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Microbial Safety of Wood in Contact with Food: A Review

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Abstract: Food packaging is multifunctional: it protects from harvest to table. Four main groups of materials for direct food contact are mentioned in the literature: wood, glass, plastic, and metal. In this review, the focus is on wooden packaging for direct contact with food. In Europe, wood as a food contact material is subject to European Regulation (EC) No 1935/2004 states that materials must not transfer their constituents to food. Today, wooden packaging, like other packaging materials, does not have a Europe-wide harmonized specific regulation, so member countries legislate at different levels. Wood has been safely used for centuries in contact with food but is usually questioned because of its microbiological behavior compared with smooth surfaces. Based on a review of published conclusions from scientific studies over the last 20 y and after a description of the general properties of wooden packaging, we focus on the microbiological status of natural wood. Then, we discuss the parameters influencing the survival of microorganisms on wood. Finally, we report on the transfer of microorganisms from wood to food and the factors influencing this phenomenon. This review demonstrates that the porous nature of wood, especially when compared with smooth surfaces, is not responsible for the limited hygiene of the material used in the food industry and that it may even be an advantage for its microbiological status. In fact, its rough or porous surface often generates unfavorable conditions for microorganisms. In addition, wood has the particular characteristic of producing antimicrobial components able to inhibit or limit the growth of pathogenic microorganisms.

Keywords: food, microorganisms, packaging, safety, wood

Introduction

Food packaging is multifunctional: it protects from harvest to table, preserves, transports, distributes, and informs the consumer. Four main groups of materials for direct food contact are mentioned in the literature: wood (all forest-based material, paper/cardboard included), glass, plastic, and metal. In this review, the focus is on wooden packaging for direct contact with food.

Wood took off as a packaging material during the Roman Empire. Succeeding the amphora, the wooden barrel has now been used for 2000 y almost exclusively for maturing, storing, transporting, and selling wine. The magnitude of the volume of wine once transported in wooden tuns is evidenced by the fact that large quantities are still measured in "tons." In the twentieth century, other materials for barrels emerged, such as concrete, fiberglass, and stainless steel, but the use of wooden barrels has remained in the areas of wine and distilled wine, balsamic vinegar, and olive oil. The wooden barrel is not only a storage container but also directly affects the body and aroma of the wine. In addition to barrels for wine and distilled wine, wood in direct contact with food is found in other forms such as kitchen utensils, cutting boards, and crates and baskets for harvesting, storage and transportation. In particular, "light wooden packaging" is used for crates, baskets, boxes for fruit and vegetables, seafood, fish, and dairy products. Today, wooden light packaging is made from raw material obtained from sustainably managed forests. In Europe, there are 80 million hectares of forest, 80% of which are managed sustainably and only 64% of the annual increment of these forests is taken. This packaging responds to consumer requirements, such as sustainable development, as well as in terms of natural packaging and food protection by ensuring food safety.

In Europe, wood as a food contact material is subject to "Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC." (Anonymous 2004c) This is the reference text that lays down the general principles (Anonymous 2004a). It concerns materials and articles already in contact with

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food; materials and articles intended for food contact, and materials and articles which can reasonably be brought into contact with food or transfer their constituents to food under normal or foreseeable conditions of use. Article 3 of this regulation states that "materials and articles, including active and intelligent materials and articles, shall be manufactured in compliance with good manufacturing practice so that, under normal or foreseeable conditions of use, they do not transfer their constituents to food in quantities which could: (a) endanger human health; or (b) bring about an unacceptable change in the composition of the food; or (c) bring about a deterioration in the organoleptic characteristics. The labelling, advertising and presentation of a material or article shall not mislead the consumers." In Annex I to this regulation, there is a list of 17 materials and articles, including wood, which may be subject to specific measures. To date, specific measures have been harmonized and adopted at the European level for plastics, epoxy derivatives, active and intelligent materials, regenerated cellulose, and ceramics, but not yet for wood.

The Commission Regulation "(EC) N° 2023/2006 of 22 December 2006 on good manufacturing practice for materials and articles intended to come into contact with food" shall apply to all groups of materials and articles listed in Annex I to Regulation (EC) 1935/2004 (Anonymous 2006). They must be manufactured according to the rules on good manufacturing practices (GMP). Article 3 of this regulation states that "good manufacturing practice (GMP) means those aspects of quality assurance which ensure that materials and articles are consistently produced and controlled to ensure conformity with the rules applicable to them and with the quality standards appropriate to their intended use by not endangering human health or causing an unacceptable change in the composition of the food or causing a deterioration in the organoleptic characteristics thereof."

Except for these general principles, specific regulations for wood have not been provided at the European level. However, the Commission's in-house science service, the Joint Research Centre, has recently shown interest in food contact material sectors by providing EU policies on food contact materials using independent, evidence-based scientific and technical support and references. Adding to the European regulations, we now outline in more detail the French, Spanish, and German regulations as these 3 countries are among the major wooden packaging producers and customers in Europe.

In France, the use of wood as a material suitable for food contact is regulated by the French Arrêté of November 1945 (Anonymous 1945) and the information note of the French General Directorate for Competition Policy, Consumer Affairs and Fraud Control (DGCCRF) in n° 2012–93 (Anonymous 2012). The French Arrêté of 15 November 1945 authorizes the use of oak, chestnut, ash, hornbeam, and acacia for contact with any food; and walnut, elm, and poplar for contact with solid food. This text, written for measurement tools with no relationship to the problem of "food contact material," continues to apply in the absence of a text repealing or modifying it. The information note of the French DGCCRF n° 2012–93 is a form of recommendations to stakeholders in the timber industry (Anonymous 2012). This note allows other wood species (fir, spruce softwood, and so on) for any food.

In Spain, the specific regulation was included in the Decree 2484–1967 (Anonymous 1967) and the Royal Decree 888–1988 (Anonymous 1988). The Spanish decree of October 1967 authorized the use of wood as packaging, without differentiation by species. Materials with foreign bodies or parasites and resinous wood for fish smoking were excluded. In the same regulation, the reuse of wood was accepted, after cleaning and disinfection. However, in the RD 888–1988, Article 5 declares that wooden packages are not reusable materials, assuming then that, like cardboard and polystyrene packaging, they cannot be cleaned and sanitized after use.

The German regulation on meat hygiene "Fleischhygiene-Verordnung-FlHV" (Anonymous 1997a) from 1997 states that "surfaces (. . .) that are in contact with food products have to be in good repair and must be easy to clean or to disinfect, if necessary" (Chapter II, 1.6). In Appendix 2, it is asserted that the use of wood is *only* allowed in smoking or ripening chambers, as chopping boards, or for transportation of *packaged* meat products (Chapter I and 2). Appendix 2a defines for EU-approved food establishments that "the use of wooden pallets is permitted only for the transportation of *packaged* meat or *packaged* meat products." Similar legal requirements concerning the use of wood were given by the German regulation on poultry hygiene "Geflügelfleisch-Verordnung-GFLHV" from 1997 (Anonymous 1997b). Both regulations were overridden on 15 August 2007, when the German regulation on Hygiene for Food Products of Animal Origin ("Tierische Lebensmittelhygiene-Verordnung-Tier-LMHV") came into effect (Anonymous 2007a). This regulation does not mention the use of wood at all; neither does the German law on Food, Articles of Daily Use, and Feed ("Lebensmittel- und Futtermittelgesetzbuch-LFBG") from 2005, most recently amended on 5 December 2014 (Anonymous 2005). The German regulation on Hygiene in Production, Treatment, and Trading of Food Products ("Lebensmittelhygiene-Verordnung-LMHV") from 2007, with the last amendment of 14 July 2010, was put into effect to settle specific hygiene issues (Anonymous 2007b). Article 6 deals with certain traditional food products that are exempt from the requirements of Regulation (EC) No. 852/2004 concerning rooms, tools, and equipment (Anonymous 2004b). These food products are listed in Annex 3 of this regulation: for milk products, *wooden tools* may be used; for naturally fermented sausages *wooden bars* may be used for hanging the product during fermentation or smoking. The same applies to cured raw meat products. For food preparations, sweets, soups, and stews, *wooden tools* may be used. For fruit and vegetables in acidic or sweet-sour brine, fermented vegetables or vinegar, *wooden barrels* may be used for production. Finally, for bread and other bakery products, *wooden tools* may be used for production. Other than these exceptions, the use of wood is not mentioned in this regulation. Thus, in Germany, the use of wood in contact with food is not regulated by national law except for the specific food products mentioned above.

Wood has been safely used for centuries in direct contact with food. Fruit and vegetables as well as fresh or smoked fish have been stored in wooden crates. In cheese- and wine-making, wooden boards and barrels have been indispensable in traditional production. There are many other examples where wood has been used as a lightweight and still rough or porous packaging material from natural sources. Nevertheless, there are objections to the use of wood in direct contact with food, as it is usually considered less hygienic than other smooth or synthetic materials. To date, there is no evidence that any food-borne disease has been fostered by the proper use of wood, considering hygienic standards for production, storage, and applications. However, since the 1990s, research on wood in contact with food has led to a partial reversal of this image. Since this period, a number of scientific studies on wood and its microbiological status have been performed to investigate the impact of cleaning, disinfection, moisture content, and wood timber on the survival and transfer of microorganisms. For instance, in 1992 to 1993, the U.S. Food and Drug Administration sponsored a national telephone survey that collected data to assess consumer food-handling practices and awareness of microbiological hazards (Klontz and others 1995). Survey respondents were required to speak English, to be at least 18 y old, and to live in a household with food-cooking facilities. A total of 1620 surveys were completed, representing a response rate of 65%. The principal results of this survey suggested that certain high-risk practices were rather common, for example eating raw eggs, undercooked hamburgers, raw mollusk shellfish, and failing to be vigilant about the potential for cross-contamination of foods as a consequence of inefficiently cleaned cutting boards. Since then, although studies have differed in their results regarding the recovery of bacteria from wooden cutting board surfaces (Abrishami and others 1994; Ak and others 1994a), authors have recommended safer practices, such as cleaning the surface of cutting boards.

In this review, we first present the general properties of wooden packaging for direct food contact: wooden material and its mechanical, physical, and natural chemical properties, the use of wooden packaging in the food industry, and some examples of the perception of wooden packaging by consumers. In the second part, we describe the microbiological status of natural wood for the timber used in the manufacture of wooden packaging and methods of microbiological analysis of wooden material available to date. Then we discuss the parameters influencing the survival of microorganisms on wood such as food processing, cleaning, and disinfection and also the antibacterial compounds from wood. Finally, we report on the transfer of microorganisms from wood to food and the factors identified as influencing this phenomenon: the intrinsic properties of the wooden material, the contact time between wood and food in direct contact, and the wood moisture content.

Wooden Packaging

Wood characteristics

Wood is perhaps the oldest material used by man. Its structure is cellular and porous. It is also a heterogeneous material, highly anisotropic and somewhat hygroscopic. Anisotropic means that its physical properties vary according to the orientation of the fibers. Hygroscopic means that water may be bound in the wood cells by either molecular or capillary forces. Wood is an important constituent of trees, which can be roughly divided into 2 categories: coniferous or softwood trees, and hardwood trees. Each of these 2 groups contains thousands of different species.

Wood structure forms during the growth of a tree. The structure and properties of wood are affected by genetic and environmental factors (Wodzicki 2001). Its mechanical strength and the transport of water and nutrients are provided by a unique structure formed by biological cells. Wood cells are mostly orientated longitudinally, that is, in the direction of the stem. In softwood, these cells are called *tracheid*. They are 3 to 5 mm long and have a diameter of 20 to 80 μm. In hardwood, the cells are shorter (0.7 to 3 mm), narrower (up to 20 μm) and are not used for fluid transport (Monteiro 2014). These cells are called wood fiber. Water transport in hardwood is provided by special structures called vessel elements. Only the outer part of a stem, called the sapwood, is involved in water transport, while the inner part, called the heartwood, is not. Due to mineral deposits, gums and resins, the heartwood appears darker in color than the sapwood. The typical growth rings of wood are due to seasonal effects. During winter, a tree does not grow while in spring, thin-walled cells with large cavities are formed (springwood). In summer, cell walls increase and the diameter of the cavities decreases (latewood). These annual rings are visible in wooden surfaces, and depend on the orientation of sawing (Monteiro 2014).

Water can be held in the wood structure as nonfreezing bound water or as free water (Engelund and others 2013). Free water is situated in cell cavities, if the water content is above the so-called fiber saturation point (FSP). The FSP varies from species to species, but is around 28% to 30% water content (weight of water/weight of dry wood). The physical properties of wood (for example, mechanical strength, elasticity, thermal conductivity) are strongly related to the water content below the FSP, but hardly above the FSP (Monteiro 2014). Dry wood, as used in most technical applications, has a moisture content below 19%, while green wood contains from 60% to 200% water (Greer and Pamberton 2008). Thus, water in dry wood must be considered to be bound water and is adsorbed in amorphous regions of the cell walls consisting of cellulose, hemicellulose and lignin. A considerable swelling of these amorphous regions can be noticed during uptake of water (Engelund and others 2013).

The relationship between water content and equilibrium relative humidity (RH, which is related to water activity by a factor of 100) is given by the sorption isotherm. A typical sorption isotherm shows water content of 7% at 30% RH, of 10% at 60% RH, and of 14% at 80% RH (TIS 2015). Thus, wood can take up a considerable amount of water and release it again, depending on the relative humidity of the environment. The uptake of water causes a swelling of the wood structure due to water adsorption, while the removal of water leads to shrinking. This macroscopic shrinkage has to be taken into account whenever wood is exposed to variable humidity.

Wood contains in its porous structure a number of free low-molecular-weight compounds, called extractables (Stevanovic and others 2009). This generic name comes from the fact that these components can be extracted by solvents due to their chemical nature. They include volatile organic compounds and nonvolatile organic compounds (Stevanovic and others 2009). Although volatile extractables represent only a small percentage of wood extractives, they influence wood acidity (Stevanovic and others 2009), hygroscopicity, color (Gierlinger and others 2004; Amusant and others 2007), odor, mechanical properties, and also the natural durability of wooden material (Schultz and others 2000; Aloui and others 2004).

Trees are very diverse. Wood properties vary according to the species, growth conditions and moisture content. Thus, the following fundamental characteristics distinguish wooden material from other materials: heterogeneity, hygroscopicity, anisotropy, elasticity, impregnability and acidity.

Wooden packaging: properties and uses

According to its function, any packaging can be classified as primary, in direct contact with food, or secondary or tertiary depending on the number of layers between the packaging and the food it contains. The primary packaging contains the product; it is the container that is in direct contact with food. The secondary packaging consists of a number of primary packagings to facilitate product delivery to the sales shelves and may then constitute consumer sales units. Tertiary packaging is used to protect and transport the product between stores.

Wood has favorable mechanical, physical, and natural chemical properties for a packaging material. Its mechanical properties are its resistance to forces, that is to say compressive, tensile, and flexural strength, impact and splitting ability and hardness. Other physical properties are its low thermal and electrical conductivity. However, wood has a large hydration capacity. This can be shown by a rather large uptake of moisture according to the environmental conditions. At equilibrium, there is a given moisture content in wood depending on the relative humidity of its environment. In fact, water is everywhere in fresh or harvested wood; in the pores of wood, inside wood cells and within the cell walls. The trunk is heavily soaked in water although the heartwood contains less free water than the sapwood, representing the living and functional part of the shaft. Freshly cut wood or *green wood* contains an amount of water that depends on the timber species. Apart from its natural moisture content, the wood and timber structure also has the capacity to absorb or release water in equilibrium with the ambient atmospheric humidity or with the food in direct contact. This is one of the characteristics sought by stakeholders in the seafood, fruit, and vegetable, and cheese sectors. In addition, the majority of wood species are characterized by an acidic pH due to the presence of naturally occurring acid. The pH can range from 4.3 (European larch) to 5.2 (Parana pine) (Fengel and Wegener 1989). This characteristic influences the survival of bacteria on wooden surfaces.

Wooden packaging consists mostly of raw wood elements, sawn, sliced, or peeled thinly, veneer, and engineered wooden boards, which are associated with manufactured crates, trays, baskets, or cheese boxes. Light wooden packaging is used during harvesting, storage, conservation and transport. Depending on the specific country regulation and marketing chains, they can be considered both for single-use and reusable packaging.

Table 1 below gives examples of how widely used wood is as a packaging material for different foods and sectors.

Perception of wooden packaging

According to consumers and clients, some of the main commercial advantages of wooden packaging are its natural and sustainable character, its light weight, strength, and good preservation qualities, even in moist conditions because of its porosity and absorption capacity. However, from a hygienic point of view, the latter can be considered a disadvantage.

In this respect, there are a number of studies available about consumer perception of wooden packaging, such as the one conducted by the French Lille 1 Univ. and the French Technical Cheese Institute (ITFF) (Gigon and Martin 2006). In the specific case of cheese ripening, this work provided information on the perception of products matured on wood from a sensory and food safety viewpoint. This survey was based on a questionnaire sent by mail to some consumers who then sent it to another one, thus ensuring random sampling. The panel consisted of 322 individuals (50% women, 50% men) aged 18 to more than 55 y, 30% were students, 19% staff, and 12.7% employees. The others were friends or relatives. Seventy-eight percent of respondents felt that wood was synonymous with warmth, friendliness, well-being, and tradition. The consumers under 25 y old were less receptive to the use of wood for cheese-ripening. It was observed that about 70% of people preferred to buy cheese in wooden packaging rather than in plastic wrapping. This trend was confirmed by about 60% of individuals who believed that “a product in a wooden packaging is more attractive.” Over 60% of respondents also said that “food products in wooden packaging seem healthy,” although only 30% thought that “wood is a material that preserves the food.” These trends show that wooden packaging guarantees food quality and food safety. A last important point is that the concept of “respect for the environment” was strong: more than 80% of respondents thought that “the use of wood for food packaging helps to protect the environment.” Thus, this investigation highlighted that the majority of these consumers reflected a positive image of wood used in contact with food.

Another study developed in Spain in 2002 conducted 1004 telephone interviews, nationally representative, with men and women aged 15 to 74 y, in order to know whether they were worried about the hygiene of boxes of different materials for fruit and vegetables, and which offered them a more natural image, quality, and environmental advantages (FEDEMCO and Partner Espana S.A. 2002). The study concluded that hygiene worried more than 80% of the people. 70% of them thought that wood was the most natural-looking material when compared to others. However, only 60% said that wood was positively a hygienic material, whereas 30% thought it was not. Besides, wood packaging was considered slightly better positioned with regard to the other materials in terms of sustainability and association with quality products.

Wood and Food Safety Assessment

Food can be contaminated in many different ways and may even pose a health risk. In 2007, the Codex Alimentarius Commission published a guideline (CAC/GL 62–2007) to “provide guidance to national governments for risk assessment, risk management and risk communication with regard to food-related risks to human health” (Anonymous 2007c). This guideline recalls that the objective of risk analysis studies applied to food safety is to ensure human health protection and to produce safe food. The WHO also recommends that “Governments should make food safety a public health priority, as they play a pivotal role in developing policies and regulatory frameworks, establishing and implementing effective food safety systems that ensure that food producers and suppliers along the whole food chain operate responsibly and supply safe food to consumers” (Anonymous 2014). Food products could pose health risks due to chemical, microbiological, and other hazards.

For example, food could be contaminated by chemical substances that are responsible for acute poisoning or cancers. The causes of microbiological contamination of food are due to various microorganisms. In 2015, the European Food Safety Authority and the European Centre for Disease Prevention and Control published a report about “Trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013” (EFS and Authority 2015), which presented the most important outbreaks in 32 European countries. Since 2005, *Campylobacter* has continued to be the most commonly reported gastrointestinal bacterial pathogen in humans in the European Union and 31.4% of samples (single or batch) of fresh broiler meat were found to be *Campylobacter*-positive, whereas its detection in other food was at low to very low levels. In 2013, 414 *Campylobacter* outbreaks were reported, of which 32 were strong-evidence outbreaks from broiler meat and products thereof; others involved mixed or unspecified poultry meat and products thereof, and also milk and mixed foods. Salmonellosis is the second most widespread infection across Europe with a total of nearly 83000 confirmed cases reported. *Salmonella*-contaminated foodstuffs include fresh broiler, pig, and bovine meat, but the most important source of *Salmonella* is eggs. This study reported that the prevalence of target *Salmonella* serovars has decreased in all poultry populations. Nevertheless, in these 32 European countries, *Salmonella* remained the most frequently detected causative agent in food-borne outbreaks (22.5%

Table 1–Examples of using raw, engineered, and untreated wood in the food industry.

Sector	Wooden product	Use	Quality of wood	Wood species
Domestic	Cooking utensils	Culinary	Longevity, comfort, and safety for the user, easy maintenance	Boxwood, olive, beech
Domestic	Cutting boards	Cutting	Comfort and safety for the user, easy maintenance	Hardwood beech
Liquid	Barrels	Technological tool, storage	Aroma, storing for aging wine and spirits	Oak, chestnut
Liquid	Marketing boxes	Spirits, oil or wine bottles	Secondary luxury packaging	Pine
Seafood	Baskets for oysters and shellfish	Marketing, transport	Maintaining humidity exchanges	Poplar, white wood
Seafood	Boxes for fish and shellfish	Marketing, transport	Maintaining humidity exchanges	Poplar, white wood, pine
Cheese	Ripening cheese boards	Technological tool, storage	Conservation of the biofilm for ripening cheese	Spruce, beech
Cheese	Boxes, cheese ring, box base	Transport, marketing	Inducing humidity exchanges	Poplar, pine
Fruits, vegetables	Crates	Transport, marketing	Inducing humidity exchanges	Poplar, pine
Meat	Cutting boards, blocks	Cutting	Comfort and safety for the user, easy maintenance	Hardwood
Meat	Terrines	Marketing	Luxury packaging	Poplar, pine
Pastries, bread products	Mould, baking tray, tart ring, box base	Cooking, marketing	Cooking in a heat oven or microwave, maintaining exchanges (moisture, flavor and heating) and crispness	Poplar
Food contact	Gift boxes	Chocolate, salt, cakes, meats, canned food	Luxury packaging	Poplar, pine
Transport	Crates	Storing vegetables, fruits	Long-term storage, stacking	Pine
Transport	Pallets	Primary/secondary packaging	Long-term storage, stacking	Pine

of total outbreaks). The third most common pathogen responsible for human cases and outbreaks is *Listeria*. 1763 confirmed human cases of listeriosis were reported in Europe resulting in 91 deaths. Foodstuffs contaminated by *L. monocytogenes* were, first, certain ready-to-eat (RTE) foods at retail level (positive samples at retail were highest in fish products (mainly smoked fish), soft and semisoft cheeses, RTE meat products, and hard cheeses. In 2013, 13 listeriosis outbreaks were reported including 8 whose source was evident, such as from crustaceans, shellfish, mollusks, and products thereof. In fourth place is verocytotoxigenic *Escherichia coli* (VTEC), responsible for 6043 cases in Europe with the most common VTEC serogroup being O157. This was primarily detected in ruminants (cattle, sheep, and goats) and meat thereof. In 2013, 73 VTEC-related outbreaks were reported across the EU and the main vehicle was bovine meat and products thereof, then "vegetables and juices and other products thereof" and cheese. Other microorganisms responsible for food-borne outbreaks in the EU were *Brucella* and *Trichinella*.

With regard to wooden products,Abdul-Mutalib and others (2015) identified bacteria present on 26 kitchen cutting boards (plastic or wood) collected from different grades of food premises in Malaysia. They used pyrosequencing and quantitative-PCR techniques to study microbial diversity in these 26 different samples and to identify the food-borne bacteria present. Abdul-Mutalib and others (2015) showed that each sample contained a highly diverse microbial community, and 40 bacteria were identified. They also demonstrated that the microbial abundance on cutting boards from different grades of food premises was very similar. In addition, these authors did not reveal a correlation between the cutting board material and the bacterial abundance identified on it. Abdul-Mutalib and others (2015) recommended correct food handling in every kitchen to avoid food-borne illnesses.

To date, wood in contact with food has not been found responsible for any food-borne outbreak, and yet wood tends to be considered less hygienic than other materials in contact with food, such as plastic, stainless steel, and glass, all used in the food industry. This concept stems from the fact that wood is known to be a porous material, which is difficult to disinfect. Although it is less common today, the use of wood in contact with food has generally been considered to be hygienic and safe. It is still used extensively in some traditional sectors worldwide, such as the wine industry, cheese manufacturing, fruit and vegetable storage, as well as seafood and meat transportation. As a result, techniques must be available to study wooden material in different "microbiological environments" according to the different food sectors using wood in direct contact with food. Thus, in this section, after a note on the natural microbial flora of wood, we describe methods for removing microorganisms from wood to carry out certain studies on wooden material, in particular. First, we discuss the survival of certain microorganisms on timber after the manufacturing steps, disinfection, or in the presence of wood-derived antibacterial compounds. If microorganisms survive on surfaces in contact with food, they can be transferred to food and even be responsible for cross-contamination. This important point for the food industry will be addressed in the case of wooden material in contact with food. Clearly, the concept of hygienic work surfaces intended for direct food contact is important because these same surfaces, whatever their material, may be responsible for health crises. For the consumer, the main risks come from the diversity of food brought into contact with working surfaces. For example, a specific microbial population of food A can contaminate food B by cross-contamination on the working surface (Brown and others 1988). For the food industry, the risk comes from the amount of products prepared and dispatched on a large scale, which may cause a significant food-borne illness outbreak.

Natural microbiological status of wooden surfaces

It is well known that wood naturally contains a microbial population according to its moisture content, its decay status, duration of storage after cutting the tree (Dutkiewicz and others 1992), and after contact with water as demonstrated by Beyer and others (2002) with wooden pallets. The microorganisms described in different studies are usually not food pathogens but parts of the total flora of microorganisms commonly found in soil and on plants. This natural population of microorganisms, such as total coliforms, could stem from different sources, for instance, natural soil microflora (Cosenza and others 1970) or root systems.

Methods of microbiological analysis of wood for food contact

Wooden surfaces are generally not considered smooth because they are rough or porous. Quantitative methods to analyze the microbial contamination of surfaces, such as the agar-contact plate and swabbing methods (Miller 1996; Lortal and others 2009), have been used on wooden surfaces in accordance with the international standard ISO 18593:2004 (Anonymous 2004a). However, these methods show poor recovery rates on this type of porous material (Carpentier 1997). Other techniques, such as stomacher and ultrasonic sound methods (Le Bayon and others 2010) and brushing methods (Mariani and others 2007), have also been used, but no standard recovery method has been described for wooden surfaces because of the difficulty of recovering microorganisms from this natural material (Ismaïl and others 2013). Ismail and others (2014) demonstrated that a higher yield of microorganisms present on the wooden surface was obtained by destructive methods such as grinding or planing. These authors showed that grinding was the most reliable method for recovering microorganisms from poplar, pine, and spruce samples, with an average yield of 30.1% for *Listeria monocytogenes* on spruce and *Escherichia coli* on poplar and 30.4% for *Penicillium expansum* on poplar at 37% wood moisture content. Planing was shown to be an efficient method for thicker wooden samples. However, there is no scientific evidence that microorganisms trapped within the cavities of wooden surfaces are likely to be transferred to the surface again. More studies must be carried out on this point.

Survival of microorganisms on wooden surfaces: significant parameters

Impact of antibacterial compounds of wood. Like all plants, trees may be subject to microbial attack against which they have developed a number of defense strategies. The first is constituted by their structure and the existence of protective surfaces, such as the periderm and rhytidome, preventing the entry of microorganisms from the outside (Pearce 1996). This first line of defense is completed inside the tree by other mechanisms, such as active or passive low availability of oxygen in the deeper tissues, the presence of other anatomical barriers, limited access to those nutrients required for microorganism survival, the synthesis of lytic enzymes and gum, and the presence of antimicrobial compounds (Pearce 1996). The latter are an important defense mechanism because they persist, even when the tree is transformed to become food contact material for example (Canillac and Mourey 2001).

In fact, several studies have been carried out on the antimicrobial properties of wood compounds. Most of the time, the target microorganisms are in contact with either purified components or wood extracts obtained after extraction with a solvent. These microorganisms are usually bacteria, yeasts, and molds that are relevant to food hygiene. The compounds most frequently studied belong to a small number of classes: phenols, lignans, tannins, stilbenes, flavonoids, and terpenoids (Pearce 1996). Their effects are described as antimicrobial against bacteria but it is not clear whether these are bacteriostatic or bactericidal (Mourey and Canillac 2002), usually depending on the concentration of the antimicrobial component and the microorganism strain. The effects of molds are controversial. Some studies have observed no inhibitory effect of flavonoids and phenolic compounds on *Aspergillus niger* (Rauha and others 2000), while others have shown an inhibitory effect of stilbenes (pinosylvin) and flavonoids (pinocembrin) on *Aspergillus fumigatus* and *Penicillium brevicompactum* (Välimaa and others 2007). Another stilbene, resveratrol, showed a significant inhibitory effect on molds found on human skin, as well as *S. aureus,* another natural occupant on human skin (Chan and others 2002). Overall, yeasts are usually inhibited by extracts of wood or purified compounds (Lee and others 2005). For example, Valimaa and others (2007) showed strong inhibitory activities for *Candida albicans* and *Saccharomyces cerevisiae* with wood extract or purified compounds (pinosylvin and pinocembrin). This result was later confirmed for pinosylvin by Plumed-Ferrer and others (2013) on *Saccharomyces cerevisiae*.

As for bacteria cells, studies have shown an inhibitory effect of wood extracts or purified compounds on a wide range of bacteria of interest in food hygiene. Thus, Karaman and others (2003) carried out extensive screening of a large number of wood species (54) and bacterial strains (143). They employed extracts from *Juniperus oxycedrus* and demonstrated that methanol was the best solvent to extract compounds from wood with an inhibitory effect on the strains tested, whereas the aqueous extracts revealed no inhibitory effect. This is due to the capacity to concentrate some substances inside plants. If their solubility in water is high, the antibacterial property is lost after washing. When antibacterial chemicals are apolar, they may migrate inside the polymer in resins or other organic polymers, leading to a stable property over time. The data also showed that the 143 strains reacted in very different ways, and finally, only 54 strains belonging to 24 different bacterial species were inhibited. It should be noted that none of the tested fungi proved sensitive to wood extracts (Karaman and others 2003).

This diversity of actions is also found in other works. For example, Canillac and Mourey (2001) tested essential oils extracted from maritime pine on *Listeria monocytogenes*, *Staphylococcus aureus,* and fecal coliforms (*Escherichia coli, Klebsiella oxytoca, Enterobacter cloacae*). Only *L. monocytogenes* and *S. aureus* were inhibited by these extracts, coliforms were not (Mourey and Canillac 2002). Conversely, for Chacha and others (2005), *E. coli* as well as *Bacillus subtilis* and *S. aureus* were inhibited by both flavones and isoflavones extracted from wood. *L. monocytogenes* is a bacterium that has been tested in several studies, each time showing sensitivity to extracts or purified compounds (Mourey and Canillac 2001; Plumed-Ferrer and others 2013).

The mechanism of action of compounds involved in such effects is not clear. In fact, it is difficult to find an explanation for the diversity of behavior of bacteria mentioned above. For instance, Plumed-Ferrer and others (2013) reported that pinosylvin has a real inhibitory effect on Gram-positive bacteria (*L. monocytogenes, S. aureus, B. cereus*) but not on lactobacilli and *Lactobacillus plantarum* in particular. Differences in the membrane and its ability to depolarize could explain this difference (Plumed-Ferrer and others 2013). Clearly, wood contains several compounds with antimicrobial effects on a large number of microorganisms (bacteria, yeasts, molds) of interest in food hygiene but whose mechanism of action is not known. To our knowledge, no research has tested these compounds on viruses. Further research is necessary to identify precisely the different compounds and their mechanisms of action, although some are already used in medical treatments (resveratrol) or in the development of food packaging film (Chana-Thaworn and others 2011).

It is necessary to discuss the normalized procedures to evaluate wood antibacterial properties: ISO-22196:2011 is a norm for plastics and nonporous substances (Anonymous 2011) and ISO-20743:2013 is a norm for textiles (Anonymous 2013). ISO 22196 is an effective norm but the polymeric matrix of wood may frequently retain microorganisms, with the possibility of overevaluating the antibacterial activity (Anonymous 2011). According to Ismaïl and others (2014), using a destructive method leads to better microbiological results. To evaluate the antibacterial effect of a porous material like wood, it may be possible to obtain an accurate result by grinding the surface of the material, in the same way as for porous textile fibers. Then, ISO 20743 would be the appropriate norm (Anonymous 2013). However, for an antifungal evaluation, a different norm would be needed. ISO-846:1997, which evaluates the action of microorganisms for plastics (Anonymous 1997c), may be a better choice, considering the possibility of analyzing a sample of wood and exposing it to the action of molds and yeasts for 4 wk in a saturated moist atmosphere. In this case, it is possible to obtain a visual analysis of the final antifungal effect.

Assessment of new and used food wooden surfaces. Impact of cleaning and disinfection. In the 1990s, wooden cutting boards were suspected of being harder to clean because of the porosity of wooden material. During this period, USDA's Food News for Consumers recommended that consumers use plastic instead of wooden cutting boards, but today this institution recommends both types (USDA 2013). In 2014, guidelines were prepared by consumer organizations: Léo Lagrange, an association for the protection of consumers, and the "Confédération Syndicale des Familles et Familles Rurales," according to the principles of good hygiene practice guidelines (Association Léo Lagrange 2014). They have been evaluated by the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) and approved by the French public authorities (Association Léo Lagrange 2014). The experts recommended using 2 cutting boards, 1 for meat and 1 for fruit and vegetables, to avoid cross-contamination between different raw foods and replacing excessively worn cutting boards. They advised washing a wooden cutting board after each use with dish-washing liquid, rubbing it well, and rinsing it with warm water. Finally, the cutting board should be left to dry in the open air or wiped with a clean dry cloth.

In 1994, Ak and others (1994b) compared the cleaning and decontamination of plastic and wooden cutting boards. The objective was to prevent cross-contamination at home, and also in restaurants, retail butcher shops, and the meat industry. The authors used new and used plastic and wooden boards cut into 5-cm square blocks. Wooden cutting boards were made of ash, basswood, beech, birch, butternut, cherry, hard maple, oak, and American black walnut. Plastic cutting boards were made of polyacrylic, polyethylene, foamed polypropylene, polystyrene, and hard rubber. The experimental conditions were based on home kitchens, except that the contaminants were generally monocultures. Ak and others (1994a) studied cross-contamination with 3 strains of *E. coli*, including *E. coli* O157:H7, and 2 strains of *Listeria,* including *L. monocytogenes* and *Salmonella typhimurium*. Surfaces were inoculated by pressure on Petri dishes for low level bacterial concentrations and by direct bacterial deposit on surfaces for high-level bacterial concentrations. To enumerate bacteria on surfaces, they were recovered by pressing a block directly onto a Petri dish or by soaking the contaminated surface in nutrient broth. In the case of *E. coli* O157:H7, the results showed a large reduction in bacteria inoculated on new wooden boards, where a loss of 3 log 10 CFU was observed within 2 h, whereas on plastic the *E. coli* O157:H7 population remained stable. The authors expressed a first hypothesis that the nature of the wood was responsible for the lethality of the bacteria tested in this study, whereas the nature of the plastic allowed bacteria to survive or even grow. A second hypothesis was that the wood had antimicrobial properties, whereas the plastic cutting board did not. Ak and others (1994b) concluded that with reasonable cleaning effort, new or used wooden cutting boards can be used safely in home kitchens. Wooden cutting boards are not synonymous with a high risk of cross-contamination of food. In the case of commercial use (food service kitchens, retail meat-cutting establishments, and meat and poultry processing plants), the authors recommended identifiying the critical points that might affect the safety of cutting board materials.

Another study by Miller (1996) compared the recoveries of beef bacterial microflora from plastic (polyethylene) and wooden (maple and/or beech laminated along the longitudinal direction) cutting boards. The ground beef was in contact with plastic and wooden cutting boards for 0, 30, 60, and 90 min. At the end of the contact, the boards were rinsed with water or scrubbed with 4 different chemical cleaners. Boards were contaminated with *E. coli* O157:H7 and enumerated after each test. The major result was: no statistical difference ($P > 0.05$) between the cleaning step with water or chemical cleaners on wooden and plastic boards. In this study, the authors used cleaners atypical of commercial use (Liquid-Nox and Ajax) and a smaller quantity of water than used commercially. Thus, Miller (1996) suggested washing cutting boards (wooden or plastic) with hot water and using a chemical cleaning agent to minimize the residual bacterial load on these surfaces.

Gough and Dodd (1998) studied the survival of *Salmonella typhimurium* on plastic (polyethylene) and wooden (beechwood) cutting boards before and after a decontamination step, in the presence or absence of food residue. These cutting boards were untreated or scored. *Salmonella typhimurium* was in contact with the wood and plastic for 10 min, and before the boards were rinsed. *Salmonella typhimurium* cells were enumerated in the rinsing solution and also by contact plate on contaminated wooden and plastic surfaces (30, 60, 90, and 120 min). There were 2 experimental conditions: without and with residual fat (from a raw pork chop or chicken breast). The principal results were that more bacteria were counted in the rinsing liquid obtained from plastic boards than from wooden boards. They showed that more bacteria were found on the wooden surface after 2 h of bacterial contact. This study also demonstrated that more bacteria were counted on untreated than damaged surfaces (scored with a scalpel), up to 2 h after the bacterial contact. The authors concluded that bacteria stuck more strongly on wood than on plastic or bacteria were trapped in the wooden boards. In the presence of food residue, there was no significant difference in the recovery of *S. typhimurium* between wooden and plastic boards. The authors suggested that plastic cutting boards should be used in preference to damaged wooden cutting boards. Actually, it was easier to recover bacteria from plastic than from wood, suggesting that *Salmonella* were strongly attached to the wooden cutting boards and not to the plastic ones. However, they also stated that cutting boards are a potential vehicle for cross-contamination whatever the material.

Gehrig and others (2000) compared hygienic aspects of wood and polyethylene boards to determine the risk of food contamination in household and commercial kitchens. Wooden boards were cut in either a longitudinal or transversal direction to simulate cutting boards or chopping blocks, respectively, and the samples were examined either new or after use in commercial and household kitchens. They were compared with samples of new and used polyethylene boards. Surfaces were examined using scanning electron microscopy (SEM). Polyethylene surfaces also appear rugged after use, similar to wooden surfaces. Half of the samples were coated with a fine layer of vegetable fat (containing 10% butter), while half were used as they were after initial cleaning and disinfection. All samples were contaminated with bacteria (*E. coli*) in aqueous solution and cleaned either in a lab dishwasher (commercial use) or manually using a brush (household use). Automatic cleaning at 60 °C for 2 min and rinsing at 65 °C for 1 min was comparable to a commercial dishwashing process. In this case, a commercial detergent containing chlorine was used. Manual cleaning was done at 50 °C using an all-purpose detergent for household use. Most samples were cleaned immediately after contamination; a small part was stored for 15 h at 21 °C in moist conditions to allow germination of bacteria. All wooden samples kept under moist conditions showed considerable bacterial growth, whether the surface was greasy or not. The same was true for polyethylene boards. In the case of household use, polypropylene boards showed a higher amount of bacteria, compared to wooden boards. This could be due to the fact that the wooden boards took up less water during the manual cleaning step and were allowed to dry slightly after cleaning. Greasy surfaces showed more growth of bacteria than clean surfaces in all cases. The highest number of bacteria was found on polyethylene boards after storage. Automatic cleaning in a dishwasher did not totally reduce the number of bacteria: only about 15% of the wooden samples could be considered sterile after the cleaning step as described above. However, only 1 polyethylene sample (out of 60) was found sterile after automatic cleaning. Manual washing using a clean brush resulted in much better results: the bacterial count was reduced to a minimum in all cases, independent of the type of material and conditions of use. The authors concluded that a humid environment, found in many commercial kitchens, will foster the growth of bacteria in all cases. A greasy surface or a polyethylene surface may enhance bacterial growth even more. Thus, cleaning wooden boards does not seem to be more difficult than cleaning polyethylene boards. Hygienic conditions are provided by proper drying after the cleaning step. Nongreasy surfaces of new or used wood enable rapid drying and result in the most hygienic conditions.

Snyder (2008) compared the absorption parameters of wooden (hard maple) and plastic (acrylic) cutting boards. In this study, the author applied Glo Germ® fluorescent powder in mineral oil on both surfaces to perform a simple visual test (Glo Germ TM, Moab, Utah, U.S.A). The powder particle size was about 5 microns in diameter, like bacteria. Before the fluorescent powder application, the cutting boards were scarred. Then the excess powder was removed with a paper towel and cutting boards were taken to a sink, rinsed with hot water, washed, and scrubbed twice. Finally, dried cutting boards were exposed to ultraviolet light and photographs were taken. The author demonstrated that a small quantity of the fluorescent powder had become trapped in the fibers of wood compared to the plastic board which was more fluorescent, especially in the cracks (Figure 1).

Another study was carried out on the efficacy of disinfecting kitchen cutting boards and working food-processing surfaces by soaking with electrolyzed oxidizing (EO) water (Chiu 2006). The aim was to inactive *Vibrio parahaemolyticus*, responsible for large numbers of human gastroenteritis cases associated with raw or cooked seafood consumption. Therefore, *V. parahaemolyticus* may be responsible for cross-contamination from working food surfaces. Materials compared were bamboo (bamboo is not considered a wood but a plant), wood and plastic, representing cutting boards, stainless steel, and glazed ceramic tile, representing food processing plants. Surfaces were inoculated with *V. parahaemolyticus* (5.5 log 10 CFU cm², sample of 25 cm²). Disinfection tests, by soaking with EO water, were carried out for different times: 1, 3, and 5 min. A greater reduction in *V. parahaemolyticus* was noted on wooden and plastic surfaces after 5 min of soaking, whereas *V. parahaemolyticus* was still detected on bamboo. In the case of stainless steel and glazed ceramic tile, *V. parahaemolyticus* was not detected after only 45 s. These results showed that more time was needed to penetrate into wood tissue to inactivate *V. parahaemolyticus* and also that bamboo might contain substances that could interact with compounds in EO water and neutralize its antibacterial activity. Thus, rinsing and cleaning of cutting boards is essential to reduce the risk associated with the intrinsic pathogens of raw fish.

The most recent study comparing wooden and plastic cutting boards after proper cleaning was carried out by Lücke and Skowyrska (2015). They used 3 types of cutting boards: NSF® certified hardwood cutting boards (made of maple), beechwood cutting boards commonly used in homes, and polyethylene hard plastic boards. All boards were originally new. Some of them were used for laboratory tests and others for tests in a real gastronomy environment. Plastic boards were washed in an industrial dishwasher and wooden boards were hand-washed with ordinary detergent. In laboratory tests, boards were cut and inoculated with a food mixture to simulate normal usage at home. In the gastronomy environment, boards were used for 2 mo in a small gastronomy unit as usual (cutting, washing). These boards were returned to the laboratory and were also inoculated with a food mixture. The results after the cleaning step were all acceptable, that is to say wooden boards were microbiologically safe for direct contact with food. No significant differences in microbiological counts on wooden and plastic cutting boards were detected after proper cleaning. No evidence was found for an increased microbiological risk when properly maintained wooden cutting boards are used at home or in gastronomic units (Lücke and Skowyrska 2015). The authors underlined the importance of following the instructions of the manufacturers of wooden cutting boards for cleaning and first use to ensure food safety.

Zangerl and others (2010) evaluated the survival of *Listeria monocytogenes* following cleaning and heat disinfection processes of wooden cheese-ripening shelves to guarantee the safety of cheeses. The survival of *Listeria monocytogenes* on the surface and inside the wooden shelves was studied. Destructive methods were used to recover the maximum *L. monocytogenes* from the wooden structure. One-year-old ripening blocks were inoculated with a mixture of 6 *Listeria monocytogenes* strains (2 ATCC strains and 4 cheese isolates) at a concentration of 5.5×10^7 CFU/mL. Before inoculation, the surface of the wooden blocks was treated with UV light overnight. Then, wooden blocks were dried for 1 h in a laminar-flow cabinet. After 20 to 24 h inoculation, wooden blocks were soaked for 15 min in a solution of hot (50 °C) alkaline detergent (0.5% P3 Gamo plus ST solution, Henkel Ecolab, Düsseldorf, Germany), brushed for approximately 30 s and rinsed with hot water (50 °C). Some of these cleaned blocks were subsequently heated

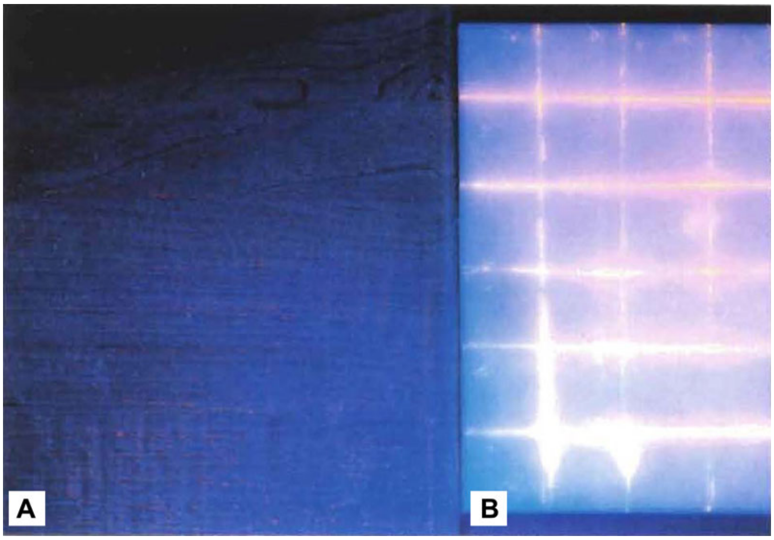

Figure 1–Pictures of a hardwood cutting board (A) and a high-density polyethylene cutting board (B) after wiping them with fluorescent powder, washing and exposing them to long-UV radiation. Copyright agreement from Snyder (2008).

at 80 °C for 5 min and at 65 °C for 15 min, respectively, in a water bath (Julabo, Seelbach, Germany). Survival of *L. monocytogenes* after the cleaning process and heat treatment was evaluated from the wooden shavings and the RODAC plate sampling (agar contact plate method). *L. monocytogenes* was not recovered from shavings of spruce blocks (2 mm of wood layer) after cleaning and subsequent heat disinfection at 80 °C and 65 °C, respectively. These results confirmed the effectiveness of the decontamination process usually applied in Austrian alpine pastures as well as in other countries like France and Switzerland. The authors reported that wooden shelves do not affect the hygienic safety of cheeses in direct contact. Wooden ripening shelves have to be in good repair and thoroughly cleaned by heat treatment. They 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.

In 2000, a French survey carried out among many cheese manufacturers found that brushing wood with water (cold or <35 °C) and then subjecting it to water at high pressure at 85 °C were the most frequently used methods. This cleaning process was responsible for a reduction of more than 5 logs of the total microbial flora (Actia 2000).

Impact of wooden surfaces in food processing. The majority of publications on wooden food packaging are from the 1990s with most appearing after 1998. In 1997, when Carpentier (1997) wrote an overview of the research on the survival of microorganisms on cutting boards, from various materials, domestic or used by professional butchers, there were only 12 scientific papers on this subject. From the beginning of the article, Carpentier (1997) warned about comparing the results between scientific studies. In fact, the researchers were not using the same experimental parameters: (i) method of surface contamination; (ii) origin and condition of microorganisms used to contaminate surfaces; (iii) contact time between microorganisms and the surface before evaluation of contamination; (iv) type of wood used; (v) orientation of wood fibers; (vi) wood moisture content; (vii) surface state of wood; (viii) fouling of wood prior to contamination; (ix) method of sampling to count microorganisms. Whatever the mode of inoculation of the wood, and whatever the extraction technique, the number of microorganisms may decrease after 30 min of contact. This report showed that the hygiene of chopping boards depends mostly on the moisture content of wood, which plays a major role in decreasing the number of microorganisms. In fact, dry wood causes a drastic reduction in living microorganisms, while survival is greater on wood that is wet or covered with organic matter, as is the case on meat chopping boards.

Dervisoglu and Yazici (2001) studied the production process of Kulek cheese to standardize it. Kulek, a ripened acid-curd cheese with rennet added, is one of the most important cheeses consumed in Turkey. During 3 mo, they analyzed the effect of packaging materials and examined microbiological changes during ripening. They compared the ripening process in wooden containers constructed from dry poplar boards, 1.5 cm in thickness, and plastic containers, 3 mm in thickness, purchased from a local store. First, all field samples analyzed in this study did not contain coliforms. Next, specific cheese microorganisms were followed during the ripening period. It was noted that microorganisms rapidly increased during the first 30 d. Total bacteria counts gradually increased in wood and plastic, but the difference was not significant. Yeast and mold counts increased up to 2 log CFU/g in wood and plastic, but the increase was only significant for wood compared to plastic ($P < 0.05$). Cheese samples on wood had more proteolytic microorganisms and psychotropic bacteria than cheese samples on plastic ($P < 0.05$). In general, the microbial results indicated that wooden material had a better permeability to air and moisture to enhance microbial growth. Thus, the authors recommended using wooden packaging for ripening Kulek cheese to obtain better results.

Mariani and others (2011) carried out a study to characterize the development of *Listeria monocytogenes* on wooden cheese boards for ripening. The wooden shelves for the production process of the Protected Designations of Origin (PDO) "Reblochon de savoie" were cut lengthwise in spruce wood (*Picea abies*). In this study, the traditional ripener collected cheeses from different farmhouse origins. The authors compared inoculations on native or autoclaved wooden samples after cleaning-drying, and also on native wooden samples before cleaning-drying after 2 incubations. Two strains of *Listeria monocytogenes* were selected according to their behavior after inoculation on wooden shelves: the most and the least resistant. Then, wooden shelves were inoculated with a static deposit for 30 min and the wooden samples were re-incubated at 15 °C in boxes in 98% saturated humid air. After 1 or 12 d of incubation, microorganisms were recovered by the ultrasound method for enumerating bacteria. The results demonstrated that, whatever the conditions tested, a significant reduction in *L. monocytogenes* on native wooden shelves was noticed after 12 d of incubation. However, a clear pathogen growth occurred when the resident microorganisms were heat-inactivated on wooden shelves. This effect was observed on wooden samples harvested before and after cleaning-drying, whatever the cheese origin. Mariani and others (2011) concluded that the resident microbial biofilm living on wooden ripening shelves displayed a stable anti-Listeria effect according to the experimental ripening conditions.

In the food plants making dried egg pasta (such as screw-shaped pasta, spirals, and so on), wooden trays are used to dry the fresh pasta. Only a part of them is in direct contact with the pasta, and wood is now gradually being replaced by plastic. This step in pasta production is difficult and expensive and there are national regulations for reducing the water content. Filip and others (2012) counted the total number of microorganisms on wood and plastic material used for pasta trays by a swabbing method. The authors analyzed 105 samples from wooden trays (*Abies spp.*) and 105 from plastic trays (PET). They evaluated with regard to total aerobic counts (TAC), Enterobacteriaceae, *Escherichia coli*, molds, yeasts, and *Staphylococcus aureus*. The aim was to answer the question, "Does the tray material and/or location of the swab sample influence the colony-forming unit (CFU)/20 cm²?" First, the results showed that the total number of microorganisms (CFU/20 cm²) was significantly lower on wooden frames compared to plastic frames and that 30% of swabs sampled from plastic frames exceeded 200 CFU/20 cm², whereas the value for wooden frames was only 3%. Second, concerning microorganism counts, *Escherichia coli* was not detected in the 210 samples. Between wooden and plastic frames, there was no difference for Enterobacteriaceae counts. *Staphylococcus aureus* was significantly lower on wooden than on plastic frames: 54% and 3% of swabs were positive on plastic and on wooden frames, respectively. The counts of molds and yeasts were significantly lower on wooden frames compared to plastic ones. With these field test results of surfaces in contact with food, the authors concluded that wood is appropriate in the pasta food industry from a hygienic and technological point of view.

Another food industry process using wood is cider production. Swaffield and others (1997) were the first to describe biofilms on wood in this environment. This study identified bacteria (lactic acid and acetic acid bacteria) and yeasts isolated from wooden cider fermentation vats. The authors showed that microorganisms penetrated the porous wood to a depth of 1.2 cm within 2 wk. They concluded that these kinds of stable biofilms influenced the organoleptic profiles of cider.

Mariani and others (2007) described the natural biofilms on wooden shelves used for the ripening of Protected Designation of Origin (PDO) "Reblochon de Savoie" to provide further data. In fact, the safety of using wood during cheese ripening has frequently been questioned. The amount of cheese ripened on wood is estimated to be greater than 350000 tons per year in France, especially in Registered Designation of Origin productions. The ripening shelves were cut lengthwise from spruce wood (*Picea abies*) and had been used from 6 mo to 14 y in cheese ripening. Mariani and others (2007) analyzed 50 wooden shelves of 3 different ages (from 4 y old to 8 y old) at the end of the cheese ripening process and cheeses came from 8 farm producers. First, wooden shelves were used on the producer's farms at 17 °C with 95% relative humidity. Second, wooden shelves were taken from the traditional ripener for a 2-stage ripening in 2 ripening rooms (i) 13 °C and 95% relative humidity and (ii) 14 °C and 95% relative humidity. Then biofilm was removed by the ultrasound method to count bacteria and yeast populations from different wooden shelves. Pseudomonas cells were detected only at low levels (3.0 log 10 CFU/cm^2), and coliforms at very low levels from the shelves analyzed in the summer (<2.2 log 10 CFU/cm^2). Microbial characterization of wooden shelves supporting cheese during ripening was performed during 2 different seasons (summer and autumn). The authors showed that the dominant microflora was constituted of Micrococci, Corynebacteria, and yeasts and was homogeneous for cheeses of different origins. They also found Leuconostocs, facultative heterofermentative Lactobacilli, Enterococci, Staphylococci, and Pseudomonas at lower levels. These populations were not statistically different between wooden shelves of different ages and there was no seasonal effect on the microflora enumerated. The authors determined the physicochemical properties (pH, water activity [aw], and salt concentration) of the wooden shelves. This study underlined that the origin of the cheese had a statistically significant impact on the physicochemical properties of wooden shelves, whereas the age of the shelves did not influence these parameters. These data demonstrated the stability of the technological biofilms present on wooden shelves.

"Cantal" and "Salers" are PDO (Protected Designation of Origin) cheeses produced in France. The raw milk is placed directly into a traditional wooden vat called a "gerle." This cheese is made without lactic starters, and the use of the wooden "gerle" is mandatory in its production regulation. It is a cylindrical or conical wooden vat made of chestnut wood with a capacity of 100 to 1000 L (Lortal and others 2014). In 1997, Richard (1997) was the first to observe the biofilm of wooden "gerles," compounds of yeasts and bacteria, using scanning electron microscopy. Didienne and others (2012) analyzed the characteristics of microbial biofilms on these wooden "gerles" used for "Salers" production. They explored "gerles" from 10 different farms and showed that biofilm was dominated by Lactobacilli, Leuconostocs, Gram-negative bacteria, yeasts, and molds. They also described a large biodiversity between these 10 wooden vats, which correlated with management procedures. In the light of these results, the authors concluded that wooden "gerles" were very efficient in the development of desirable lactic acid bacteria and thus safe for cheese ripening.

Menendez and others (1997) assessed the presence of *Listeria* spp. in a Spanish cheese factory. A total of 311 samples (liquids [10 mL] and surfaces [400 cm²]) were analyzed within a period of 10 mo. Forty-six samples were positive for *Listeria* spp., of which 36 were *L. innocua*-positive, 8 positive for *L. monocytogenes,* and 2 positive for *L. welshimeri*. Twenty percent of raw milk samples were *Listeria*-positive. *L. monocytogenes* was detected in a sample taken from a wooden board out of 5 samples in total. Conversely, no *Listeria* was detected on stainless steel ripening shelves. However, it should be noted that the samples were taken on arrival of the "raw" milk before any transformations and *L. monocytogenes* was also detected. How can we be certain that the wood was responsible for contamination? The authors speculated that changing the old machines, preventing workers from going outside, changing the disinfection system, and removing wood from the ripening cellars were the criteria for improving the quality of this cheese. Thus, a hypothesis can be advanced that milk initially contaminated with *Listeria monocytogenes* was the one used in the manufacture of cheese in contact with wooden ripening shelves and not with stainless steel ripening shelves. In fact, *Listeria monocytogenes* was also detected on the press, floors, and packaging equipment. Thus, a cross-contamination event could have occurred between the wooden ripening shelves and one of these contaminated plant elements.

For centuries, wood has been considered a natural package for the ripening of various food products, especially cheese. New food safety regulations are contributing to the substitution of wood by other materials, like polypropylene, high-density polyethylene or stainless steel (Galinari and others 2014; Scatassa and others 2015). However, the replacement of wooden utensils by other materials changes the characteristics of cheese, affecting the traditional flavor and texture (Galinari and others 2014).

In Brazil, Galinari and others (2014) analyzed the biofilm composition on wooden utensils used for the production of a Brazilian artisanal cheese: Minas. In fact, from 2002, the cheese makers had to respect new GMP in which wood was replaced by other materials. However, this resulted in changes in the traditional characteristics of this cheese. Biofilms on wooden surfaces were evaluated by the swabbing method. This study concluded that biofilms are responsible for microbiological safety but also play an important role in cheese ripening. It was shown that 2 out of 6 ripening shelves were detected positive for *Staphylococcus aureus* and 1 was detected positive for *E. coli* prior to contact with the cheese. However, the authors underlined that cheeses in contact with all these 6 wooden ripening shelves were not contaminated by *S. aureus*, *E. coli*, *L. monocytogenes*, and *Salmonella*. Then, the authors made the link between milk, initially contaminated with *S. aureus* and coliforms, with biofilms found on the wooden utensils that were analyzed. Actually, milk is the main source of microbial flora responsible for biofilm formation on wooden surfaces and cheese rind. Thus, this research confirms that the microbiological quality of cheese is directly linked to the microbiological quality of milk, and GMP such as the maintenance of wooden surfaces rather than their replacement.

In the south of Italy, wood has been used as a traditional material for cheese production from raw milk without the inoculation of starter cultures (Gaglio and others 2015; Scatassa and others 2015). Scatassa and others (2015) evaluated the capacity of bacteria to colonize the inner surface of the wood, assuring a specific microbial composition, which is transferred to the milk and the curd during milk production. The authors studied 20 wooden vats (13 made of chestnut and 7 of Douglas fir) used for the production of Caciocavallo Palermitano cheese and the Vastedda della valle del Belice cheeses. Biofilms on wooden surfaces were collected by the brushing method. The enumeration results showed that the lactic acid bacteria (LAB) isolated from the wooden cheese vats were predominantly *Lactobacillus casei*, *Enterococcus faecium*, *Lactobacillus rhamnosus*, *Streptococcus thermophilus* and *Pediococcus acidilactici*. Moreover, the efficacy of the sanitation procedures applied during cheese production was demonstrated, as no indicator microorganisms (coliforms and *E. coli*) or pathogens, such as *Listeria monocytogenes*, could be detected (Scatassa and others 2015). Scatassa and others (2015) concluded that GMP of these Sicilian cheeses and good maintenance of wooden vats are 2 very important conditions for the achievement of food safety objectives.

Gaglio and others (2015) evaluated the development of a stable biofilm on the virgin wooden surfaces of vats used for Sicilian cheese production. Four chestnut vats were inoculated with a natural whey starter culture (*Lactococcus lactis* subsp. *cremoris* PON36, PON153, PON203 isolated from cheeses). In fact, the authors wanted to grow specific bacteria, as a biofilm on the surface of the wood, which could be transferred to the milk during the cheese making process. However, the antibacterial properties of wood had to be overcome. To avoid the prevention of bacteria attachment and enable biofilm formation, the wooden surface was washed daily with hot water (75 to 80 °C) for 30 d prior to biofilm activation (Gaglio and others 2015). *Lactococcus lactis* subsp. *cremoris* showed a high capacity to form stable biofilms on the wooden surface, with counts higher than 6 log CFU/cm^2. This amount of Lactic Acid Bacteria ensured the correct inoculation of the milk, maintaining the traditional organoleptic characteristics of the cheese (Gaglio and others 2015; Scatassa and others 2015). The microbiological analysis of the neo-formed biofilms on the wooden surfaces did not reveal *Clostridia*, coagulase-positive *Staphylococci*, *Salmonella* spp., *L.* monocytogenes, *E. coli* or *Pseudomonas* spp. The authors suggested that this was probably due to the specific acid conditions on the wooden surfaces with *Lactococcus* new biofilms formed by *Lactococcus lactis* spp. They concluded that wooden surfaces may be used as controlled inoculation surfaces for the safe production of traditional cheese.

Microorganism transfer resulting from wood-food contact. Influential factors

Influence of wood properties. Above we described the study performed by Ak and others (1994b) comparing wooden and plastic cutting boards intentionally contaminated with 3 different microorganisms. They tested the impact of storage temperature (room temperature and 4 °C) and humidification by placing wooden and plastic blocks at room temperature and +4 °C overnight with humidification in both conditions. *E. coli* were not or only somewhat recovered from wooden blocks (2.7% for maple and 1.6% for birch with oil at room temperature), whereas *E. coli* could be recovered from plastic (from 3.9% to 158.3% indicating bacterial growth at room temperature or 4 °C). A multifactorial analysis of variance confirmed that recoveries from wooden and plastic blocks were significantly different ($P < 0.01$).

The anatomy of wood, particularly its porous nature, plays an important role in microorganism survival. Gilbert and Watson (1971) demonstrated that scored wood was more contaminated than new wood. Ak and others (1994a) showed that wooden cutting boards covered with a multilayer of food residue did not absorb bacteria as quickly as new wooden cutting boards.

Schonwalder and others (2000, 2002) performed 2 studies concerning *Escherichia coli* and *Enterococcus faecium* survival on different wood species including Scots pine (*Pinus silvestris L.*), Norway spruce (*Picea abies Karst.*), European larch (*Larix deciduas Mill.*), beech (*Fagus silvatica L.*), and black poplar (*Populus nigra L.*) compared to plastic. Scots pine had an antibacterial effect on *E. coli* and *Enterococcus faecium*, which could be due to antibacterial substances from Scots pine and also its hygroscopic properties. The authors concluded that the hygienic characteristics of the wood depended strongly on the penetration and absorption of the material. In fact, when bacteria were rapidly transferred into the wood, the surfaces were quickly free of bacteria. This absorption characteristic depended on the wood species and varied considerably.

Microorganisms can penetrate transversal cuttings more deeply (more than 4 mm deep) than wood cut longitudinally as reported by Prechter and others (2002). Their study dealt with the hygienic aspects of wooden cutting boards for household use and compared the results with plastic boards. The penetration depth of bacterial cells (*E. coli*) and bacterial spores (*B. subtilis*) was determined in cutting boards of different kinds of wood, cut in a longitudinal or transversal direction. Microorganisms could enter more than 4 mm in transversal cuttings, much deeper than in longitudinal cuttings where the maximum penetration depth was less than 1 mm for viable bacteria and less than 2.5 mm for spores. Cleaning was more effective when the wooden boards had a longitudinal orientation (that is, along the stem axis) and when the surface was smooth. Even for rugged surfaces, more than 95% of bacteria could be removed, which is quite sufficient for household use. Cleaning plastic boards made of polyethylene was slightly more effective. In most cases, brushing the surface and using a household detergent proved sufficient for elimination of hygienic risks. The sanitation could be improved, if necessary, by using bleaching agents (sodium percarbonate plus bleaching activator TAED) or 5% acetic acid. The authors concluded that wooden cutting boards do not pose a risk in private households if handled properly. Quick drying of cleaned cutting boards was the most effective in reducing residual bacteria.

Soumya and others (2013) aimed to predict microbial potential adhesion on different wood species using a thermodynamic approach. They studied the adhesion of 12 microorganisms, including 6 species of bacteria: *Bacillus subtilis, Bacillus* sp., *Pseudomonas pseudoalcaligenes, Klebsiella* sp, *Acinetobacter lwoffi,* and *Oceanobacillus picturae,* and 6 species of molds: *Aspergillus niger, Penicillium expansum, P. granulatum, P. commune, P. chrysogenum,* and *P. crustosum*. The timber species tested were cedar, beech, pine, ash, oak, and teak. The authors showed that *Bacillus subtilis* was the only hydrophobic bacterial strain, whereas a similar hydrophilic character was found for the other strains. The highest hydrophilicity was shown for *Penicillium commune*, *Penicillium crustosum,* and *Penicillium chrysogenum* spores, while *Penicillium chrysogenum* was the most hydrophilic among all the mold species. This study demonstrated that bacterial cells have a greater ability than mold spores to adhere on wood. However, bacterial adhesion was dependent on the bacteria studied, for example, the adhesion process of *Klebsiella* sp. was unfavorable to beech but favorable to oak. Except for teak wood, generalizations about the adhesion of microorganisms on wood species cannot be made because it proved to be dependent on the wood species and the microorganisms tested in this study.

Di Grigoli and others (2015) studied the influence of the wood during the manufacture of Caciocavallo Palermitano cheese. They evaluated the variations in physicochemical characteristics and microbial populations during the ripening step, comparing cheeses produced traditionally using wooden equipment to a standard production using stainless steel equipment. The wooden equipment used at each step of the Caciocavallo Palermitano cheese production were a vat for milk coagulation, a stick for curd breaking, a bowl for curd pressing, a cane lattice for residual whey loss by pressing, a horizontal stick for curd acidification testing, a truncated conical vat for curd stretching, and a form for molding. Di Grigoli and others (2015) showed the microbial evolution during ripening according to the cheese-making conditions and ripening time. For example, they compared the lactic acid bacteria (LAB) populations from both the traditional and the standard productions. Only 1 LAB strain *Lactobacillus delbrueckii* was found in the traditional production, highlighting the higher LAB biodiversity of the traditional cheese productions compared to the standard ones. This study demonstrated that *Enterococcus faecalis, E. casseliflavus,* and *E. gallinarum* were present during cheese maturation only in the traditional production. In particular, *E. faecalis* was found to dominate the Enterococcal population at the end of the ripening step, corroborating the role of the wooden vat in the variation of LAB populations during Caciocavallo Palermitano cheese ripening. They showed that chemical and physical qualities of these cheeses were highly influenced by the cheese manufacture using wooden equipment.

Influence of contact time. As described above, Chiu (2006) carried out a study on the survival of *Vibrio parahaemolyticus* on cutting boards and working food processing surfaces. The materials compared were bamboo, wood, and plastic, representing cutting boards, and stainless steel and glazed ceramic tile, representing food processing plants. Surfaces were inoculated with *V. parahaemolyticus* for different contact times: 5, 10, 20, and 30 min for wood, bamboo, and plastic, and 15, 30, 45, and 60 min for stainless steel and tile. The authors noticed that, after 20 min, *V. parahaemolyticus* decreased rapidly on bamboo and wooden boards but seemed to survive better on plastic. After 30 min, viable cells of *V. parahaemolyticus* were not detected on bamboo or wooden cutting boards but were present on plastic boards. The results obtained for food processing surfaces showed that *V. parahaemolyticus* was still present on stainless steel and ceramic tile after 30 min and decreased rapidly after 45 min. After 1 h, *V. parahaemolyticus* was not detected on stainless steel, whereas it was not detected after 30 min on wooden surfaces. The authors concluded that *V. parahaemolyticus* survived better on food processing surfaces than on cutting boards made of wood, bamboo, and plastic.

Influence of moisture content. Residues on food worktops can be the cause of the presence and proliferation of bacteria in food contact material. These residual organic materials easily impregnate porous or damaged surfaces and protect microorganisms due to the moisture content.

Above we described the study performed by Ak and others (1994b) comparing wooden and plastic cutting boards intentionally contaminated with 3 different microorganisms. They tested the impact of air-drying, which influences the surface moisture content. Uncovered and contaminated wooden and plastic blocks were placed under a laminar-flow hood for a short time. *L. monocytogenes* decreased significantly on the plastic blocks (from 6.8 log 10 CFU to 5.8 log 10 CFU within 3 h) and more significantly on wooden blocks (from 6.8 log 10 CFU to 4.5 log 10 CFU within 3 h). When the same test was repeated, but with the blocks covered, *L. monocytogenes* increased slightly on the plastic, while declining again on the wooden blocks.

In the study described above carried out by Chiu (2006) on the survival of *Vibrio parahaemolyticus* on different surfaces, rough and porous materials (bamboo, wood) were compared with smooth materials (plastic, stainless steel, and glazed ceramic tile). *V. parahaemolyticus* was shown to survive better on stainless steel, plastic, and ceramic tile, representing smooth surfaces, and not so well on rough and porous surfaces such as bamboo and wood. The authors hypothesized that it was probably because smooth surfaces could maintain high moisture content and favor the survival of *Vibrio parahaemolyticus*. In fact, porous surfaces may trap and make unavailable liquid, so the moisture content on the wooden surface decreases and becomes an unfavorable environment for the survival of microorganisms.

The objectives of Abrishami and others (1994) were to evaluate bacterial adhesion and survival on plastic and wooden cutting boards in order to identify the bacterial growth-promoting or -inhibiting properties of these 2 surfaces. For this purpose, *Escherichia coli* was inoculated on new wood (hard maple) and plastic (clear acrylic) cutting boards in dry or wet conditions. The first results about the impact of moisture content revealed the different levels of absorption of the inoculums when wood was wet or dry. For example, 5 min after the inoculation, more *E. coli* cells were recovered (nonadherent) from wet wood than from dry wood. These data suggest a better penetration of liquid into dry wood allowing bacterial adhesion to wooden surfaces and survival of *E. coli*. Under the same conditions, *E. coli* was detected on plastic boards even after 24 h in the dry condition.

Discussion

Wooden packaging in direct contact with food protects it from being wasted on its way from harvest to table. It is important to know that other wooden surfaces, such as boards, and wooden tools are frequently used in traditional food manufacturing. In addition, it should be underlined that the wood timber and products tested in the studies cited above were untreated with chemical products as is commonly done in food industry use. On one hand, new wood is perfectly suitable for food contact if appropriate storage conditions are chosen. On the other hand, reused wooden surfaces must undergo an appropriate cleaning process. For instance, kitchen worktops are generally considered a critical point in food processing. Regardless of the surface material, working surfaces need to be constantly maintained and monitored for cleaning and disinfecting.

Most of the studies described in this review are about the first use and reuse of wooden boards. This seems to be the consequence of an opinion that links the porous nature of wood with a hygienic trouble. Indeed, there is a great deal of evidence that porosity is an advantage for the microbiological status of wood in contact with food, even when processing food. In fact, its structure generates surface cavities that can trap bacteria in a state unfavorable for their survival, so bacterial growth is extremely limited. The rough or porous surface of wood is also an advantage for controlling the level of surface moisture. This was particularly highlighted by the French Agency for Food, Environmental and Occupational Health and Safety (ANSES, France) in the case of maturing wooden planks, which enable the regulation of the moisture content required for biofilm development on cheeses (AFSSA 2008). This same French agency authorizes wooden boards in direct contact with food. In 2014, Lortal and others (2014) described the role of wood as a "reservoir of microbial biodiversity for traditional cheeses" according to the results of safety assessments. Moreover, the natural biofilms which form on wooden surfaces have been proved safe and able to inhibit pathogenic bacteria with mechanisms that need to be further explored (Mariani and others 2011). Thus, according to the studies described above, different untreated timber species can be used for direct contact in the food industry.

Conclusion

As described in this review based on 86 references, wood is suitable for direct food contact. In the case of light-weight wooden packaging, its single use is an additional argument for the safe nature of the wood used in the food industry. Wood represents ecological ideas that are attractive to consumers and these have resulted in a new interest in wood for use in food packaging. In addition, some food products, such as vegetables, fruits, seafood, and cheese, depend strongly on the use of wood as an indispensable packaging material. It is clear that wooden packaging and wooden tool surfaces contribute beneficially to the final quality, safety, and character of many food products.

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