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1 **Survival of *Listeria monocytogenes* on conveyor belt material with/without antimicrobial**
2 **additives**

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16 Running title: Survival of *Listeria monocytogenes* on conveyor belt

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19 Keywords: *Listeria monocytogenes*, survival, conveyor belt, antimicrobial additives

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25 **ABSTRACT**

26 Survival of *L. monocytogenes* on conveyor belt material with and without antimicrobial
27 additives was investigated. In these experiments, also the effect of food debris (meat, fish and
28 vegetables) and temperature (10, 25 and 37°C) was evaluated. The pathogen survived best at
29 10°C, followed by 25°C and 37°C on both conveyor belt materials. The reduction rate of the
30 pathogen on conveyor belts with antimicrobial additives in the first six hours at 10°C (0.6 log
31 unit) was significantly higher than that on the surfaces without the additives, or in presence of
32 food residue (0.2-0.3 log unit). At high temperature (37°C) and low humidity (20%), a rapid
33 decrease in numbers of the pathogen on both conveyor belt surfaces was observed. Under
34 these conditions, an effect of antimicrobial substances could not be noticed. However, at
35 ambient (25°C) and low (10°C) temperature and high humidity (60-75%), a rapid decrease in
36 cell counts was observed. These results demonstrated that temperature and relative humidity
37 play an important role in the reduction of the bacteria on the surfaces. Antimicrobial conveyor
38 belts reduced numbers of *L. monocytogenes* faster than normal conveyor belts. However, this
39 effect is limited on dry surfaces, where a reduction took place at lower rate than that in the
40 first six hours when the surfaces were still wet. Moreover, the presence of food debris
41 neutralized the effect of antimicrobials. The results suggested that the antimicrobial additives
42 in the conveyor belt could help to reduce numbers of microorganism in particular at low
43 temperature and in an absence of food residues.

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50 **1. Introduction**

51 Contamination of *Listeria monocytogenes* on ready-to-eat cooked meat products most likely
52 occurs after thermal processing. Many studies report that *L. monocytogenes* attaches to
53 various types of surfaces in food processing plants (Beresford et al., 2001; Lunden et al.,
54 2002) such as stainless steel, glass, polypropylene and rubber (Mafu et al., 1990; Wong,
55 1998). The bacteria survive and grow on spots that are difficult to clean and disinfect due to
56 their poor designs, such as slicing machines, conveyor belts (Lin et al., 2006), and narrow
57 openings between surfaces and apparatus (Tompkin et al., 1992). Of the many processing
58 surfaces, conveyor belts are of great concern in food processing because during transfer of
59 food products the belts are often contaminated with high numbers of microorganisms
60 including foodborne pathogens such as *L. monocytogenes* (Thévanot et al., 2005) and
61 *Escherichia coli* O157:H7 (Keeratipibul et al., 2009). Inadequate cleaning and disinfection
62 procedures can cause accumulation of bacteria on surfaces and formation of biofilms, which
63 are difficult to control or remove by conventional cleaning methods (Chmielewski and Frank,
64 2003; Smoot and Pierson, 1998). However, recontamination from contaminated surfaces to
65 food products can be prevented by hygienically designed apparatus, cleaning and disinfection
66 and zoning (Anonymous, 1991). At present, it is possible to add antimicrobial additives (e.g.
67 triclosan, silver) to surface materials to control the survival or growth of microorganisms on
68 the surfaces. Triclosan and silver are GRAS (generally recognized as safe) and approved by
69 the U.S. Food and Drug Administration for their safety (Jones et al. 2000) and are currently
70 used in medical devices, food processing, and building materials (Feng et al., 2000). Several
71 studies have been published about the effect of antimicrobial additives in surface materials
72 used in the food processing industry against microorganisms, e.g. antimicrobials incorporated
73 in cutting boards, food containers (Wijnhoven et al., 2009) and the inner liner in refrigerators
74 (Kampmann et al., 2008). However, few studies have investigated survival of microorganisms

75 on antimicrobial surfaces in comparison to normal surfaces in practical situations of low and
76 ambient temperatures or with food residues.

77 In this study, the survival of three strains of *L. monocytogenes* was studied on conveyor
78 belt materials with and without antimicrobial additives at different temperatures and in the
79 presence and absence of food residues.

80

81 **2. Materials and methods**

82 *2.1 Bacterial strains*

83 *L. monocytogenes* LF 38 (serotype 1/2a isolated from cooked ham), LF 36 (serotype
84 1/2b isolated from cooked sausage) and LF 29 (serotype 4e isolated from cooked sliced
85 sausage) obtained from the Food and Consumer Product Safety Authority (Zutphen, the
86 Netherlands) were used in this study. Stationary-phase cultures of all strains were stored in 1
87 ml cryo vials (Greiner Bio-one, Frickenhausen, Germany) with 25% (v/v) glycerol (Fluka-
88 Chemica, Buchs, Switzerland) and glass beads (2 mm, Emergo, Landsmeer, the Netherlands)
89 at -20°C. For each experiment, one glass bead of each strain was transferred to tryptone soy
90 agar plates (TSA, Oxoid, UK), which were incubated at 30°C for 24 h. A single colony was
91 picked, inoculated into 10 ml brain heart infusion broth (BHI; Difco, Becton Dickinson,
92 Maryland, USA) and incubated for 72 h at 10°C or for 24 h at 25°C and 37°C.

93

94 *2.2 Conveyor belt material (US Patent 6994209)*

95 Two types of food grade conveyor belt materials were used in this study. The control
96 conveyor belt material without antimicrobial additives (CM-) (FNB-6EZCT, Habasit
97 Netherlands BV, Nijkerk, the Netherlands) was composed of polyester fabric impregnated
98 with thermoplastic polyurethane (TPU). The conveyor belt material with antimicrobial
99 additives (CM+) (FNB-6EZCT+H14, Habasit Netherlands BV, Nijkerk, the Netherlands) had

100 the same composition and was impregnated with thermoplastic polyurethane (TPU) and the
101 antimicrobial compound HyGUARD[®]. According to the US patent 6994209 (Cediél et al.,
102 2006), antimicrobial substances used are silver zeolite, aluminum oxide, calcium oxide,
103 magnesium oxide, zinc pyrithione, oxybisphenoxarsine or a combination of those. The
104 conveyor belt surfaces were cut from the roll in sheets of 10 x 10 cm.

105

106 *2.3 Food debris*

107 Raw food (minced meat, smoked salmon and chopped lettuce) were bought from a local
108 supermarket, kept in a refrigerator (< 7°C) and used within 24 h. To prepare a 10% suspension
109 of the food debris, 10 g of each selected food was aseptically placed in Stomacher[®] bags and
110 90 ml peptone saline solution (PSS: 8.5 g/l NaCl (Merck, Darmstadt, Germany) and 1 g/l
111 Neutralised Bacteriological Peptone (Oxoid, Basingstoke, England)) was added. The food was
112 then homogenized in a stomacher (Stomacher[®] 400 Circulator, England) for 1 min at 260 rpm.

113

114 *2.4 Artificial contamination of the surfaces*

115 The conveyor belt sheets were washed with anionic-active detergent, rinsed with hot
116 water (70-80°C) and air dried. Before each experiment, the belt surfaces were disinfected with
117 70% ethanol for 15 min and air dried for 15 min in a laminar flow cabinet.

118 Appropriate serial dilutions of the overnight cultures were prepared in PSS. For the
119 selected final dilutions (approximately 10⁶ CFU/ml), PSS containing 0.1% Tween 80 (Merck,
120 Hohenbrunn, Germany) was used to lower the surface tension in order to obtain an even
121 spread of bacteria on the surfaces. The influence of food debris on the antimicrobial action
122 was investigated by preparing the final dilution of the test cultures in a 10% suspension of the
123 food products (with 0.1% Tween 80). From these tubes with and without food debris, 250 µl
124 was spread over 100 cm² of the belt surfaces by using a cotton swab (Sterile wooden/cotton

125 swab, LP Italiana SPA, Milano, Italy). The conveyor belt sheets were then placed at 10°C,
126 25°C and 37°C for 72 h. Relative humidity (Rh) was measured at each storage temperature
127 with a digital hygro-thermometer (VWR International BV, Amsterdam, the Netherlands).

128

129 *2.5 Enumeration of test strains from the conveyor belt sheets*

130 The conveyor belt surfaces were sampled after 0, 6, 24, 48 and 72 h by rubbing 10 x
131 10 cm areas with sterile cotton swabs (EUROTUBO® collection swab, Delta lab, Rubi, Spain)
132 stored in 5 ml of buffered peptone water (BPW) according to ISO 18593:2004 (Anonymous,
133 2004). The swabs were reinserted in the BPW, vortexed and resuscitated for one hour. After
134 that, 2 x 1 ml of the swab solution was added to 2 tubes with 2 ml peptone saline solution
135 (PSS). The contents of these tubes (3 ml) were plated onto two Petrifilm™ Environmental
136 *Listeria* plates (3M Company, St.Paul, MN, USA.). All plates were incubated at 37°C for 24-
137 30 h, and the numbers of recovered *L. monocytogenes* were determined and transformed into
138 log CFU per 100 cm².

139 Reduction rates of *L. monocytogenes* (log CFU/100 cm²/h) were calculated by
140 dividing the reduction in cells counts on the surfaces in times by lapse times (h), which were 6
141 h from the first six hours and 66 h from 6 to 72 h.

142 *2.6 Statistical analysis*

143 The results of triplicate experiments were combined. The significance of difference in
144 survival of *L. monocytogenes* ($P < 0.05$) on types of the surfaces, *Listeria* strains, food
145 residues, and temperatures was determined with ANOVA and a general linear model using
146 SPSS for Windows 98/NT/2000 release 15. A balance of input (initial concentration of
147 inoculum) and output (number of bacteria on the tested surfaces before and after sampling)
148 was calculated.

149

150 **3. Results and discussion**

151 As can be seen in Fig. 1 (A, B and C), a rapid decrease of 2.5 - 4 log CFU/100 cm² of
152 the test strains on the test surfaces was observed during the first six hours. Thereafter, the
153 reduction was only 0.1 log unit in 6 h. These results were similar with those of
154 Kusumaningrum et al. (2003) and may be attributed to the stress conditions during the rapidly
155 decreasing water activity. Despite the rather high standard deviations found in our
156 experiments, it becomes clear from Fig. 1 (A-B) that at lower temperatures (25 and 10°C), the
157 cells were significantly faster inactivated on the antimicrobial belt surfaces compared to the
158 normal belt surfaces ($P<0.05$). The high reduction rate during the first 6 h demonstrated that
159 the antimicrobial additives in the test surfaces were more active in wet than dry conditions.
160 From the moment the surface was dry, the remaining cells were protected by the low water
161 activity (McEldowney and Fletcher, 1988). Another explanation could be the fact that in a
162 broth culture, a fraction of the population might be resistant against detrimental influences
163 such as low pH, low water activity, and low concentration of preservatives or biocides
164 (Wesche et al., 2009). A temperature close to the optimum growth temperature of the
165 microorganism gave a more rapid inactivation and no extra effect of the antimicrobial belt
166 could be demonstrated (Fig.1C). At 37°C, the surfaces were dry within one hour, whereas
167 these took 2 and 4 hours to dry at 25 and 10°C, respectively. This emphasizes the benefit of
168 thorough drying of surfaces directly after cleaning and disinfection.

169 The ability of bacteria to survive on food contact surfaces has been reported previously
170 (Bremer et al., 2001; Wilks et al., 2006; Wong, 1998) and is influenced by bacterial strain,
171 temperature, time, humidity, availability of nutrients, pH, presence of inhibitors and surface
172 materials (Allan et al., 2004; Beumer and Hazeleger, 2003). All three *L. monocytogenes*
173 strains used in this study showed the same pattern in survival on the two surfaces. The
174 recovery of the test organisms from the surfaces was approximately 70-75%, whereas 15%

175 was lost during spreading of the test organism and a small proportion of the cells (10%)
176 remained on the surfaces after sampling (data not shown).

177 During the experiments we measured the Rh; however, it was not a set variable. At high
178 temperature (37°C), the Rh was low at around 20%. This combination of high temperature and
179 low Rh, together with the fast drying of the surfaces, resulted in a rapid decrease in numbers
180 on both types of the conveyor belt surfaces (Fig. 1C). This phenomenon was also described in
181 a rapid reduction of *Pseudomonas fluorescens* at 35°C on antimicrobial-containing inner liners
182 in refrigerators (Kampmann et al. 2008). At ambient (25°C) and low (10°C) temperature, the
183 Rh in our experiments showed high values (60-75%). These combinations resulted in a better
184 survival of the target organism on the belt surfaces, probably because more time was needed
185 to obtain a completely dry surface (2-4 h). Furthermore, it is known that *L. monocytogenes*
186 can survive in stress conditions such as at the low temperature (2-4°C) (Rocourt and Cossart,
187 1991) or in low water activity (0.92) (Petran and Zottlola, 1989) or in less oxygen in vacuum
188 package (Walker et al., 1990). Regarding to survival of *L. monocytogenes* at low temperature,
189 it is due to the fact that changes in temperature results in changes in membrane composition to
190 maintain the optimum fluidity for growth at low temperature (Beales, 2004). This is an
191 important aspect, since these conditions can be found in many food production areas. Similar
192 results were found in a study of antibacterial substances in a kitchen, where the bacterial
193 counts on antimicrobial surfaces were significantly lower than those on conventional surfaces
194 (Sadako et al., 2001).

195 Presence of 10% food debris (minced meat, smoked salmon and chopped lettuce)
196 decreased the antimicrobial effect and protected the cells. In table 1, the results obtained at
197 10°C are presented. On the antimicrobial belt, a decrease in numbers of the test strains on the
198 antimicrobial belts (log 0.63/100 cm² per h) was faster than that on the normal belts (log
199 0.38/100 cm² per h). In the presence of food debris, however, even in the low concentrations

200 (10%) used in this experiment, the decrease is reduced to only log 0.2-0.3/100 cm² per h and
201 no statistically significant difference ($P>0.05$) was found in the survival of *L. monocytogenes*
202 on both types of the materials.

203 In comparison to lettuce, *Listeria* survived better in the presence of meat and fish
204 residues. This may be attributed to a higher concentration of protein and fat in these products
205 (Vorst et al. 2006). Moreover, amino acids in meat and fish can bind to the antimicrobial
206 additives released from the surfaces, and in this way enhances the survival of the pathogen
207 (Liau et al., 1997). Similar results were found in several studies, where milk and chicken or
208 meat suspensions provided a protective effect on *Salmonella enteritidis* and *Campylobacter*
209 *jejuni* on stainless steel surfaces (Kusumaningrum et al., 2003), on *L. monocytogenes* and
210 *Yersinia enterocolitica* on a mortar (Allan et al., 2004) and on *L. monocytogenes* and
211 *Salmonella typhimurium* on stainless steel and buna-N rubber (Helke and Wong, 1994).

212 It is not fully clear which antimicrobial additives were present in the test surfaces in our
213 study. The US Patent (Cedeil et al., 2006) indicated that the additives used in the conveyor
214 belt might be silver zeolite, aluminum oxide, calcium oxide, magnesium oxide, zinc
215 pyrithione or oxybisphenoxarsine. Some of these substances demonstrated an antimicrobial
216 effect against *E. coli* and *L. monocytogenes* on surfaces (Cowan et al., 2003) and in
217 suspensions (Chang et al., 2008). Relevant data obtained from the literatures demonstrated the
218 effect of those antimicrobial additives in surface materials (Kampman et al. 2008, Cowan et
219 al., 2003). However, the antibacterial effect was considerably greater in suspension (Chang et
220 al., 2008) than that on surfaces (Cowan et al., 2003). Furthermore, longer contact time and
221 lower temperature also resulted in a greater reduction. Studies on the antimicrobial effect of
222 silver-containing inner liners in refrigerators demonstrated that reduction of bacterial numbers
223 on the silver-containing surfaces at 35°C was one log unit faster than at 5°C, when compared
224 24 h with 72 h (Kampman et al. 2008). Our results agreed with the previous study, in which

225 the test at 10°C required a longer contact time (72 h) than at 37°C (24 h) to achieve a similar
226 reduction after exposure to the antimicrobial surfaces. However, it becomes clear from the
227 previous studies that even for the same antimicrobial agent results may vary considerably
228 (Cowan et al., 2003, Chang et al., 2008). With the set up of the testing procedure, choice of
229 test strain, concentration of the antimicrobial agent in the material, temperature, relative
230 humidity, suspension-/carrier tests, the reducing effect of the antimicrobial agents will be
231 influenced.

232 In conclusion, the presented results demonstrate that the antimicrobial additives in the
233 conveyor belt material can help to reduce survival of microorganism on the surfaces.
234 However, the effectiveness of the antimicrobial additives was reduced in presence of food
235 debris. Although the additives in the surfaces are not known, the results were comparable with
236 previous studies. Additional studies are required to examine the effectiveness of the
237 antimicrobial conveyor belt on the other microorganisms such as *E. coli* and *Salmonella* spp.

239 **Acknowledgements**

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241 conveyor belt materials used in this study.

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348 **Figure captions**

349

350 Fig. 1. Survival of *L. monocytogenes* on conveyor belt material with (◇: CM+) or without
351 (▲: CM-) incorporated antimicrobial additives at 10°C (A), 25°C (B) and 37°C (C). (Means
352 of three *L. monocytogenes* strains ± standard deviation (n = 3), DL: detection limit).

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373 Fig. 1.

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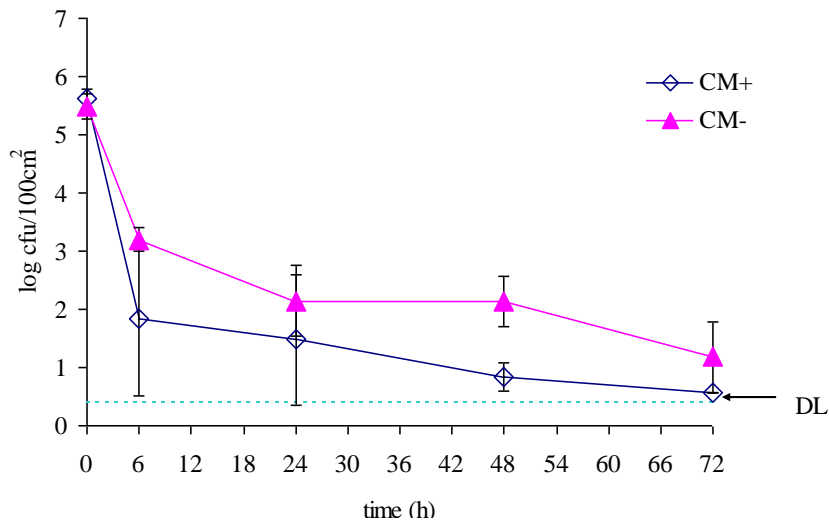
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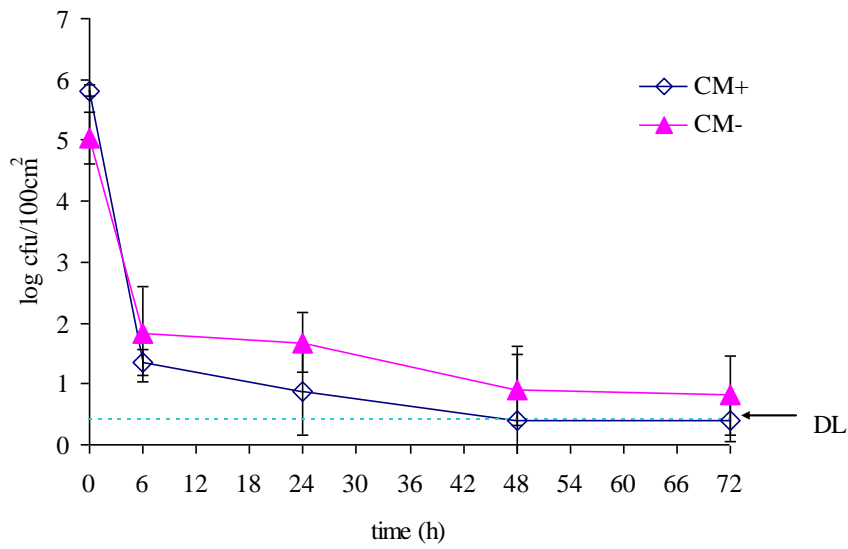
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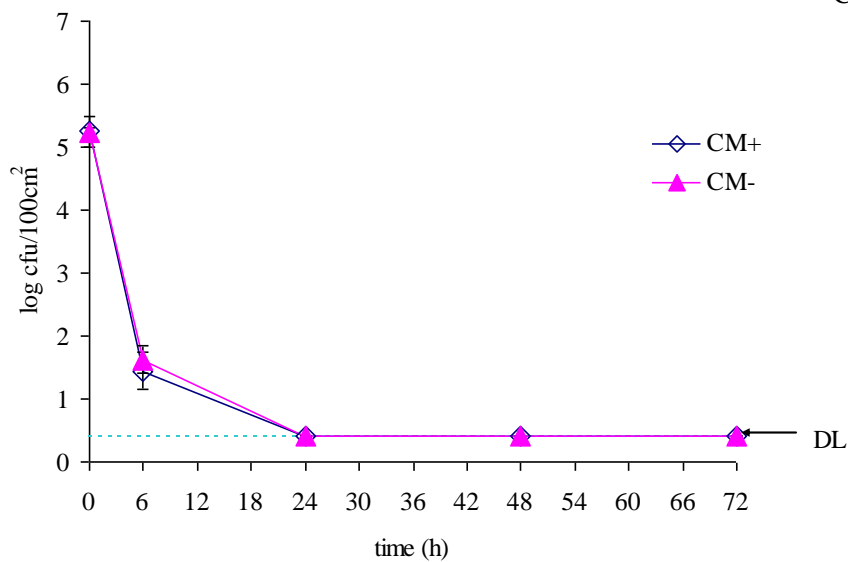
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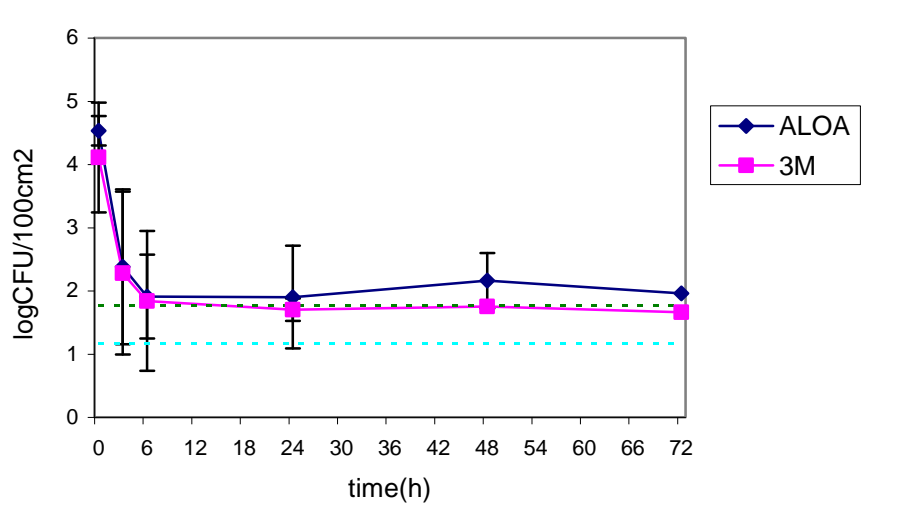
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0 cm²/h, mean of three L.

por belt material with (CM+) and

ood debris stored at 10°C for 72 h.

CM-	
0-6 h ^{**}	6-72 h ^{**}

Control	0.63 ± 0.02 ^a	0.02 ± 0.00	0.38 ± 0.06 ^b	0.03 ± 0.00
Minced meat	0.22 ± 0.05 ^c	0.03 ± 0.01	0.26 ± 0.03 ^c	0.02 ± 0.01
Smoked salmon	0.23 ± 0.01 ^c	0.03 ± 0.01	0.26 ± 0.03 ^c	0.02 ± 0.01
Chopped lettuce	0.33 ± 0.04 ^c	0.03 ± 0.01	0.31 ± 0.11 ^c	0.03 ± 0.01

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* Different letters in the same row or column are significantly different ($P = 0.05$)
 ** Reduction rates were calculated at two different time intervals (first 6 h and 6-72 h).