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Wooden Tools: Reservoirs of Microbial Biodiversity in Traditional Cheesemaking

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ABSTRACT Today, wooden shelves are used for the ripening of about 500,000 tons of cheese per year in Europe, including about 350,000 tons in France, such as most of the famous cheeses with the protected designation of origin (PDO), e.g., Comté, Reblochon, Beaufort, Munster, Cantal, and Roquefort. For some PDO cheeses, the use of wooden tools is mandatory. Many cheesemakers believe that wooden tools improve the organoleptic and typical characteristics of their final products. Wood is a natural and sustainable material which has been used for centuries in traditional cheese production in a wide variety of forms (vats, shelves, and packaging). Wood is important in the cheesemaking process, interacting with the milk in vats or with the cheeses placed on shelves for ripening. Wood is viable due to its ability to exchange water but, above all, because it is covered by a rich microbial biofilm. As wood is porous and difficult to clean, the European Commission regularly highlights the question of its safety when in contact with food and calls for deeper scientific investigation. In this review, knowledge about the multiple technological roles of wood in dairy technology is discussed. The crucial role of wood as a reservoir of microbial biodiversity for traditional cheeses is reviewed, along with results of safety assessments. As a conclusion, the numerous questions remaining about this natural inoculating system are discussed.

WOODEN TOOLS IN TRADITIONAL CHEESEMAKING

For centuries, farmers and cheesemakers have used wooden tools to collect and transform milk (vats, spoons, molds, cream separators, hoops for Swiss cheese, shelves for ripening, and boxes for direct packaging such as for Vacherin Mont-d'Or) (Fig. 1). Unfortunately, as wood is a biodegradable material (compared to ceramic, for example), there are very few historical artifacts documenting proof of its use in the past, in contrast to objects such as wine barrels, which are often represented on frescoes and in paintings.

Today, wooden shelves are used for the ripening of about 500,000 tons of cheese in Europe annually; of this total, approximately 350,000 tons of cheese is produced in France, including many well-known cheeses with the protected designation of origin (PDO), such as Comté, Reblochon, Beaufort, Munster, Cantal, and Roquefort. The use of wooden tools is mandatory for some PDO cheeses (e.g., the *gerle* vat in the production of Salers cheese). In Ragusano PDO cheese production, included in European Union (EU) directive 96/536/EC (1), the use of a spontaneous microflora and the specific cheesemaking technology refer to natural processes avoiding the use of starters, reflecting traditional methods in the area of production, including the use of a wooden vat known as a *tina*. Many cheesemakers consider wooden tools to improve the organoleptic and typical characteristics of their final products. Wood is composed of cellulose (40 to 50%), hemicellulose (10 to 30%), lignin (15 to 30%), and minerals. Its structure is heterogeneous, anisotropic,

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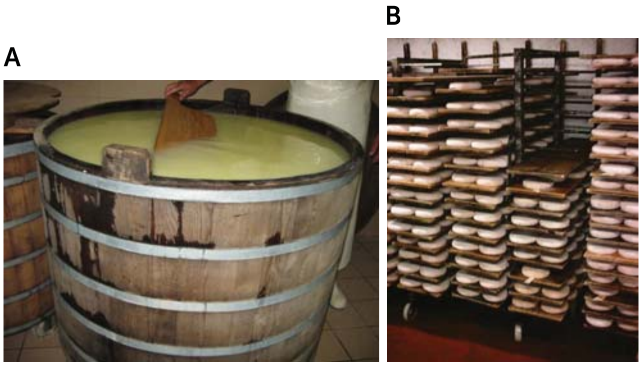

FIGURE 1 Examples of wooden tools. (A) A wooden *gerle* vat; (B) shelves used for cheese ripening. Both are used in French PDO cheesemaking. doi:10.1128/microbiolspec.CM-0008-2012.f1

porous, and hygroscopic. Electron microscopy reveals a very specific structure with tubular fibers that can be easily seen in a tangential cut (Fig. 2).

The density of wood is extremely variable from one species of tree to another (350 to 1,100 kg m^{-3}). Spruce is most frequently used for dairy products, in particular for shelves, whereas for vats, Douglas fir and chestnut are the most cited woods. Due to the presence of organic acids in its composition, wood is generally acid (pH between 4.0 and 6.0); it is a natural, plentiful, and renewable raw material which holds specific antifungal compounds whose efficiency depends on ambient humidity. Ideally, wood should be cut in the winter to limit the quantity of sap inside. Wood is very resistant from a mechanical point of view and has several technological roles as detailed below, explaining why it is still used in many parts of the world, including Europe and the United States, despite the growing hygienic regulations. Very few data exist regarding the inoculation of wood by microorganisms: recently, El abed et al. (2) explored the physicochemical interactions between the surface of bacteria and the surface of wood. These authors concluded that the microbial adhesion was dependent on the wood species and microorganisms tested.

LEGISLATION CONCERNING WOOD IN CONTACT WITH MILK OR CHEESES

Wood in contact with raw milk and cheeses is rapidly covered by a microbial biofilm. Because of its irregular surface (crevices, cracks, etc.) and its high porosity, wood is not easy to clean. Even though wood has never been documented to be involved in any food-borne disease outbreak, the *Codex Alimentarius* (3) does not approve the use of wood in contact with food; thus, the question of its safety must be absolutely raised. In Europe, the first effort to harmonize the member rules about materials in contact with food was directive 76/893/EEC of 23 November 1976 (4), but wood was not cited. It was then replaced by directive 89/109/CEE of 21 December 1988 (5), which evokes the case of wood for the first time. Likewise, decision 96/536/EC of 29 July 1996 (1), regarding milk-based products with traditional characteristics, mentioned that the instruments and equipment, whatever their nature, could be used if constantly maintained in a satisfactory state of cleanliness and regularly cleaned and disinfected. Finally, it was replaced by regulation 1935/2004 (6), in which wood is listed with other materials for which specific measures can be adopted, but member states can maintain or adapt national decisions. Lastly, the recent and very complete regulation 853/2004 (7), called the "Hygiene Package" in Europe, defined specific hygienic rules for the production of food from animals, in particular from milk (section IX: raw milk and dairy products). It is clearly stated that surfaces in contact with milk (tools, vats, etc.) should be easy to clean and well maintained. This requires smooth, washable, and nontoxic surfaces. After use, these surfaces should be cleaned and, if necessary, disinfected. Wood is not specifically pointed out

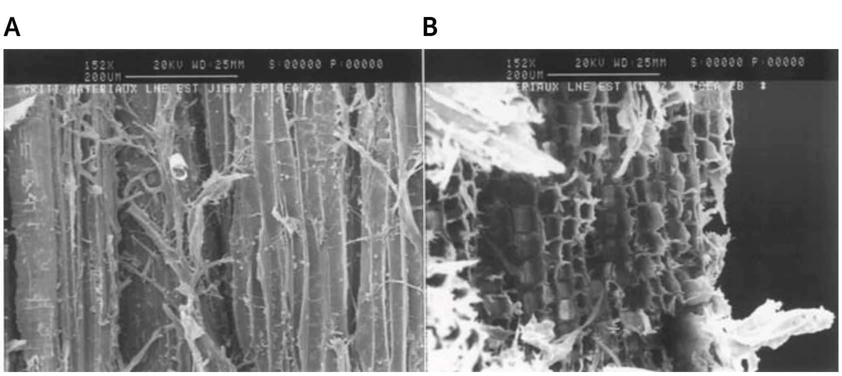

FIGURE 2 Scanning electron microscopy of the surface of spruce ripening shelves showing wood tubular structure. Cross sections are in the direction of wood fibers (A) and perpendicular to the fibers (B). Courtesy of E. Notz. doi:10.1128/microbiolspec.CM-0008-2012.f2

in this text, however: its porosity clearly does not fit the requirement of smooth surface, and plastic and stainless steel utensils and equipment are preferred. In France, a favorable provisory judgment was reaffirmed for the use of wood in dairy technology by the French Agency for Food, Environmental and Occupational Health and Safety (ANSES; opinion no. 2007-SA-0206 [8]), which, on the other hand, asked for more scientific data, as very few are available about these natural systems. The use of wood is mandatory in several French PDO cheeses, such as Comté and Beaufort. Wood is widely used in Italy and Spain. Outside Europe, the situation is variable. Like in Europe, in the United States, regulations specify that equipment and utensils used to process milk and manufacture dairy products must be constructed to allow for easy removal for cleaning and sanitizing (9). Product contact surfaces of all utensils and equipment must be constructed of stainless steel or other corrosion-resistant material, and other nonmetallic parts (other than glass) which have product contact surfaces must comply with 3-A Sanitary Standards. Use of wood in cheesemaking is nevertheless allowed, but this varies from state to state (10). Wood is allowed in central Asia. Interestingly, despite the suspicion of safety authorities, the use of wood in cheesemaking has maintained over the past decades a positive consumer image, at least among French consumers. It is spontaneously regarded as a natural and authentic material.

Wood from many species of trees (oak, spruce, various species of pine, chestnut, beech, walnut, etc.) is allowed for contact with food, including milk and cheeses, as long as no chemical preservative treatments were previously applied to it; this list was given for the first time in France in a document officially published on 15 November 1945. The kind of tree species used broadly depends on the region and altitude. The microbial contamination of new spruce shelves when they leave the sawmill was shown to be negligible. The procedures of cleaning after use involve most of the time a brushing step with water, or whey, and an adequate period of drying. A French survey in 2000 (11) revealed that brushing with water (cold or <35°C) and then subjecting the wood to water at high pressure at 85°C were the most frequently used methods. By these two successive steps, a reduction of more than 5 logs of the total microbial flora can be reached. When the microbial ecology of plastic versus wood kitchen cutting boards was explored, several authors highlighted that the results do not support the frequent assertion that plastic cutting boards are safer than wood (12). Moreover, cleaning of wooden kitchen cutting boards with hot water and detergent generally removed contaminant bacteria, regardless of the bacterial and wood species and regardless of the age of the wood (new versus used) (13).

Currently no legislation or guidelines exist in Europe regarding the cleaning of wooden tools, and each cheesemaker has, on the same general basis, his own recipe. The only mandatory aspect is to guarantee the efficiency of the cleaning regarding potential contamination by pathogen species.

MICROBIAL ECOLOGY OF WOODEN VATS

In the scientific literature, only two wooden vats have been explored: the *tina* used in Sicilian Ragusano cheese production and the *gerle* used in French Cantal cheesemaking. Both of these cheeses are seasonally produced PDO cheeses. Ragusano cheese is a brine-salted pasta filata cheese from the Hyblean region of Sicily. Raw milk is directly placed in the traditional wooden vat (tina) for cheesemaking, without the addition of any starter. The microscopic observations of the biofilm from many tinas revealed a continuous layer of microorganisms entrapped in a thick polysaccharidic matrix (Fig. 3). Molecular characterization performed on 5 different tinas demonstrated the predominance of lactic acid bacteria, in particular *Streptococcus thermophilus*, and the presence of thermophilic lactobacilli, lactococci, and a few high-GC-content bacteria, such as coryneform bacteria (14). Variable but usually very low levels of molds and yeasts were detected. The microbial profiles of the tina had common and specific features depending on the farm; they exhibited in any case 2 to 10 codominant species, thus representing a valuable source of biodiversity. The tina's biofilm was also shown to be a very efficient delivery system for lactic acid bacteria, releasing in a few minutes 10^5 to 10^6 CFU of lactic acid bacteria per ml of milk poured into the vat (15) (Fig. 4). Lactic acid is thus produced by a combination of the natural raw milk ecosystem inoculated by the lactic flora from the tina biofilm.

The tina is not used continuously during the year, as most of the farms stop cheese production during the summer when pasture is not available, leading to interesting questions regarding survival of bacteria entrapped in the biofilm within the wood. In one study, it was found that during the season of production, the predominant species, *S. thermophilus*, was also the most metabolically active, as shown by reverse transcription-PCR–temporal temperature gradient gel electrophoresis (Valence F, Delorme C, Pediliggieri C, Madec M-N, Chuat V, Parayre S,

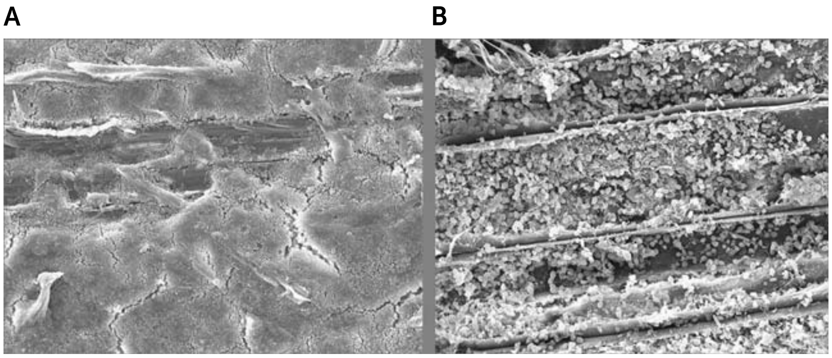

FIGURE 3 Microbial biofilm covering the wooden vat called a tina used in Ragusano cheesemaking. Shown are scanning electron micrographs of bacterial cells embedded in the polysaccharides at the surface of wood (A) and inside the wood fibers (B). doi:10.1128/microbiolspec.CM-0008-2012.f3

Carpino S, Renault P, Lortal S, Licitra G, unpublished data). More than 200 clones of *S. thermophilus* were isolated from four tinas, and after identification by 16S sequencing, they were analyzed by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) in order to assess the number of strains. Several strains were shown to cohabit each tina, and seven new sequence types were found. By comparing these new strains isolated from tinas to the MLST profile of a collection of 160 other strains of *S. thermophilus*, it was found that the Sicilian strains formed a completely separate cluster (Valence et al., unpublished) and were thus unique. This might suggest a selection pressure exerted by the life within the wooden biofilm. Regarding the contribution of the strains to the ripening, the PFGE profiles of several clones of *S. thermophilus* isolated from Ragusano cheese during ripening were identical to the ones of the corresponding tina.

Cantal and Salers are PDO cheeses produced in central France. The raw milk is directly placed into a traditional wooden vat called a *gerle*; in the seasonal production of Salers, the cheese is made without the addition of any lactic starters, and the use of the wooden gerle is mandatory in its production regulation. The gerle is a cylindrical or conical wooden vat made of chestnut wood with a capacity of 100 to 1,000 liters. The presence of yeasts and bacteria within the biofilm of wooden gerles was first observed by Richard (16) using scanning electron microscopy. The biofilm microbial composition was then extensively explored in different gerles from 10 different farms, using several samples per gerle at four different periods of the year (17). In contrast to that of the tina, the gerle biofilm is dominated by lactobacilli (4 to 6 log cm^{-2}), leuconostocs (1.4 to 5.2 log cm^{-2}), gram-negative bacteria, yeasts (3 to 5.5 log cm^{-2}), and molds (1.7 to 4.5 log cm^{-2}), which can be explained

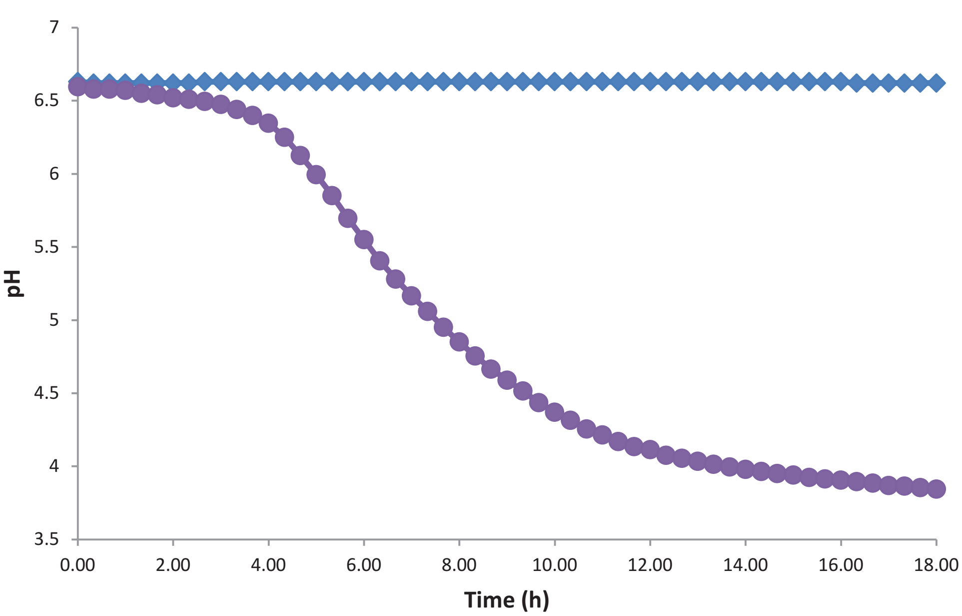

FIGURE 4 Spontaneous acidification at 37°C of microfiltered milk before (diamonds) and after contact 10 min with the tina wooden vat (circles) showing the efficient inoculation of lactic acid bacteria into the milk (15). doi:10.1128/microbiolspec.CM-0008-2012.f4

by a different cheese technology. Again, a large biodiversity in the biofilm composition was observed among gerles and was correlated with management procedures. As with tina wooden vats, the gerles were shown to very efficiently inoculate lactic acid and ripening bacteria into milk, contributing to acidification and the final cheese character.

WOODEN SHELVES, MICROBIAL ECOLOGY, AND TECHNOLOGICAL ROLES IN WATER EXCHANGES

It must be underlined that the use of wooden shelves versus wooden vats raises rather different scientific questions. In vats, the interface between wood and milk is between a solid and a liquid, is quick (a few hours), and can include heating steps. In the case of shelves, the interface between wood and cheese is between a solid and a semisolid, occurs after cheesemaking, is long (weeks or months), and occurs in the cold and in humid ripening cellars. For these reasons, wooden shelves have two different roles: one related to the microbial ecology of the cheese surface and the other one very important from a technological point of view, related to hydric exchange with the cheese curd and with the cellar air humidity. Lastly, the physicochemical local conditions within the wood are generally different between vats and shelves (acid versus neutral).

Hydric Exchanges of Wood with Cheeses and Ripening Cellars

Users of wooden shelves agree that wood possesses exceptional technological value when used to regulate cheese and cellar humidity. Indeed, wood is hygroscopic and can lose or retain humidity depending on the temperature and ambient humidity. By using shelves with various levels of hygrometry (10 to 19% humidity), cheesemakers modulate with an ancestral empirical knowledge the kinetics of cheese drying, which has a strong influence on the correct setup of the microbial rind. Very few publications are available on this key practice. In France, research in this area was explored by the dairy technical institute Actilait (18). For example, for wet shelves (27 g of H_2O/100 g of wood) used to ripen Reblochon cheese in a cellar (12°C and 96% humidity), at least 5 days is necessary to stabilize the flux of humidity migrating from the cheese to the wood (equilibrium reached at about 55 g of H_2O/100 g of wood). Most likely, the water flux from the cheese to the shelves also carries with it nutrients and microorganisms, but this hypothesis was never really explored with accurate analytical and molecular tools. Dumont et al. (19) and Bosset el al. (20) demonstrated the presence of specific volatile compounds in particular terpenic molecules after contact with wood.

Cheesemakers are very careful in choosing the wooden shelves from the sawmill. Wood must be correctly dried (15 to 18% humidity), which might require 3 to 5 months when done outside. Drying under vacuum is also possible and is much quicker (a few days). No chemical treatments should have been applied to wood. There is much empirical know-how about the drying of shelves by cheesemakers themselves, with inspection and touch as their very accurate tools to qualify the shelves. A shelf which is too humid favors mold defects on the surface of cheese, and sometimes *Pseudomonas fluorescens*. If too dry, it favors the development of thick, strong rinds and red defects (*Serratia*). Of course, analytical tools also exist in parallel (e.g., the pin-type moisture meter) and can confirm the cheesemaker's estimation. When shelves are not in use within the ripening cellar (rotation), their proper drying is fundamental to reinforce food safety (water activity [a_w] from 0.955 when in use to 0.83 when stored).

Wooden Shelves, Biofilms, and Microbial Ecology of the Cheese Surfaces

Wooden shelves used for the ripening of Reblochon de Savoie were extensively characterized (21, 22). Reblochon is a cow's milk smear-ripened cheese, made most often from raw milk. After analyzing the biofilm composition of 50 shelves of three ages during two seasons, which were used to ripen cheeses from eight farms, the authors concluded that the biofilm was mainly composed of micrococci, corynebacteria, yeasts, and molds but also contained leuconostocs, lactobacilli, enterococci, coagulase-negative staphylococci, and *Pseudomonas*. This composition was very stable over time and was similar to that of the cheese surface, supporting the hypothesis that wooden shelves represent an essential source of microbial flora for the rind of cheeses, as claimed by many cheesemakers. Schuler (23), for example, demonstrated the contribution of different wooden shelves to the development of the cheese rind microflora and to the final quality of a semihard Swiss cheese. The colonization of the wood was estimated to occur in the first 2 mm of depth. The wooden shelves had a neutral pH (7 to 8.3), a high a_w, and a low salt content (14 mg/cm^2). Examination by infrared spectroscopy of the zones of wood in contact with cheeses versus zones without contact (blank) revealed obvious spectral differences (24). In contrast, spectra of the blanks and cheese zones were similar after cleaning, supporting the efficiency of the cleaning procedure.

SAFETY ASSESSMENT

The presence of pathogens (*Listeria*, *Salmonella*, *Escherichia coli* O157, and *Staphylococcus aureus*) was analyzed in more than 15 tinas (wooden vats) which came from different farms in the Ragusa region of Sicily. Except for very rare and very low levels of contamination with *S. aureus* (only seen after enrichment by the BAX system), none of these pathogens was detected within the biofilm (15). Many hypotheses can explain this resistance to the establishment of pathogens on the wood. First of all, this observation is in agreement with findings for many other positive biofilms. Second, the local pH of the wooden vat is below 5.0; the temperature cycle for Ragusano cheesemaking includes a heating step (even if thermocouples showed that it never exceeded 45°C); nutritional competition with the positive microflora can occur; and the predominant species, *S. thermophilus*, can also produce bacteriocins (25). Last, brushing and washing can also limit the potential adhesion of milk pathogens on the surface of the biofilm. All these factors are likely combined to make an efficient barrier toward pathogens. The wood itself can release antimicrobial compounds toward pathogens, as suggested by Miller et al. (26), or it can have a bactericidal effect due to some of its physical properties (26, 27). The complete absence of pathogens was also found in 10 gerles examined in Cantal cheesemaking. When raw milk before contact with the wooden vats was artificially contaminated with high levels of *Listeria* organisms and *Staphylococcus* (17), pathogens were still not able to be established within the biofilm.

In a study conducted in the technical center Actilait (11), samples (brushings of 25 cm^2) obtained from 90 different shelves were examined for the presence of pathogens. Neither *Listeria* nor *Salmonella* spp. were found, and less than 10 CFU/cm^2 was recovered for *S. aureus* and *E. coli*. On the other hand, in a study examining the distribution of *Listeria monocytogenes* within a cheese plant (28), one sample from wooden shelves was positive (of five shelf samples examined). In contrast, in a Brazilian dairy plant manufacturing fresh cheeses (29), none of the five shelves explored was contaminated by *L. monocytogenes*. Of course, if the surface of cheeses is highly contaminated, for example, by *L. innocua* (30), cross contamination of the shelves can occur. In particular, the washing solutions used on the cheese surface are notable sources.

More recently, challenge tests were performed; *Listeria* was deliberately inoculated on wooden shelves and was successfully inhibited by the resident biofilms, as long as the biofilm was viable (22). In another study concerning the survival of *L. monocytogenes* following cleaning and sanitation of wooden shelves, Zangerl et al. (31) concluded that "there is no reason to replace wood employed in cheese ripening processes with other materials" as long as cleaning procedures are appropriately followed.

MAIN CONCLUSIONS AND PERSPECTIVES

As wood is obviously a natural material, very few questions about its safety were addressed by scientists until 1970, when increased regulatory activity placed general hygienic pressure on food production. Even though no food-borne disease outbreak has ever been attributed to the use of wood, wood was suspect because of its porosity, and without any demonstration of obvious risk, it was replaced as often as possible by other materials (which ironically have been documented to carry risks). However, from the literature surveyed here, it seems clear that wooden vats and shelves act as a reservoir of microbial biodiversity contributing to the final quality, safety, and character of dairy products. Moreover, the natural biofilms which form on wooden surfaces are safe and able to inhibit and limit pathogen implantation with mechanisms that still need to be further explored. Wood, as a tool to regulate cheese and cellar humidity, has also been proved to be difficult to replace by any other synthetic materials. Its role is crucial in the hydric balance and drying of cheese, which are subsequently crucial for the development of the expected microbial ecosystem on the rind; this ability has never been equaled by any other kind of shelving material.

All these results would support the use of wood without restriction, provided that appropriate cleaning and drying procedures are utilized. General guidelines for wood management in the traditional dairy sector are missing and would be of great value to the future of artisan cheese production. Many scientific questions remain and deserve exploration in order to understand the sustainability and microbial dynamic of this natural inoculating system as well as new tools for physicochemical microexploration within the depths of the wood interior. Among the most important questions still to be examined are the following. What are the mechanisms of biofilm establishment and species selection? Does the wood, depending on the tree species, exert a kind of selection of the bacteria able to adhere and survive? Do the wood biotype and the cycles of technological use and washing exert any kind of selective pressure on the microbial biofilm? How does the biofilm survive during long periods without use? Is the biodiversity observed in terms of species and strains stable over time? By which mechanisms are these positive biofilms able to prevent pathogen establishment: nutrient competition, inhibitory compounds, or a set of environmental local parameters such as low pH and low a_w? Does the wood itself, via specific inhibitory components, contribute to this protection? Last but not least, the impact of the microbial flora inoculated from wood on the typical character of the product needs to be further explored.

In conclusion, this fascinating natural inoculating system, providing obvious microbial diversity in dairy traditional products, deserves much more investigation before being abandoned because of excessive and scientifically unjustified hygienic considerations. Globalization represents a real risk of eliminating traditional systems (32), and wooden tools are part of these systems all over the world. It is the responsibility of scientists to explore these systems and provide data, avoiding blind and ignorant regulatory decisions. Wood should be considered in its natural technological environment as an essential component of traditional practices which provide the cheesemaking ecosystem with its original complexity and balance. Scientific exchanges with other food sectors using wood would also be of high added value.

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