 9.35 C	964.48 ± 37.85 A	758.35 ± 29.76 B	509.69 ± 11.35 D	751.27 ± 22.48 B	450.19 ± 11.32 D	89.20	***	***
	1-Pentanol	8.98 ± 0.31 D	7.49 ± 0.30 D	22.92 ± 0.97 B	22.33 ± 0.56 B	27.45 ± 0.64 A	22.62 ± 1.01 B	13.15 ± 0.31 C	28.94 ± 0.78 A	2.91	***	***
	1-Hexanol	5.87 ± 0.14 G	11.92 ± 0.37 F	32.43 ± 0.77 C	77.67 ± 1.16 A	28.52 ± 0.69 D	16.96 ± 0.40 E	34.79 ± 0.95 C	39.09 ± 0.91 B	7.85	***	***
	2-Heptanol	11.06 ± 0.25 G	5.01 ± 0.04 H	73.51 ± 2.73 D	140.25 ± 3.45 B	85.74 ± 3.07 C	63.07 ± 1.41 E	150.94 ± 5.55 A	40.60 ± 0.97 F	19.05	***	***
	2-Phenylethanol	104.69 ± 1.71 E	98.39 ± 2.20 E	159.14 ± 5.00 D	254.63 ± 6.02 B	160.09 ± 4.05 D	115.53 ± 5.02 E	203.3 ± 4.23 C	276.72 ± 10.00 A	23.97	***	***
Aldehydes	Benzaldehyde	23.75 ± 0.73 E	29.52 ± 1.06 E	45.79 ± 1.08C	50.15 ± 1.10C	78.33 ± 2.45 B	36.68 ± 1.92 D	100.38 ± 1.64 A	81.75 ± 1.99 B	9.80	***	***
Aromatic hydrocarbons	p-Cymene	22.70 ± 0.68 F	10.00 ± 0.03 G	43.04 ± 1.58 C	51.24 ± 1.26 B	76.31 ± 2.99 A	43.41 ± 1.61 C	35.59 ± 0.99 D	30.12 ± 0.47 E	7.04	***	***
Aromatic ethers	Estragol	44.63 ± 1.01 D	45.92 ± 1.10 D	109.45 ± 3.21 AB	117.04 ± 4.35 A	64.94 ± 2.43 C	104.14 ± 5.20 B	112.18 ± 1.75 AB	111.79 ± 2.30 AB	11.09	***	***
Esters	Ethyl octanoate	8.57 ± 0.21 E	7.68 ± 0.17 E	29.27 ± 0.72 C	35.48 ± 1.04 B	28.41 ± 0.67 C	17.77 ± 0.53 D	88.08 ± 2.75 A	18.73 ± 0.51 D	9.09	***	***
	2-Propylfuran	6.66 ± 0.15 D	38.53 ± 1.16 A	20.32 ± 0.62 C	22.50 ± 0.68 BC	20.70 ± 0.79 C	24.74 ± 1.04 B	38.52 ± 0.87 A	21.30 ± 0.68 C	3.67	***	***
	Ethyl decanoate	10.36 ± 0.34 F	14.19 ± 0.43 F	58.64 ± 1.08 E	135.74 ± 5.05 C	101.21 ± 2.35 D	69.57 ± 3.26 E	276.77 ± 6.45A	237.63 ± 5.09 B	34.87	***	***
Ketones	Acetoin	194.88 ± 6.11 A	11.49 ± 0.32 D	13.19 ± 0.22 D	3.90 ± 0.21 E	14.80 ± 0.67 D	10.96 ± 0.34 D	144.90 ± 3.35 B	86.49 ± 1.73 C	26.12	***	***
	3,5-Octadien-2-one	15.63 ± 0.53 E	10.67 ± 0.32 F	36.12 ± 0.85 C	46.05 ± 1.10 B	59.45 ± 1.74 A	31.33 ± 1.45 D	39.01 ± 0.62 C	62.27 ± 0.97 A	6.55	***	***
	2-Nonanone	11.36 ± 0.19 E	13.17 ± 0.54 E	137.80 ± 5.41 A	50.63 ± 1.24 BC	44.01 ± 1.86 C	16.50 ± 0.50 E	57.51 ± 1.56 B	27.80 ± 0.87 D	14.66	***	***
	2-Heptanone	9.28 ± 0.21 F	12.20 ± 0.45 F	39.88 ± 1.20 D	64.07 ± 2.08 B	58.58 ± 1.70 C	75.65 ± 2.16 A	27.17 ± 0.43 E	27.60 ± 0.57 E	8.68	***	***

Abbreviation: W1 ChV_{15}, Vastedda cheese produced in Calabrian Chestnut wooden vat after 15 days of refrigerate storage; W2 ChV_{15}, Vastedda cheese produced in Sicilian Chestnut wooden vat after 15 days of refrigerate storage; W3 ChV_{15}, Vastedda cheese produced in Cedar wooden vat after 15 days of refrigerate storage; W4 ChV_{15}, Vastedda cheese produced in Cherry wooden vat after 15 days of refrigerate storage; W5 ChV_{15}, Vastedda cheese produced in Ash wooden vat after 15 days of refrigerate storage, W6 ChV_{15}, Vastedda cheese produced in Walnut wooden vat after 15 days of refrigerate storage; W7 ChV_{15}, Vastedda cheese produced in Black pine wooden vat after 15 days of refrigerate storage; W8 ChV_{15}, Vastedda cheese produced in Poplar wooden vat after 15 days of refrigerate storage. Results indicate mean values of three measurements and are expressed (in μg/kg).
SEM, standard error of means.
Results indicate mean values of four cheese per trial (carried out in duplicate for two independent productions).
*** $P < 0.001$.

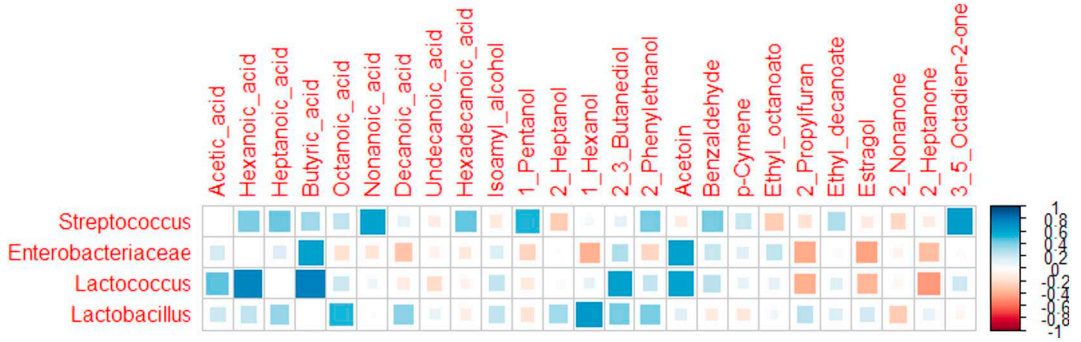

Fig. 2. Spearman's correlation between volatile compounds emitted from cheeses and microbial OTUs. Color intensity is related to the level of association.

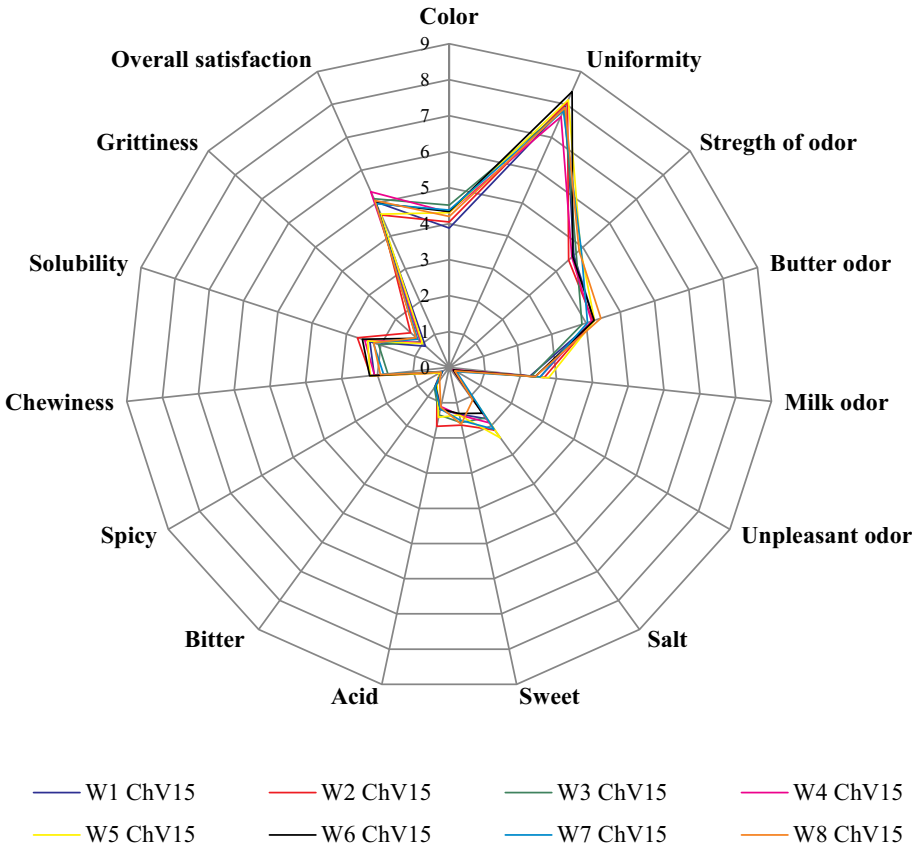

Fig. 3. Spider diagrams of descriptive sensory analysis of experimental Vastedda della valle del Belìce PDO cheeses. Abbreviation: W1 ChV15, Vastedda cheese produced in Calabrian Chestnut wooden vat after 15 d of refrigerated storage; W2 ChV15, Vastedda cheese produced in Sicilian Chestnut wooden vat after 15 d of refrigerated storage; W3 ChV15, Vastedda cheese produced in Cedar wooden vat after 15 d of refrigerated storage; W4 ChV15, Vastedda cheese produced in Cherry wooden vat after 15 d of refrigerated storage; W5 ChV15, Vastedda cheese produced in Ash wooden vat after 15 d of refrigerated storage, W6 ChV15, Vastedda cheese produced in Walnut wooden vat after 15 d of refrigerated storage; W7 ChV15, Vastedda cheese produced in Black pine wooden vat after 15 d of refrigerated storage; W8 ChV15, Vastedda cheese produced in Poplar wooden vat after 15 d of refrigerated storage.

Illumina in order to better evaluate their microbial composition. The LAB genera found were *Streptococcus*, *Lactobacillus* and *Lactococcus* and a consistent percentage of members of *Enterobacteriaceae* were revealed. These results are in agreement with plate count results. However, when microbiotas detected by culture-dependent and –independent tools were compared, barely *Lb. casei/paracasei/rhamnosus* and *Lc. lactis* were evidenced at dominant levels. Although streptococci constituted the major part of OTUs identified, no *Streptococcus* strains were among the dominant LAB isolated from the cheeses. These results are not surprising since streptococci are members of the thermophilic starter LAB and rapidly superseded by mesophilic species. Furthermore, the abundances of streptococcal DNA confirmed that the fermentation process was carried out by the typical LAB species (*S. thermophilus*) operating during *pasta filata* cheese productions performed with the traditional wooden tools (Carpino et al., 2017; Lortal et al., 2009; Settanni et al., 2012).

Several physicochemical parameters (fat, protein, dry matter and ash content, as well as pH) were within the range previously reported for VdB cheeses (Todaro et al., 2017). Water activity is the most important factor that affect cheese stability (Di Marzo et al., 2006). The mean value of a_w (0.986) was in accordance with other VdB cheeses at the same storage (Todaro et al., 2017), while the lower a_w values recorded for VdB cheeses processed with Calabrian chestnut and walnut wooden vats are not well explainable. Between color parameters, the effect of the wooden vats significantly influenced only hue angle. Chestnut wood showed significant lower hue angle parameters than other wooden vats, imputable to the slightly lower a* and slightly higher b* which give these cheeses a less intense color; these results deserve further investigations to be better explained. Cheese making factor significantly influenced several physicochemical and color parameters of cheese. Cheese making factor is strictly related to the milk composition, that might vary consistently even at 1-week interval.

Analysis of cheese fatty acids showed the typical composition of VdB cheeses (Todaro et al., 2017). The sole significant differences were found in the walnut vat, that produced a VdB cheeses with a significant reduction of branched FA (BCFA) due to lower level of C 17:0 *anteiso*.

The branched FA, known for their anti-cancer activity (Parodi, 2009), derive from animal feeding, in particular from the biosynthesis of cellulolytic bacteria in the rumen (Vlaeminck et al., 2006), but no evidence is reported in literature about the effect of dairy equipments. VdB cheeses produced with poplar vat showed a low primary and secondary lipid oxidation. This fact could be due to antioxidant residuals released from the wood. Since the phenolic compounds can increase the antioxidant capacity of the cheeses, the seven types of wood were used also to evaluate if the different content/composition of the wood phenolic compounds could affect the phenolic compound concentration of the resulting cheeses. However, the analysis of total polyphenols in the cheeses did not show significant differences. Not even the antioxidant capacity, measured by TEAC assay, showed significant differences between cheeses. Thus, it can be supposed that other residuals, resins or essential oils with antioxidant effect, may have been released from the poplar vat.

Cheese flavor is derived from a wide range of compounds that result from the hydrolysis or metabolism of carbohydrates, proteins and fats, along with compounds added during processing or directly from the milk (Fox et al., 2000). Milk fat is relevant for cheese flavor because it undergoes various reactions such as hydrolysis, oxidation, and esterification and produces free fatty acid, lactones, esters, and ketones that contribute to the overall flavor of cheese (Alewijn et al., 2005; McSweeney and Sousa, 2000). The main components of the volatile fraction of VdB cheeses analyzed in this study were free fatty acids mostly represented by hexanoic, octanoid and butyric acid, in accordance with Todaro et al. (2018). Similar free fatty acid profiles were also observed for Provola dei Nebrodi cheese, another traditional stretched Sicilian cheese (Ziino et al., 2005). These compounds derive mainly from the action of the lamb rennet used for curdling, responsible for the high amounts of short-chain free fatty acids (Virto et al., 2003). Free fatty acids can also react with alcohol groups to form esters which are volatile and odor-active and are important compounds influencing the final flavor of many cheeses (Fox et al., 2000), especially providing fruity flavors to dairy products (Urbach, 1997). Aldehydes and esters are poorly represented (Todaro et al., 2018), probably because VdB is a fresh cheese and if the ripening time of fresh cheeses would be prolonged the concentrations of these latter compounds could increase (Fernandez-Garcia et al., 2004). In general, a number of key aromatic compounds are derived from the metabolism of carbohydrates (lactose and citrate) by LAB resulting in acetate, 2,3-butanediol, acetaldehyde, acetoin (3-hydroxy 2-butanone), ethanol, propionate and lactate (Fox et al., 2000). Among these acetoin and 2,3-butanediol were detected in all VdB cheeses. Benzaldehyde was present in all samples, especially in W7 ChV_{15} and W8 ChV_{15}, this compound can be formed by enzymatic activities (proteolysis and peptidolysis) or by chemical conversion by phenyl-pyruvic acid (Smit et al., 2005).

The type of wood used to construct the vats as well as bulk milk significantly influenced the VOCs of VdB cheeses. The cheeses produced in poplar, black pine and cherry wooden vats showed the highest concentration of VOCs. The chemical classes mostly influenced by the wood type were acids (hexanoic, heptanoic, octanoic and decanoic acids), alcohols (isoamyl alcohol, 2,3-butanediol, 1-hexanol and 2-phenylethanol) and aldehydes (benzaldehyde), but further investigations are necessary to better understand how each wood type impact cheese volatile components. Regarding the contribution of LAB, the correlation between microbiota and volatilome profile of the cheeses evidenced the positive association between lactococci and hexanoic and butyric acid. In an attempt to evaluate the impact of the wooden vat biofilm microbiotas on the formation of cheese flavor compounds, Carpino et al. (2017) and Guarrasi et al. (2017) analyzed their behavior in milk and cheese based medium respectively. In particular, the last work showed the higher contribution of *Lb. paracasei* to the formation of alcohols, aldehydes and esters than *Lb. rhamnosus* and *P. pentosaceus*, while an opposite trend was found for the generation of ketones.

The significant differences in chemical compounds emitted by the cheeses were not confirmed by sensory analyses, probably because their levels were below the perception threshold of the panelists. Sensory evaluation showed that the seven woods used for the manufacture of VdB all produced cheeses with similar sensory characteristics. Furthermore, the resulting cheeses were comparable to those made in control Calabrian chestnut.

In conclusion, this study demonstrated the persistence and dominance of LAB of wooden vat origin in all cheeses at 15 days of refrigerated storage and the absence of L. *monocytogenes* and *Salmonella* spp. The use of Sicilian tree species did not negatively affect the chemical composition of VdB cheeses. Lower hue angle values of cheeses produced in chestnut was observed. The cheeses produced with poplar vat showed the lowest primary and secondary lipid oxidation. The differences in VOCs detected in the cheeses from the different trials have characterized the aromatic profiles of VdB cheeses, but this was not perceived by the panelists who recognized all cheeses as similar. This study showed the suitability of the seven Sicilian tree species in traditional dairy productions.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2018.11.017.

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