Food Control 73 (2017) 273–280

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

An assessment of *L. monocytogenes* transfer from wooden ripening shelves to cheeses: Comparison with glass and plastic surfaces

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ARTICLE INFO

Article history:
Received 6 June 2016
Received in revised form
4 August 2016
Accepted 8 August 2016
Available online 10 August 2016

Keywords:
Wooden surfaces
L. monocytogenes
Transfer
Cheeses
Cross-contamination

ABSTRACT

Food contact surfaces are subject to contamination by pathogens, which can lead to cross-contamination by transfer to other food products. However, the European regulation specifies that materials intended for safe food contact must not interfere with foodstuff characteristics. Considered a traditional and natural material, wooden boards are used as a "technological tool" during the cheese-ripening process. In France, wood is authorized for food-contact.

The aim of this study was to determine the behavior of deliberately contaminated wooden surfaces in direct contact with foodstuffs. The model studied consisted of spruce ripening shelves experimentally inoculated with a well-known potential hazard throughout the production chain of some dairy products: *Listeria monocytogenes*. Then, the transfer from deliberately inoculated wood to cheese was analyzed and compared with plastic and glass inoculated surfaces.

For this purpose, a protocol was developed and first-use spruce boards were inoculated with *L. monocytogenes* solution at a concentration of 10^5 CFU/cm^2 then the microbial transfer to pressed non-cooked cheeses was studied. Factors such as cheese contact time, wood and cheese moisture contents, were tested. We compared the transfer rate with glass plates and plastic sheets with inclined meshes, commonly used throughout the cheese production chain, in the same conditions.

The results showed a transfer yield below 3% (CFU/cm^2) in the first hour of contact for all surfaces tested and was 0.55% for the wooden boards. No differences were found for drier cheeses or for higher wood moisture content. The wooden porous surface in contact with cheeses was not a factor that increased the *L. monocytogenes* direct transfer rates. In conclusion, wooden shelves have the lowest transfer rate of *L. monocytogenes* to pressed non-cooked cheeses compared to glass and polypropylene surfaces. This results contribute to the suitability of use of wooden material for direct contact with food.

1. Introduction

Food microbiological contamination events, which are responsible for food-borne diseases, can occur at every stage of the food production chain in industry. The nature of the foodstuffs, the hygienic conditions of the premises (Tebbutt, 1991), aero-contamination, procedures and practices of transport and logistics, and transformation processes are all factors that can lead to the transfer of microorganisms, such as pathogenic bacteria, from sources to food products.

These contaminated foodstuffs can then become vectors of cross-contamination by contaminating working surfaces or other food products. Although most outbreaks result from extensive growth at unsuitable storage temperatures, undercooking, a break in the cold chain, and other poor storage conditions, many are associated with bacterial cross-contamination or recontamination.

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http://dx.doi.org/10.1016/j.foodcont.2016.08.014
0956-7135/

For instance, the role of the hygiene practices of consumers in their kitchens is highlighted by scientific studies.

Dawson, Han, Cox, Black, and Simmons (2007) showed that proper and diligent sanitation of food contact surfaces is needed to reduce cross-contamination to food, because even very short contact times result in the transfer of large numbers of bacteria, even in domestic situations. For example, De Jong, Verhoeff-Bakkenes, Nauta, and De Jonge (2008) recommended the use of two different cutting boards, one for raw food and the other for ready-to-eat food. Pérez-Rodríguez, Valero, Carrasco, García, and Zurera (2008) reported that surface to food contact is the common way for foodstuff to be contaminated during food transport and processing. They concluded that high levels of moisture, contact time and pressure could result in a higher transfer between surfaces for the most common ways of bacterial transfer to food. However, when studying transfer from contact surface materials to foodstuffs, some studies showed that the type of material is important too because of its impact on microbial adhesion. Certain studies, like that of Tang et al. (2011) showed no difference between transfers of *Campylobacter jejuni* from wood or polyethylene cutting boards to cooked chicken.

At present, wood can be used as a utensil, as packaging or as a ripening support in the production of traditional cheeses according to the European regulation n° 1935/2004 (Anonymous, 2004b) and n° 852/2004 (Anonymous, 2004a). In France, wood is authorized for food contact by the French decree of November 1945 (Anonymous, 1945) and the information note n° 2012-93 of the French consumer affairs authority (Anonymous, 2012). However, the use of wood has decreased during the last 20 years because it could be considered difficult to clean due to its porosity. Therefore, other materials like plastic and stainless steel have taken its place in the food industry. Nevertheless, several studies have shown that wood can be as hygienic as other materials that is to say wood is safe for food in direct contact as other materials (Ak, Cliver, & Kaspar, 1994; Filip, Fink, Martina, & Jevšnik, 2012; Milling, Smalla, Kehr, & Wulf, 2005). Otherwise, the efficiency of cleaning and disinfection procedures applied to *L. monocytogenes* contaminated ripening wooden shelves, had been assessed (Zangerl, Matlschweiger, Dillinger, & Eliskases-Lechner, 2010): procedures combining cleaning and a subsequent heat disinfection lead to get rid of a 4.5 $\log_{10}$ CFU/cm^2 *L. monocytogenes* contamination. Moreover, some species like spruce and pine wood even demonstrate antimicrobial properties (Schönwälder, Kehr, Wulf, & Smalla, 2002). Considered a traditional and natural material, wooden ripening shelves are used as a "technological tool" during the cheese-ripening process, in which the hygroscopic properties of wooden shelves are appreciated for drying out cheeses. Here, in this study, we choose the model "spruce ripening shelves – *Listeria monocytogenes*" because this bacterium is a well-known potential hazard throughout the production chain of some dairy products (Jemmi & Stephan, 2006). The microbial ecosystems present on wooden food-contact surfaces were also found to contribute to the acidification, diversity and protection of raw milk cheeses, such as pressed cooked Ragusano cheeses (Di Grigoli et al., 2015; Lortal et al., 2009) and pressed non-cooked cheeses (Mariani et al., 2011). Several studies, mostly on cutting boards with appropriate bacterial models, have investigated secondary transfer events in cross-contamination scenario from wood to food, for instance Cliver (2006). However, to our knowledge, the first transfer phenomena from wooden surfaces to food products have not yet been described in the literature except in the study of Montibus et al. (2016). In this recent work, an assessment *of Penicillium expansum* and *Escherichia coli* transfer from poplar crates to apples was performed. Montibus et al. (2016) showed low transfer rates from wooden crates to apples (not exceeding 0.25%), reinforcing the demonstration of the good suitability of wood for food contact.

The aim of this work is to study and quantify the *L. monocytogenes* transfer from inoculated wooden working surfaces to cheeses during their first contact. In the context of this work, ripening shelves studied are used for the first time in contact with the cheeses. As a relevant hygienic health risk in the dairy sector, *Listeria monocytogenes* was chosen as the microbial model of transfer from spruce ripening shelves to pressed non-cooked cheeses. *L. monocytogenes* can be responsible for listeriosis, a potentially fatal food-borne disease (Swaminathan & Gerner-Smidt, 2007) and has been implicated in outbreaks associated with the ingestion of contaminated food (Gandhi & Chikindas, 2007). Moreover, *Listeria monocytogenes* is known to persist on material surfaces (Chaitiemwong, Hazeleger, & Beumer, 2010; Rücker et al. 2014) and is therefore susceptible to being responsible for cross-contamination from working surfaces to food (Scott & Bloomfield, 1990). A comparison of the transfer rate from wooden shelves was carried out with two other types of material surfaces: glass and polypropylene. Different parameters were tested for their potential influence on the transfer rates, such as the cheese moisture content and the contact time between contaminated surfaces and cheeses.

2. Materials and methods

2.1. Tested surfaces

The first-use wooden ripening shelves were cut lengthwise from spruce wood (*Picea abies*) in a local sawmill and dried naturally. Then, before being sent to the laboratory, they were routinely treated like effective ripening shelves at a traditional cheese-ripener place. They were brushed with cold water, naturally air-dried, then placed in the same ripening room as used for the 20-day-old cheeses (D20) for 2–3 days, but not in contact with the cheeses. This permitted to equilibrate for moisture the experimental shelves. During transport to the laboratory for experiments (2 h), the clean dry shelves were wrapped in cling film in order to maintain their moisture content and avoid aero-contamination from the exterior environment. Then, for practical requirements, they were sawn into 20-cm-square boards large enough to carry the analyzed cheeses. They were wrapped again in cling film and put into a closed box. The cheeses boards were stored in a dry cold-room for 2–5 weeks before being used for the experiments. Their moisture level was monitored with a hygrometer and controlled at around 10.9 ± 3.3% moisture content while they were in the ripening room (n = 18). To evaluate *L. monocytogenes* transfer percentages from moistened wooden shelves to cheeses, 3 boards were wetted by immersion in sterile distilled water for 15 s followed by 1 h of drying in ambient temperature. Their moisture content was 34.5 ± 4.2%, measured by the weighing method (Schirp & Wolcott, 2005). The plastic surfaces, inclined mesh awning used in cheese industry (40 holes/cm^2), were bought from a supplier of dairy industry equipment (Sogebul, Poligny, France). These 1-mm-thick plastic sheets were perforated with 1-mm^2 holes and bordered by inclined meshes. This kind of surface is currently used during the draining step of the cheese process. The sheet, made of polypropylene plastic and flexible, was cut into 20-cm^2 specimens to fit with the cheese circumference. The plastic specimens were cleaned in a bleaching bath and dried before being individually placed and trimmed in a thin aluminum tray for UV-light treatment (30 min).

The glass surfaces were represented by 26.2*18.2* 6 cm round Pyrex® glass baking dishes. They were bought in a kitchen store and autoclaved before experiments. The thickness of these glass samples was 3 mm. They were tested for the transfer from the surface