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Survival of *Listeria monocytogenes* after cleaning and sanitation of wooden shelves used for cheese ripening

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view

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3 1 **Survival of *Listeria monocytogenes* after cleaning and sanitation of wooden shelves used**
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5
6 2 **for cheese ripening**
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9
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34 15
35 16 **Abstract**
36

37 17 The effect of cleaning and heat disinfection processes of wooden shelves used for
38 18 cheese ripening on the survival of *Listeria monocytogenes* was examined. The cut boards
39 19 were inoculated with a suspension containing 5.5×10^7 colony forming units (CFU) / ml of *L.*
40 20 *monocytogenes*. Survival of *L. monocytogenes* was investigated in the wood shavings. During
41 21 the 24-hour incubation period in a humidity chamber, *Listeria* counts increased by 0.8 log
42 22 units on average (n=9), compared to results 1 hour after incubation, indicating the absence of
43 23 antimicrobial properties of the wood in use. Additionally, after incubation for 24 hours at
44 24 room temperature, the boards were cleaned by soaking them for 15 minutes in a solution of
45 25 hot alkaline detergent followed by brushing and rinsing with warm water. Some of the

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3 26 cleaned boards were subsequently heat treated at 80°C for 5 minutes and at 65°C for 15
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5
6 27 minutes, respectively. The cleaning procedure alone was not sufficient to render *L.*
7
8 28 *monocytogenes* from the upper 2 mm wood layer inactive. In the case of both temperature-
9
10 29 time combinations for heat disinfection, however, *L. monocytogenes* was not detectable.

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12
13 30 The present study shows that the use of wooden shelves does not affect the hygienic
14
15 31 safety of cheeses if such shelves are in good repair and are thoroughly cleaned and sanitized
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17 32 by heat treatment. Therefore, there is no reason to replace wood employed in cheese ripening
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19 33 processes with other materials.
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29 37 **Holz als Reifungsunterlage für Käse - Überleben von *Listeria monocytogenes* nach** 30 31 32 38 **Reinigung und Desinfektion**

33
34 39 Der Effekt definierter Reinigungs- und Desinfektionsbedingungen auf das Überleben
35
36 40 von *Listeria monocytogenes* auf Holz als Reifungsunterlage von Käse wurde untersucht. Dazu
37
38 41 wurden handelsübliche, längsgeschnittene und gehobelte Bretter in quadratische Stücke
39
40 42 geschnitten und *L. monocytogenes* in einer Schmierwassersuspension mit einer Keimzahl von
41
42 43 $5,5 \times 10^7$ koloniebildenden Einheiten (KbE) / ml aufgetragen. Der Nachweis von *L.*
43
44 44 *monocytogenes* erfolgte durch Abhobeln der obersten 2 mm Schicht mit anschließendem
45
46 45 quantitativen bzw. qualitativen Keimnachweis in den Hobelspänen. Die Listerienkeimzahl
47
48 46 stieg bei Inkubation in einer feuchten Kammer innerhalb von 24 h um durchschnittlich 0,8 log
49
50 47 Einheiten an (n=9), im Vergleich zur Keimzahl nach 1 Stunde. Dies lässt nicht auf eine
51
52 48 antibakterielle Wirkung des Holzes schließen. Im Anschluss an die 24-stündige Inkubation
53
54 49 erfolgte eine Reinigung der Klötzchen durch Einlegen in eine alkalische Reinigungslösung für
55
56 50 15 min bei 50°C und anschließendes Bürsten für ca. 30 sek. Einige der gereinigten Blöcke
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3 51 wurden einer Hitzebehandlung in einem Wasserbad (80°C, 5 min bzw. 65°C, 15 min)
4
5 52 unterzogen. *L. monocytogenes* konnte in den Hobelspänen der obersten 2 mm Schicht nach
6
7 53 Hitzedesinfektion weder bei 80°C für 5 min noch bei 65°C für 15 min nachgewiesen werden.
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9
10 54 Bei Reinigung ohne anschließender Hitzedesinfektion wurden in allen neun Klötzchen
11
12 55 Listerien in den Hobelspänen nachgewiesen.

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15 56 Aus den Ergebnissen kann geschlossen werden, dass die Verwendung von Holz als
16
17 57 Reifungsunterlage bei sorgfältig durchgeführter Reinigung mit anschließender
18
19 58 Hitzedesinfektion die hygienische Sicherheit von Käse nicht beeinträchtigt. Es besteht daher
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21 59 kein Grund das Holz durch einen anderen Werkstoff zu ersetzen.
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29 62 **1 Introduction**

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31 63 During recent decades, the use of wood in food establishments has been all but
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33 64 eliminated for safety reasons. Due to its porous structure, wood is assumed to be harder to
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35 65 clean than plastics or stainless steel. Nevertheless, in traditional milk processing, wood is still
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37 66 used in cheese ripening processes and butter production. For cheese ripening, shelves made
38
39 67 from spruce wood are most commonly used.
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43 68 The Codex Alimentarius (Anonymous 2003) does not approve the use of wood in food
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45 69 processing because it does not correspond to the following definition: “working surfaces that
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47 70 come into direct contact with food should be in sound condition, durable and easy to clean,
48
49 71 maintain and disinfect. They should be made of smooth, non-absorbent materials and inert to
50
51 72 the food, to detergents and disinfectants under normal operating conditions”. However,
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53 73 Regulation (EC) No 852/2004 on the hygiene of foodstuffs established clarity regarding the
54
55 74 application of wood by the following definition: “surfaces (including surfaces of equipment)
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57 75 in areas where foods are handled and in particular those in contact with food are to be
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3 76 maintained in a sound condition and are to be easy to clean and, where necessary, to disinfect.
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5 77 This will require the use of smooth, washable corrosion-resistant and non-toxic materials,
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8 78 unless food business operators can satisfy the competent authority that other materials used
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10 79 are appropriate". In conclusion, the use of wood is possible provided that the safety of food
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12 80 products can be guaranteed. However, food manufacturers themselves are responsible for the
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15 81 evaluation.

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17 82 Numerous studies have evaluated the hygienic status of wood compared to plastics and
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19 83 stainless steel, predominantly in the case of cutting boards for kitchen use (Ak et al. 1994a, b;
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22 84 Park and Cliver 1996; Prechter et al. 2002; Rödel et al. 1994; Schönwälder et al. 2002). In
23
24 85 summary, it can be concluded that plastic surfaces are able to be cleaned more easily than
25
26 86 wooden surfaces. With cutting boards made of plastic, however, the surfaces roughen quickly
27
28 87 and then do not offer any advantages over wooden surfaces regarding their cleaning ability.
29
30 88 Based upon the works of Ak et al. (1994a, b), antibacterial qualities are attributed to wood.
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32 89 These antibacterial qualities are primarily dependent on the type of wood and have only been
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34 90 detected in the cases of oak (Rödel et al. 1994), pine and larch (Schönwälder et al. 2002).

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38 91 Only a few studies on the use of wood for cheese ripening have been published
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40 92 (Richard 1997; Notz 2000). The bacterial flora of the cheese surface was recovered from both
41
42 93 the surface and the inner parts of wooden shelves used for cheese ripening (Richard 1997;
43
44 94 Notz 2000; Mariani et al. 2007). Salt-tolerant micrococci, corynebacteria, yeasts and moulds
45
46 95 were found to be the dominant microflora in a magnitude of 3 – 9 log CFU/cm². The
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48 96 population decreased strongly with increasing distance from the surface; at a depth of 2 mm,
49
50 97 half as many counts as determined on the surface could be detected (Notz 2000). The author
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52 98 concluded that when using wood in cheese ripening, the microbiological risk is low but a
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54 99 complete absence of pathogenic microorganisms cannot be guaranteed. Thus, an efficient
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3 100 cleaning and disinfection of the wood is very important in order to remove contamination
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6 101 with pathogens.

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8 102 Hence, the aim of this study was to evaluate cleaning and sanitation procedures that
9
10 103 guarantee the safety of cheeses when ripening on wooden surfaces. The survival of *Listeria*
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12 104 *monocytogenes* on the surface and the inside of the wooden shelves was monitored. To
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14
15 105 achieve maximum recovery, destructive methods were used to also determine *L.*
16
17 106 *monocytogenes* in the wood structure.

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22 108 **2 Materials and methods**

24 109 **Wooden boards**

25
26
27 110 Longitudinally cut, used spruce boards were tested. These approximately one-year-old boards
28
29 111 came from three different cheese plants producing surface ripened cheeses. The boards
30
31 112 comprised: planed spruce solid wood boards, 3 cm thick, re-planed for use in this study;
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34 113 planed spruce solid wood boards, 2 cm thick; and planed three-layer laminated spruce boards,
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36 114 2 cm thick.

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38 115 The boards were cut into 7 cm x 7 cm squares (wood blocks). Only blocks without cracks or
39
40 116 knots were used. Squares used for cleaning tests were varnished (water-based lacquer, Friocryl
41
42 117 semi-gloss lacquer, pure acrylate, requiring dilution with water, manufactured by O. Fritze-
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44 118 Lacke GesmbH, Vienna) on the side surfaces in order to prevent water from penetrating into
45
46 119 the pores through the sides during the cleaning and sanitation process. Previous experiments
47
48 120 have clarified that the lacquer has no influence on *Listeria* counts. Prior to inoculation with *L.*
49
50 121 *monocytogenes*, the surface of the blocks was treated with UV light overnight. In order to
51
52 122 create moisture conditions comparable to a cheese ripening room, the blocks were stored at 21
53
54
55 123 - 28°C in a moist atmosphere (in an incubator with water bowls) for one week.

56 124 **Test strains and inoculation of the blocks**

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3 125 The wood blocks were contaminated with a mixture of six *Listeria monocytogenes* strains
4
5 126 (ATCC 1911, ATCC 13932, and four cheese isolates originating from red smear and mould
6
7 127 ripened cheese varieties), grown in smear water to simulate cheese ripening conditions with
8
9 128 cheese rind particles on the surface. The smear water was taken after cheese surface treatment
10
11 129 of red smear cheeses and sterilised at 121°C for 15 min. The strains were grown in brain heart
12
13 130 broth (Merck) at 37°C for 24 hours, followed by a twice-repeated growth in sterile smear
14
15 131 water at 37°C for 20 - 24 hours. The six strains were cultivated separately and mixed in sterile
16
17 132 smear water immediately before inoculating the blocks. On a marked surface of 25 cm² in the
18
19 133 centre of the block, 0.2 ml of the *L. monocytogenes* suspension were dispensed by means of a
20
21 134 Drigalski spatula so that counts of between 10⁵ and 10⁶ CFU/cm² were obtained. Thereafter
22
23 135 the blocks were incubated in a wet chamber [sterile glass Petri dishes (18 cm in diameter, 30
24
25 136 mm height) with moistened filter paper (3 ml sterile Aqua dest.) and toothpicks in between
26
27 137 the paper and the blocks] at room temperature (21 - 28°C) for 24 hours.
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34 138 **Removal of the wood surface**

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36 139 The blocks were dried for one hour in a laminar flow cabinet and thereafter 2 mm of the wood
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38 140 surface were planed off, resulting in 3 – 4 g shavings of 0.2 mm thickness.
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41 141 **Recovery of *L. monocytogenes* in the wood blocks**

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43 142 The shavings were homogenized in 200 ml peptone salt solution (ISO 8261) for 2 minutes in
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45 143 a Stomacher 400 Lab Blender (Seward Medical, London, UK) and investigated according to
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47 144 ISO 11290-2 using PALCAM agar as enumeration medium. All tests were done in triple
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49 145 repetition. The *L. monocytogenes* count was determined at 1 hour and 20 – 24 hours after
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51 146 inoculation from the wood shavings, respectively. Additionally, 1 hour and 24 hours after
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53 147 incubation, the *L. monocytogenes* count on the surface of the wood blocks was determined by
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55 148 using the RODAC plate sampling (agar contact plate method) with PALCAM agar. The plates
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3 149 were pressed directly onto the surfaces of the blocks for 5 seconds. The results were recorded
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5 150 as colony counts per 25 cm².

151 **Survival of *L. monocytogenes* after cleaning procedure and heat treatment**

152 After 20 – 24 hours' incubation, the contaminated wood blocks were soaked (contaminated
153 surface downwards) for 15 minutes in a solution of hot (50°C) alkaline detergent (0.5% P3
154 Gamo plus ST solution, Henkel Ecolab, Düsseldorf, Germany), brushed for approximately 30
155 seconds and rinsed with hot water (50°C). Some of these cleaned blocks were subsequently
156 heated at 80°C for 5 minutes and at 65°C for 15 minutes, respectively, in a water bath (Julabo,
157 Seelbach, Germany) with the contaminated surface downwards.

158 The shavings were homogenized in 200 ml half Fraser broth (Merck) for 2 minutes in a
159 Stomacher 400 Lab Blender and *L. monocytogenes* was investigated according to ISO 11290-
160 1 using PALCAM agar as isolation medium. After the cleaning procedure, the *L.*
161 *monocytogenes* count of the blocks was additionally determined by using RODAC plate
162 sampling.

163

164 **3 Results**

165 **Recovery of *L. monocytogenes* in the wood blocks**

166 25 cm² of the wood block surface was inoculated with 0.2 ml *L. monocytogenes* suspension
167 containing 5.5x10⁷ CFU/ml on average, resulting in a calculated count of 4.4x10⁵ CFU/cm²
168 surface on average. One hour after contamination, *Listeria* counts recovered from the wood
169 shavings were 1.8 log on average (n=9), lower than expected from the applied inoculum.
170 During the incubation period of 20 - 24 hours, *Listeria* counts increased by 0.8 log on average
171 (n=9) in comparison to the values at 1 hour, indicating an absence of antimicrobial properties
172 in the wood in use (Table 1). The recovery was independent of the type of wooden boards
173 tested. The *L. monocytogenes* counts on the surface of all wood blocks using the agar contact

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3 174 plate method were investigated after 24 hours of incubation only. From all samples, the
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5 175 method resulted in overgrown plates (> 300 CFU/ 25 cm^2).
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8 176 **Survival of *L. monocytogenes* after cleaning procedure and heat treatment**

9
10 177 The cleaning procedure alone was not able to remove *L. monocytogenes* from the upper 2 mm
11
12 178 wood layer (Table 2). *L. monocytogenes* was detected in all shaving samples. When using the
13
14 179 agar contact plate method, *L. monocytogenes* was not detectable on the surface of 5 of the 9
15
16 180 samples. The counts of the positive samples varied between 1 and 18 CFU/25 cm^2 (median
17
18 181 1.5 CFU/25 cm^2). This clearly shows that the agar contact plate method is not suitable for the
19
20 182 assessment of cleaning and sanitation methods of wood.
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24 183 In contrast, *L. monocytogenes* was not recovered in the wood shavings after cleaning and
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26 184 subsequent heat disinfection at 80°C and 65°C , respectively.
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29 185

30 31 186 **Discussion**

32 33 187 **Comparison of the *L. monocytogenes* counts 1 hour and 24 hours after contamination**

34 188 The findings in this study differ from previous ones (Rödel et al. 1994; Schönwälder et al.
35
36 189 2002) where the authors, using different wood types, partly observed a drastic reduction of the
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38 190 test microorganisms within 20 - 24 hours after contamination. Both working groups used the
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40 191 planed-off wood surface for sampling, but with different layer thicknesses (0.25 mm and 1
41
42 192 mm respectively). Whereas Schönwälder et al. (2002) and Milling et al. (2005a, b) found that
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44 193 wood, especially pine wood, has antibacterial characteristics, several reports concluded that
45
46 194 the reduction of the microbial load is due to the hygroscopic properties and porous structure
47
48 195 of the wood. Absorbed bacteria are attached to the porous wood structure and therefore
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50 196 recovery declines with time after contamination (Boursillon and Riethmüller 2007; Rödel et
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52 197 al. 1994; Lorentzen et al. 2000). The assumption, that microorganisms are adsorbed could
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3 198 explain the varying results between expected and recovered *Listeria* counts one hour after
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5 199 inoculation of the blocks.
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8 200 Furthermore, the disparities between the experimental conditions may explain the different
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10 201 findings, since the recovery of the microorganisms is also dependent on the wood
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12 202 characteristics (wood type, humidity level of the wood, orientation of the wood fibres on the
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14
15 203 contact surface (Schulz 1995)). The experimental design of our study was to simulate
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17 204 conditions found in a cheese ripening room (used boards, high humidity level of the boards
18
19 205 prior to contamination, smear water solution as inoculation medium, incubation in a humid
20
21 206 atmosphere). In contrast, Schönwälder et al. (2002) used boards that were dried for 12 hours
22
23 207 at 103°C. Rödel et al. (1994) did not specify the humidity level of the wood. In addition,
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25 208 many authors showed that the behaviour of microorganisms essentially depends on the
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27 209 presence of organic compounds on the wood surface (Ak et al. 1994 a, b; Rödel et al. 1994).
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29 210 If the wood is treated with nutrient broth, calf serum or a meat fat mixture prior to
30
31 211 inoculation, the capacity of wood to reduce germs decreases.
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36 212 **Cleaning and disinfection of the wood surface**

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38 213 The results clearly show that *Listeria* cannot be eliminated from the wood surfaces solely by
39
40 214 brush cleaning with hot water containing alkaline cleaners. A subsequent disinfection is
41
42 215 therefore necessary. Since chemical disinfectants can destroy the bacteria on the surface but
43
44 216 not inside the pores, rips or splits (Richard 1997), heat treatment seems to be the most
45
46 217 effective disinfection method. According to our findings, heat disinfection at 80°C for 5
47
48 218 minutes and at 65°C for 15 minutes, respectively, destroys *L. monocytogenes* in the upper 2
49
50 219 mm of the wood layer. This also confirms the effectiveness of the decontamination method
51
52 220 usually applied on Austrian alpine pastures: cleaning by means of high pressure, subsequent
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54 221 heat disinfection at 65°C and desiccation under the sun. Other countries also apply heat
55
56 222 disinfection treatment to wooden shelves used for cheese ripening to kill *Listeria*. In
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3 223 Switzerland, immersion of the shelves in boiling water for 2 - 3 minutes as well as steam
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5 224 treatment is recommended (Liengme 2001). In a French study comparing different cleaning
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8 225 methods, a wash cabin with high pressure (75 - 85°C for 30 - 40 minutes) proved to be the
9
10 226 most effective method for decontaminating ripening shelves (Notz 2000).
11
12 227 Generally desiccation is essential for the sanitation of wooden shelves because, beside the
13
14 228 disinfectant effect of UV light, which is more intensive with increasing height above sea-
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16 229 level, a reduction of microorganisms in the inside of the wood due to desiccation can be
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18 230 expected (Schulz 1995; Schönwälder et al. 2002). However, the antibacterial effect of wood
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20 231 due to withdrawal of available water mentioned in the literature should not be overestimated,
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22 232 because the reduction of the available water usually only leads to a reduction in damage to
23
24 233 sub-lethal levels.
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29 234 **Conclusion**

30
31 235 Even high counts of *L. monocytogenes* can be eliminated from wooden shelves if they
32
33 236 are thoroughly cleaned and sanitized by heat treatment. Thus, wood can be used in the
34
35 237 foodstuff sector especially where definite advantages in comparison with other materials exist
36
37 238 and possible disadvantages can be reduced by suitable measures. To age cheese on wood,
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39 239 shelves are required to be in good repair without cracks or knots. Wooden shelves are most
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41 240 suitable for the ripening and storage of cheeses because they regulate the humidity in the
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43 241 ripening room (Kammerlehner 1995). Furthermore, shelves made of spruce wood have the
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45 242 advantage of being relatively cheap and easily available in alpine regions, hence they can be
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47 243 substituted with little effort.
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52
53 244 Many authors show that plastic is not as hygienic as is often stated (Boursillon and
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55 245 Riethmüller 2007, Schönwälder 2002) and they disprove findings that suggest disadvantages
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57 246 associated with wood that make this material less desirable from a food safety perspective
58
59 247 (Boucher et al. 1998; Gehrig et al. 2000; Welker et al. 1997). In addition, the application of
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3 248 wood as a material involved in cheese ripening processes should be discussed in the context
4
5 249 of overall production conditions. The technical equipment required for cleaning of stainless
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8 250 steel hurdles or ripening shelves made of plastic is usually absent in alpine or small-scale
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10 251 cheese plants. Moreover, an effective manual cleaning of these tools is difficult to achieve.
11
12 252 Due to their perforation and sharp edges on the underside, ripening shelves made of plastic do
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14
15 253 not offer any advantage over wood regarding their cleaning ability. Cleaning and heat
16
17 254 disinfection of wooden shelves therefore can be regarded as more convenient than the
18
19 255 cleaning processes required for plastic shelves or stainless steel hurdles, especially in the
20
21 256 context of cheese ripening in alpine pastures or small-scale plants. Nonetheless, it must be
22
23 257 noted that, regardless of the material, all surfaces coming in contact with food need to be
24
25 258 constantly maintained and monitored for cleanliness (Carpentier 1997; Miller et al. 1996).
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33
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37 262

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1 Table 1: Recovery of *L. monocytogenes* (log CFU/cm²) in wood shavings (2 mm of the wood
 2 block surface) after one hour and 24 hours incubation in wet atmosphere (*L. monocytogenes*
 3 inoculum: mean 5.63 log CFU/cm², sd 0.14, n=3)

4
 5 Tabelle 1: Keimzahlen von *L. monocytogenes* (log KbE/cm²) in den Hobelspänen (Oberfläche
 6 bis 2 mm Tiefe) eine Stunde und 24 Stunden nach Kontamination bei Lagerung in feuchter
 7 Atmosphäre (*L. monocytogenes* Inokulum: Mittelwert 5,63 log KbE/cm², sd 0,14, n=3)

	Mean 1 h	sd	n	Mean 24 h	sd	n
Planed spruce solid wood board, 3 cm thick, re-planed	3.87	0.17	3	4.56	0.77	3
planed spruce solid wood board, 2 cm thick	3.77	0.29	3	4.51	0.37	3
planed three-layer laminated spruce board, 2 cm thick	3.76	0.62	3	4.66	0.46	3

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2
3 1 Table 2: Detection of *L. monocytogenes* in wood shavings after cleaning and subsequent heat
4 2 disinfection (2 mm of the wood block surface)
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6 3

7 4 Tabelle 2: Qualitativer Nachweis von *L. monocytogenes* in den Hobelspänen (Oberfläche bis 2
8 5 mm Tiefe) nach Reinigung und anschließender Desinfektion
9 6

cleaning	disinfection	<i>L. monocytogenes</i>	n
50°C, 15 min	-	positive ¹	9
50°C, 15 min	80°C, 5 min	negative ²	9
50°C, 15 min	65°C, 15 min	negative	9

7 ¹ Presence of *L. monocytogenes*

8 ² Absence of *L. monocytogenes*