

Modelling the influence of palmitic, palmitoleic, stearic and oleic acids on apparent heat resistance of spores of *Bacillus cereus* NTCC 11145 and *Clostridium sporogenes* Pasteur 79.3

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1 **Modelling the influence of palmitic, palmitoleic, stearic and oleic acids on apparent heat**
2 **resistance of spores of *Bacillus cereus* NTCC 11145 and *Clostridium Sporogenes* Pasteur**
3 **79.3.**

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6

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Abstract

Heat resistance of spores is affected by many factors such as temperature, pH, water activity (aw) and others. Previous studies have reported that free fatty acids can affect the germination and growth of bacterial spores. In this study, we investigated the influence of free fatty acids in heating medium or in recovery medium on the heat resistance of spores of *Bacillus cereus* NTCC 11145 and *Clostridium sporogenes* Pasteur 79.3. Four free fatty acids were studied: palmitic, palmitoleic, stearic and oleic acid. During thermal treatments, the impact of these FFA in heating media was generally low, but the presence of free fatty acids in the recovery medium highly decreases bacterial spore apparent heat resistance, particularly with unsaturated fatty acids. A mathematical model was developed to describe and quantify the influence of free fatty acids in recovery media on the D-values. The z'_{FFA} parameter values which quantify the impact of free fatty acids were determined. The variation of this parameter value according to the free fatty acid type was compared with MIC value variation given in the literature. The model enables the decrease in D-values in the presence of free fatty acids to be estimated. The high concentrations of free fatty acids in liver or canned duck may explain the microbial stability with low sterilization values applied.

Key words: free fatty acids, bacterial spores, heat resistance, recovery medium.

37 Introduction

38 Free fatty acids are a large group of non toxic compounds which present antimicrobial
39 activity. It is well known that soaps composed of free fatty acid salt have a bactericidal effect.
40 The bactericidal effect of free fatty acids has been extensively investigated and presented by
41 Nieman (1954). More recently Kabara (1978) provided and analysed minimal inhibitory
42 concentration values of saturated and unsaturated fatty acids for vegetative cells of pathogenic
43 bacteria. For long chain fatty acids, the influence of free fatty acids varied according to acid
44 type but did not correlate with carbon chain length. However, the carbon chain degree of
45 unsaturation greatly influenced the MIC values.

46 The application of the bactericidal effect of fatty acids, given in the literature, mainly
47 concerns soaps and the cosmetics industry. More recently, studies concerning the influence of
48 free fatty acids on pathogens have examined food products. These studies focus on pathogens
49 such as *Salmonella*, *Listeria* and *Staphylococcus*. However some spore-forming species have
50 been studied such as *Bacillus cereus* (Ababouch et al., 1994; Lee et al., 2002), *Bacillus*
51 *subtilis* (Tsuchido et al., 1992), *Clostridium botulinum* (Grecz et al., 1959) and *Clostridium*
52 *perfringens* (Skřivanova et al., 2005).

53 In foods, free fatty acid concentrations are low, however in some foods containing fats and
54 natural oils their concentrations are sufficient to affect bacterial growth. Free fatty acids in
55 food can be considered as a hurdle usable for food preservation. In compliance with
56 Leistner's concept (2000), this hurdle can be associated with other stresses such as
57 temperature during heat treatment. The combination of heat treatment with pH, water activity
58 or acids on bacterial heat resistance have been studied, modelled and presented in different
59 publications (Blackburn et al., 1997; Coroller et al., 2001; Couvert et al., 1999; Davey, 1993;
60 Juneja and Eblen, 1999; Leguérinel et al., 2005). These combinations affect surviving bacteria
61 in two stages: during heat treatment and during recovery. In these publications, a Bigelow-

62 like model and z parameters were developed to quantify the impact of these environmental
63 factors on the D-values.

64 Few studies quantify the influence of free fatty acids during heat treatment on bacterial
65 spores. Tremoulet et al. (2002) observed for different free fatty acids (C16:0, C16:1, C18:1,
66 C18:2 and C18:3) in heating media that their increasing concentration decreased the D-value
67 for *Geobacillus stearothermophilus*. Concerning the presence of free fatty acids in recovery
68 media following heat treatment Ababouch et al. (1994) have observed some influences.

69 The aim of this study was to quantify and model the influence of the concentration of palmitic
70 acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0) and oleic acid (C18:1), major free
71 fatty acids in food, in heating and recovery media for *B. cereus* and *C. sporogenes* spores.

72

73 Material and methods

74 2.1 Microorganism and spore production

75 Spores of *B. cereus* NTCC11145 were obtained as follows: cells were precultivated at 37 °C
76 for 24 h in Brain Heart Infusion broth (Difco). The preculture was used to inoculate nutrient
77 agar plates (Biokar Diagnostics BK021) to which 40 mg/l of MnSO₄ and 100 mg/l of CaCl₂
78 were added to the surface. The plates were incubated at 37 °C for 5 days. Spores were then
79 collected by scraping the surface of the agar. They were then suspended in sterile distilled
80 water and washed three times by centrifugation (10,000 g for 15 min) (Bioblock Scientific,
81 model Sigma 3K30). The final suspension (approximately 10¹⁰ spores/ml) was finally
82 distributed into sterile Eppendorf microtubes and kept at 4 °C. For *C. sporogenes* Pasteur
83 79.3, spore production was obtained using the Third Method described by Goldoni et al.
84 (1980).

85

86 2.2 Source and preparation of fatty acids

87 Palmitic and oleic acids were obtained from Alfa Aesar (Strasbourg, France) and palmitoleic
88 and stearic acid from Fluka and reported as being more than 96%, 90%, 95% and 97% pure
89 respectively. Stock emulsions with different concentrations were obtained by mixing
90 (Polytron[®] PT-MR 2100, Kinematica AG, Switzerland) and microsonication (Branson
91 Sonifier 250, Branson Ultrasonics, USA) in distilled water with 0.1% Tween[™]80 as a
92 dispersant. These solutions were added to a nutrient broth (Biokar Diagnostic BK003HA) for
93 heating media or a nutrient broth with Bacteriological Agar (15g) (Biokar Diagnostic
94 A1010HA) for recovery media. For the different FFA, the concentrations studied were lower
95 than their critical micelle concentration values (Freeman 1969, Mukerjee and Mysels, 1971).
96 The concentrations of free fatty acids added to the heating media were 0.8mM or 2mM. In the
97 recovery media, different concentrations were added from 0 to 2 mM according to their
98 critical micelle concentration values and their influence on the D values determined. After
99 sterilization by autoclaving at 110°C for 45 minutes as described by Marounek et al. (2003),
100 the pH was adjusted to 7 and the aw value was also regulated. For better visibility of bacterial
101 communities, 2,3,5-Triphenyl-2H-Tetrazolium Chloride (TTC) was added to the recovery
102 medium containing free fatty acids. Tween[™]80 and TTC were obtained from Alfa Aesar
103 (Strasbourg, France). The absence of influence of TTC and Tween[™]80 on the bacterial heat
104 resistance was checked (data not show).

105

106 2.3 Spore heat treatments

107 Firstly, 30 µl of spore suspension was diluted in 3 ml of adjusted heating medium. Capillary
108 tubes of 200 µl (vitrex) were filled with 100 µl of sample, sealed, and subjected to a thermal
109 treatment in a thermostated glycerol bath for different heating times. The heat treatment was
110 stopped by cooling capillary tubes in a water/ice bath. Then they were broken at both ends
111 and their contents poured into a tube containing 9 ml of sterile tryptone salt broth (Biokar

112 Diagnostics BK014HA) by rinsing with 0.9 ml tryptone salt broth. The viable spores were
113 counted by duplicate plating in the recovery media and incubated 72h at 37°C for *B. cereus*
114 and *C. sporogenes* strains.

115

116 2.4 Experimental design and data analysis

117 For each heating and recovery media condition, classical D-values and their associated
118 standard deviation were estimated from survival kinetics. One concentration level was studied
119 to assess the influence of free fatty acids in heating media. Concerning the influence of free
120 fatty acids in recovery media, monofactorial design, without repeat, was carried out for each
121 acid studied. The influence of free fatty acid concentrations in recovery media on the D-
122 values was modelled by a simple Bigelow-like model as a secondary model (Eq1)

$$123 \log D = \log D^* - \frac{[acid]}{z_{FFA}} \quad (\text{Eq1})$$

124 In this model z'_{FFA} values correspond to an increase in free fatty acid concentrations in the
125 recovery media, leading to a 10-fold reduction in the D-value (D^* corresponds to the D-
126 values without free fatty acids in recovery media). This parameter quantifies the influence of
127 fatty acid concentration in the recovery media on the heat resistance of bacterial spores.

128 The parameter values and their associated standard deviation were fitted using a non-linear
129 module (“nlinfit” and “nlparci” Matlab 6.1, The Mathworks). The “nlparci” function used to
130 assess standard deviation was based on the asymptotic normal distribution for the parameter
131 estimates (Bates and Watts, 1988).

132

133 Results

134 For the two species studied, *B. cereus* and *C. sporogenes*, most of the surviving cell kinetics
135 showed a linear relationship between heating time and the logarithm of survival cell numbers.
136 Hence bacterial heat resistance values were quantified using the classical D-values.

137 Concerning heat treatment, D-values were determined in heating media in the presence of 4
138 free fatty acids (C16:0, C16:1, C18:0 and C18:1). Table 1 shows a low decrease in the D-
139 values when free fatty acids were added whatever the carbon chain length or number of chain
140 bonds for the two species studied.

141 The influence of free fatty acids in the recovery media on the apparent D-values was
142 determined for different concentrations of each acid type and for each strain studied. The
143 results are shown in Figures 1 & 2 and Tables 2 & 3. Concerning D values determined in
144 media without fatty acid variability was observed. Bacterial spores coming from different
145 productions explain this variability. The presence of free fatty acids in recovery media greatly
146 reduced the apparent D-values for the two species studied. For the four free fatty acids
147 studied, the evolutions in the D-values according to their concentrations are presented Figure
148 3. A linear relationship is observed between free fatty acid concentration and log of D-values
149 for the 4 acids studied.

150 The z'_{FFA} values for the different acid types and for the two species studied are presented in
151 Table 4. The results presented in this table show that the presence of unsaturated free fatty
152 acids has a greater effect on D-values than saturated free fatty acids, with a greater sensitivity
153 for *C. sporogenes* Pasteur 79.3 compared to *B. cereus* NTCC 11145. During food heat
154 treatment, the environmental factors of heating and recovery media are identical. Table 5
155 presents the specific effects of palmitic and oleic acid in heating and recovery media on the
156 D-value of *B. cereus* spores and their associated effects in heating and in recovery media.
157 These results show the cumulative effect of free fatty acids during heat treatment and
158 recovery.

159

160 Discussion

161 Free fatty acids in heating media enhance thermal inactivation of bacterial spores. The
162 influence of the free fatty acids studied (palmitic, palmitoleic, stearic and oleic acid) reduced
163 the D-value down to 30% for *B. cereus* and *C. sporogenes*. The D-values obtained appear
164 lower than those observed by Tremoulet et al. (2002) for *G. stearothermophilus*. For 0.5mM
165 of free fatty acid added to heating media, these authors observed D-value reduction ratios
166 ranging from 50% to 75% according to the acid type used. As shown by the results presented,
167 the chain length or unsaturated bond does not appear to influence this moderate effect. To the
168 best of our knowledge, the literature gives no information to explain the mechanism of free
169 fatty acid effects during heat treatment.

170 The benefit of the association of different stresses on bacterial inactivation has been described
171 by Leistner (2000) through the hurdle concept. The combination of heat treatment with
172 unfavourable environmental conditions of recovery media, low pH or aw, decreases the
173 apparent heat resistance of bacterial spores. During heat treatment, the proportion of dead and
174 injured spores increases with the heating time. In favourable and optimal environmental
175 recovery conditions, most injured bacterial cells survive and grow, thus D-values appear
176 higher. When recovery conditions are unfavourable, bacteria cells are stressed by the
177 environmental conditions, low pH or aw, resulting in growth inhibition followed by death.
178 Thus in unfavourable recovery conditions, D-values are lower than the D-values observed in
179 optimal recovery conditions. The influence of low pH or aw values of recovery media on the
180 apparent D-values has been quantified and modelled by Couvert et al. (1999) and Coroller et
181 al. (2001). Like these two environmental factors, free fatty acids in growth media represent a
182 stress for bacterial cell development and their presence in recovery media reduces the
183 apparent D-values. It can be noted that for the different free fatty acids studied, their
184 concentrations were below the CMI values and did not modify the initial population size
185 observed without heat treatment. Thus for the concentrations studied, free fatty acids added

186 must be associated with a heat treatment or high thermal stress to show an inhibition activity.
187 Free fatty acids present in recovery media affect thermally injured bacteria. This observation
188 was made by Ababouch et al. (1994) on *B. cereus* heat stressed cells for additions of stearic
189 and oleic acid.

190 This effect of fatty acid concentration is described and quantified by a Bigelow-like model
191 (Eq1) where the z'_{FFA} parameter quantifies the impact of free fatty acid concentrations on the
192 decrease in apparent heat resistance of bacterial spores. These effects of free fatty acids in
193 recovery media have never been quantified in the literature. However z'_{FFA} values for
194 different acids and for the two bacterial species studied can be compared to data concerning
195 the impact of free fatty acid concentrations on bacteria spores or vegetative cells such as MIC
196 values.

197 The z'_{FFA} values for the saturated free fatty acids palmitic and stearic acid show a limited
198 effect. This observation for stearic acid corresponds to the observation of Ababouch et al.
199 (1994) on *B. cereus* unheated and heat-shocked spores. Concerning the *Clostridium* species,
200 the high MIC values for palmitic and stearic acid, greater than 5mM for *C. botulinum*
201 (Skřivanova et al., 2005) and close to 10mM for *C. perfringens* (Kabara, 1978), concur with
202 our observation of a moderate influence of saturated fatty acid in recovery media to reduce D-
203 values. The impact of added unsaturated fatty acid on recovery media is higher than that of
204 saturated fatty acid.

205 For the same carbon chain length, C18, the presence of unsaturated bonds in the carbon chain
206 decreased the z'_{FFA} from 3.19mM (C18:0) to 0.89mM (C18:1) for *B. cereus* and from
207 2.36mM (C18:0) to 0.36mM (C18:1) for *C. sporogenes*. While no data is available in the
208 literature concerning the influence of these unsaturated free fatty acids in recovery media on
209 D-values, data exists for the MIC concentration concerning the impact of these acids on
210 *Bacillus* or *Clostridium* growth. The presence of unsaturated bonds in the carbon chain

211 reduces the MIC values: 10mM (C18:0) to 0.75mM (C18:1) for *G. stearothermophilus*
212 (Tremoulet et al., 2002), 0.4mM to 0.05mM for *B. megaterium* (Galbraith et al., 1971)
213 11.4mM (C18:0) to 1.1mM (C18:1) for *C. perfringens* (Kabara, 1978) or 20mM (C18:0) to
214 0.48-1.48mM (C18:1) for *C. perfringens* (Skrivanova et al., 2005). For C16 chain length, the
215 influence of unsaturated bonds in the carbon chain on the decrease in z'_{FFA} values was also
216 observed. This observation concurs with the difference in MIC values determined by
217 Tremoulet et al. (2002): 10mM for palmitic acid, C16:0, and 1.25mM for palmitoleic acid,
218 C16:1, for *G. stearothermophilus* strain. For these acids, similar observations were presented
219 by Kabara et al. (1972) for different bacterial species such as *Streptococcus* or *Pneumococcus*.
220 The presence of long chain free fatty acids, particularly unsaturated free fatty acids, in
221 recovery media creates unfavourable conditions for the growth of injured heat-treated spores.
222 Different mechanisms affect the physiology of bacterial spores or vegetative cells.
223 High adsorption of free fatty acids onto bacteria was observed by Maxcy and Dill (1967) and
224 Galbraith and Miller (1973a). The accumulation of free fatty acids on the cell or spore surface
225 is the first stage of the inhibition mechanism (Nieman 1954). Concerning bacterial spores,
226 Foster and Wynne (1948) observed an inhibition of germination of *C. botulinum* spores in the
227 presence of oleic acid. In 1982, Yasuda et al. assessed the inhibitory activity of short and long
228 chain fatty acids on spore germination. The percentage inhibition of unsaturated oleic acid is
229 higher than saturated fatty acids which present the same effect. These authors suggest that
230 hydrophilic environments inhibit L-alanine receptors, keys to germination mechanisms
231 described by Johnstone in 1994.
232 The difference in solubility of saturated and unsaturated fatty acids may explain the higher
233 inhibitory effect of unsaturated fatty acids. In addition to germination inhibition, free fatty
234 acids have long been recognized as growth inhibitors for Gram-positive bacteria (Kabara,
235 1978). Ababouch et al. (1994) studied the influence of stearic and oleic acids both on spore

236 and vegetative cells of *B. cereus* growth. For acid-treated spore or vegetative bacteria,
237 morphological modifications appeared, giving long thin cells. Knapp and Melly (1986) made
238 the same observations for *Staphylococcus aureus* in the presence of arachidonic acid. The
239 influence of higher concentrations of free fatty acids in growth media causes membrane
240 disruption and cell lysis (Tsuchido et al., 1992). Zheng et al. (2005) demonstrated specific
241 action of palmitoleic and oleic acid on enol-acyl carrier protein reductase (Fab1) which
242 inhibits fatty acid synthesis for *S. aureus* and *Escherichia coli*. Stearic acid does not present
243 this effect.

244 Galbraith and Miller (1973b) showed that for *Bacillus megaterium* cells, low concentrations
245 of fatty acids stimulate oxygen uptake, whereas high concentrations (0.2mM) of oleic acid
246 inhibit oxygen intake. These authors observed that unsaturated fatty acids were more active
247 than saturated acids. A similar decrease or inhibition of nutrient uptake was observed for
248 amino acids glutamic acid and lysine for *B. megaterium* and *C. perfringens* vegetative cells
249 (Galbraith and Miller, 1973b) and glucose and glycerol uptake for *Brochothrix thermosphacta*
250 (Macaskie, 1982).

251 All these effects of free fatty acids on unheated bacteria were enhanced by the injury caused
252 by heat treatment. Free fatty acids are present in natural oils such as olive oil or in fish and
253 animal fats, for instance in canned fat duck liver. Thus the additive influences of free fatty
254 acids in heating and particularly in recovery media can be taken into account to safely reduce
255 heat treatment of bacterial spores. These major reductions in thermal resistance by fatty acids
256 in recovery media may explain the sterility of canned fat duck liver which is heat treated with
257 very low sterilization values, lower than 1. According to our results, the high concentrations
258 of free fatty acids in fresh fat duck liver, 33.3mM of oleic acid and 2.4mM of palmitoleic acid
259 (Tremoulet et al., 2002), are sufficient to greatly reduce the apparent heat resistance of
260 bacterial spores. With these acid concentrations, the D-value calculated using equation 1 is

261 theoretically reduced by a factor of 10^{33} with oleic acid concentrations (33.3mM) and by a
262 factor of 3×10^{10} with palmitoleic acid concentrations (2.4mM) for *B. cereus* NTCC11145
263 spores. The presence of free fatty acids in food associated with a heat treatment may be used
264 to reduce heat treatment or increase food safety.

265

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269

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350 Titles of figures and tables

351 Figure 1: Log N (colony-forming units, cfu) vs. time (min) for *Bacillus cereus* NTCC 11145
352 to 100°C (A & C) and *Clostridium sporogenes* Pasteur 79.3 to 90°C (B & D) with, in
353 recovery media, different concentrations of palmitic acid (A & B) or stearic acid (C & D) at
354 different concentrations: 0mM ●, 0.2mM □, 0.4mM ▲, (C18:1) 0.6mM ▽, 0.8mM ◆,
355 1mM ○, 1.5mM ■, 2mM △.

356

357 Figure 2: Log N (colony-forming units, cfu) vs. time (min) for *Bacillus cereus* NTCC 11145
358 heated to 100°C (A & C) and *Clostridium sporogenes* Pasteur 79.3 heated to 90°C (B & D)
359 with, in recovery media, different concentrations of palmitoleic acid (A & B) or oleic acid (C
360 & D) at different concentrations: 0mM ●, 0.05mM □, 0.1mM ▲, 0.2mM ◆, 0.4mM ○,
361 0.6mM ■, 0.8mM △.

362

363 Figure 3: Log D (minutes) vs. FFA concentration (μM) with respectively palmitic ●,
364 palmitoleic ○, stearic ▲ and oleic △ acid in recovery medium for: spores *Bacillus cereus*
365 NTCC 11145 heated to 100°C (A) and *Clostridium sporogenes* Pasteur 79.3 heated to 90°C
366 (B).

367

368

369 Table 1: D-values (minutes) for *Bacillus cereus* NTCC 11145 heated to 100°C and
370 *Clostridium sporogenes* Pasteur 79.3 heated to 90°C with palmitic, palmitoleic, stearic and
371 oleic acids in heating medium.

372

373 Table 2: D-values (minutes) for *Bacillus cereus* NTCC 11145 heated to 100°C in the presence
374 of different concentrations of palmitic, palmitoleic, stearic and oleic acid in recovery medium.

375

376 Table 3: D-values (minutes) for *Clostridium sporogenes* Pasteur 79.3 heated to 90°C in the
377 presence of different concentrations of palmitic, palmitoleic, stearic and oleic acid in recovery
378 medium.

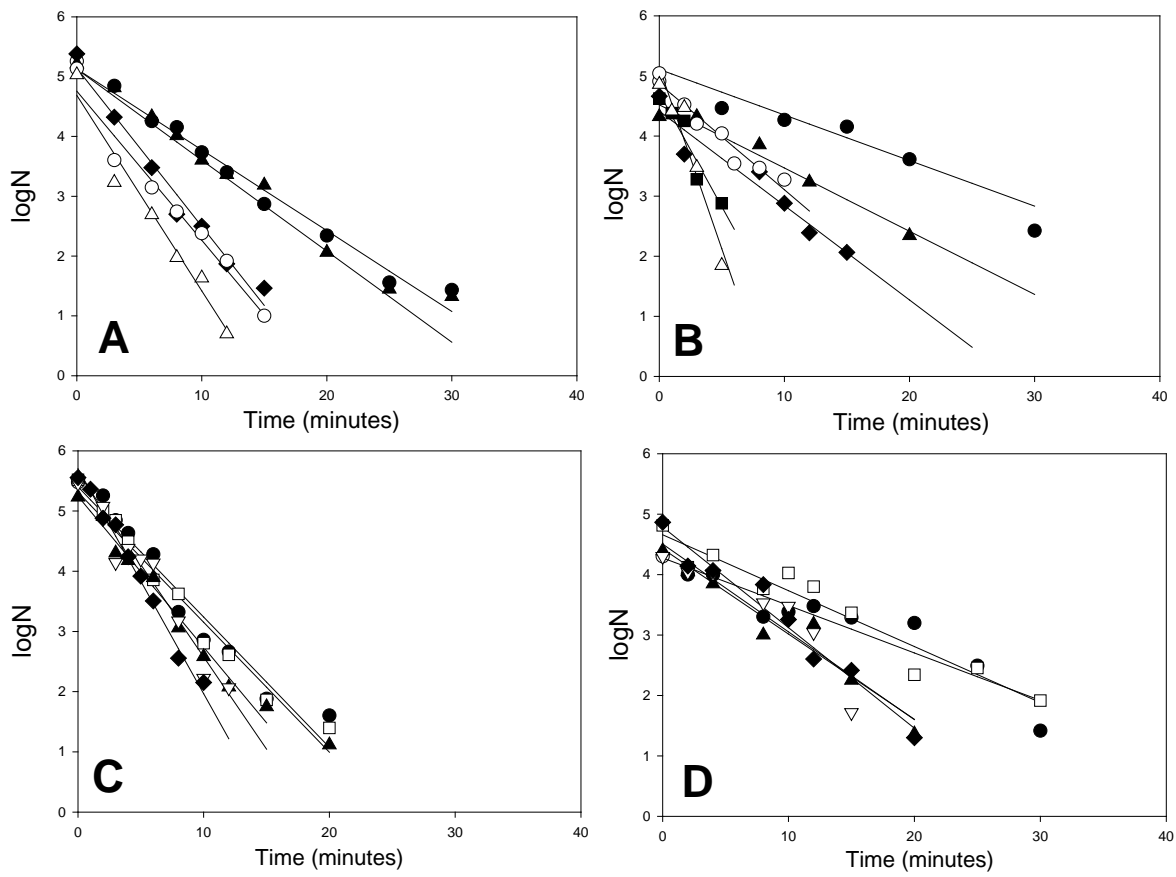
379

380 Table 4: z-values for palmitic, palmitoleic, stearic and oleic acids in recovery medium for
381 *Bacillus cereus* NTCC 11145 and *Clostridium sporogenes* Pasteur 79.3.

382

383 Table 5: Specific effect of the addition of 0.8mM of palmitic and oleic acid in heating and
384 recovery media on the D-value of *Bacillus cereus* spores heated at 100°C and their associated
385 effect in heating and recovery media.

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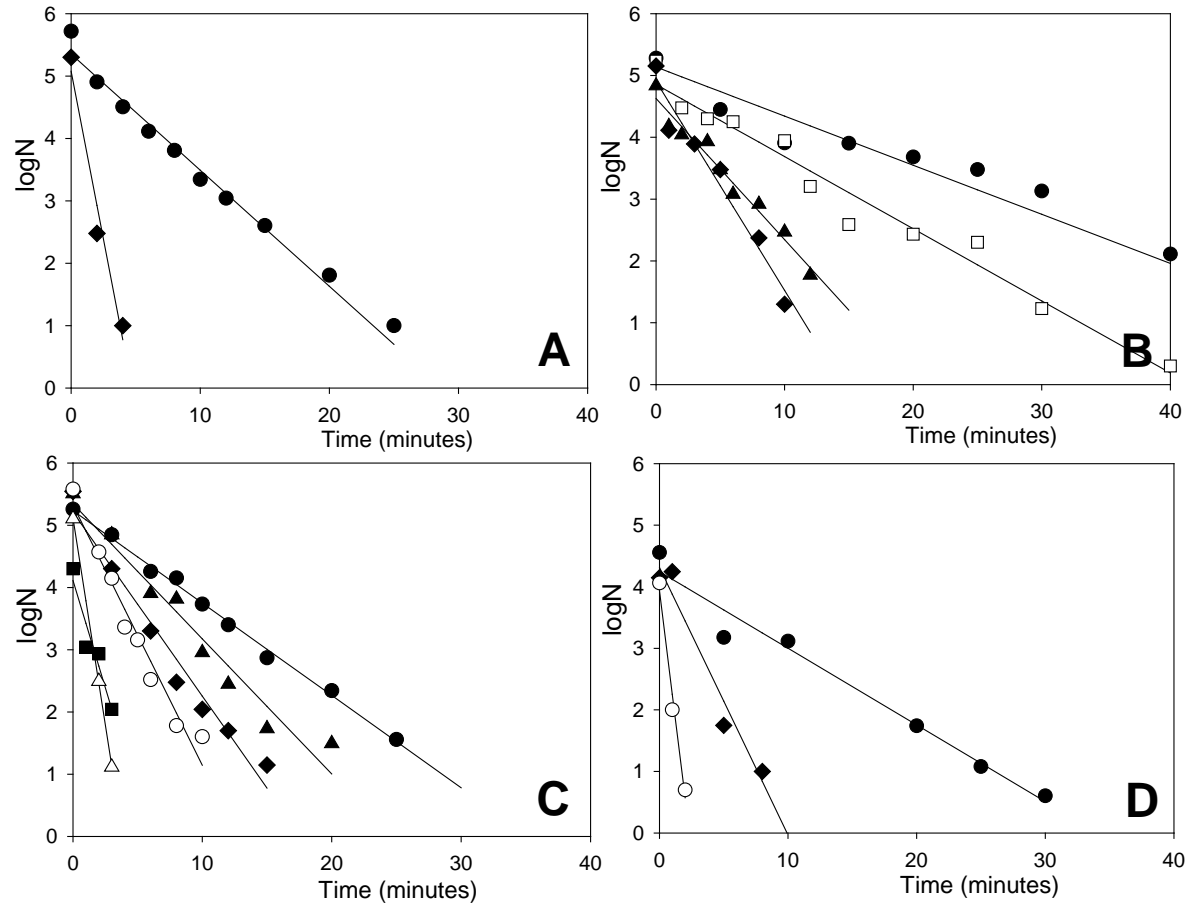
390 Figure 1

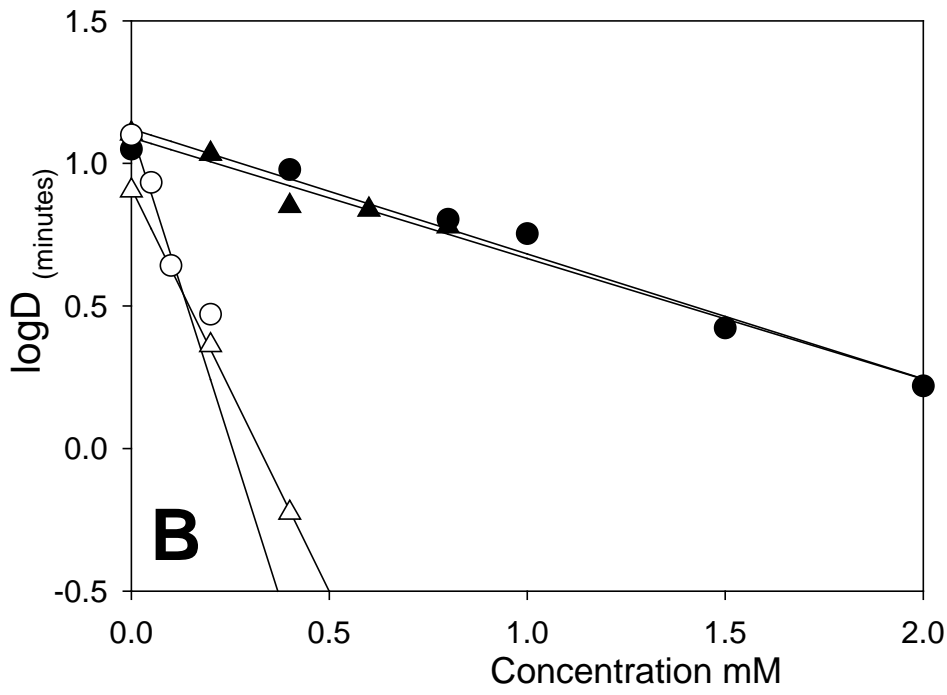
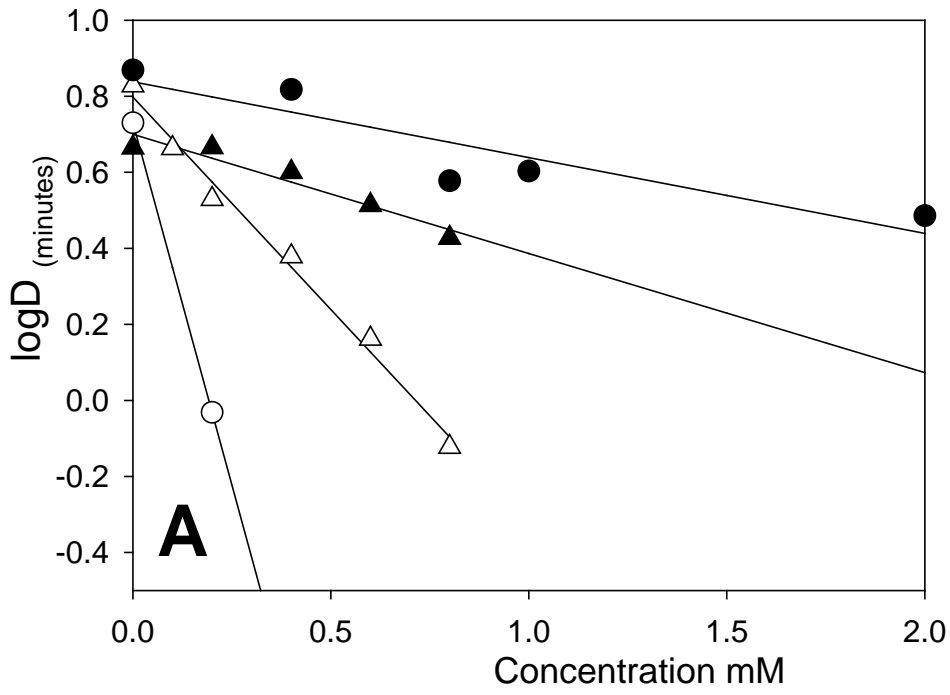
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400

401 Figure 3 A&B

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| | concentration mM | Palmitic acid C16:0 | | Palmitoleic acid C16:1 | | Stearic acid C18:0 | | Oleic acid C18:1 | |
|------------------------|---------------------|---------------------------|------|------------------------------|------|--------------------------|------|------------------------|------|
| | | D (min) | SD | D (min) | SD | D (min) | SD | D (min) | SD % |
| <i>Bacillus cereus</i> | 0 | 6.74 | 0.21 | 6.74 | 0.21 | 6.99 | 0.21 | 6.74 | 0.21 |
| NTCC11150 | 0.8 | ND | | 5.21 | 0.32 | ND | | ND | |
| Heating T°C: 100°C | 2.0 | 3.55 | 0.27 | ND | | 4.63 | 0.35 | 4.78 | 0.29 |
| <i>Clostridium</i> | | | | | | | | | |
| <i>sporogenes</i> | 0 | 13.20 | 3.37 | 13.89 | 1.64 | 12.73 | 1.93 | 12.59 | 0.95 |
| Pasteur 79.3 | 0.8 | ND | | 11.44 | 0.59 | 12.19 | 0.64 | 7.47 | 0.95 |
| Heating T°C: 90°C | 2.0 | 4.78 | 0.29 | ND | | ND | | ND | |

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Table 1

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| Concentration μM | Palmitic acid | | Palmitoleic acid | | Stearic acid | | Oleic acid | |
|--------------------------------|----------------------------------|------|----------------------------------|------|----------------------------------|------|----------------------------------|------|
| | C16:0 | | C16:1 | | C18:0 | | C18:1 | |
| | $D_{100^\circ\text{C}}$ (min) | SD | $D_{100^\circ\text{C}}$ (min) | SD | $D_{100^\circ\text{C}}$ (min) | SD | $D_{100^\circ\text{C}}$ (min) | SD |
| 0 | 7.40 | 0.42 | 5.37 | 0.35 | 4.62 | 0.47 | 6.74 | 0.72 |
| 0.1 | ND | | ND | | ND | | 4.61 | 0.49 |
| 0.2 | ND | | 0.93 | 1.09 | 4.63 | 0.35 | 3.38 | 0.35 |
| 0.4 | 6.57 | 0.26 | | | 3.99 | 0.28 | 2.39 | 0.24 |
| 0.6 | ND | | | | 3.26 | 0.28 | 1.45 | 0.69 |
| 0.8 | 3.78 | 0.34 | | | 2.68 | 0.16 | 0.75 | 0.07 |
| 1.0 | 4.01 | 0.44 | | | | | | |
| 1.5 | ND | | | | | | | |
| 2.0 | 3.06 | 0.70 | | | | | | |

415

416 Table 2

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418

| Concentration μM | Palmitic acid | | Palmitoleic acid | | Stearic acid | | Oleic acid | |
|--------------------------------|---------------------------------|------|---------------------------------|------|---------------------------------|------|---------------------------------|------|
| | C16:0 | | C16:1 | | C18:0 | | C18:1 | |
| | $D_{90^\circ\text{C}}$ (min) | SD | $D_{90^\circ\text{C}}$ (min) | SD | $D_{90^\circ\text{C}}$ (min) | SD | $D_{90^\circ\text{C}}$ (min) | SD |
| 0 | 11.22 | 2.27 | 12.59 | 0.95 | 12.73 | 1.93 | 8.03 | 0.98 |
| 0.05 | ND | | 8.57 | 0.61 | ND | | ND | |
| 0.1 | ND | | 4.38 | 0.36 | ND | | ND | |
| 0.2 | ND | | 2.95 | 0.45 | 10.81 | 1.22 | 2.30 | 0.34 |
| 0.4 | 9.51 | 1.61 | | | 7.09 | 0.96 | 0.59 | 0.51 |
| 0.6 | ND | | | | 6.88 | 1.78 | | |
| 0.8 | 6.36 | 1.21 | | | 6.01 | 0.60 | | |
| 1.0 | 5.67 | 0.86 | | | | | | |
| 1.5 | 2.65 | 0.74 | | | | | | |
| 2.0 | 1.66 | 0.46 | | | | | | |

420

421 Table 3

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423

| | Palmitic acid | | Palmitoleic acid | | Stearic acid | | Oleic acid | |
|---|---------------|------|------------------|------|--------------|------|------------|-----|
| | C16:0 | | C16:1 | | C18:0 | | C18:1 | |
| | z' mM | SD | z' mM | SD | z' mM | SD | z' mM | SD |
| <i>Bacillus cereus</i> NTCC11145 | 5.01 | 2.10 | 0.26 | - | 3.19 | 0.85 | 0.89 | 0.7 |
| <i>Clostridium sporogenes</i> Pasteur 79.3 | 2.28 | 0.27 | 0.23 | 0.04 | 2.36 | 0.68 | 0.36 | 0.5 |

424

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426 Table 4

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| | Palmitic acid | | Oleic acid | |
|--|--------------------------|------|--------------------------|------|
| | 2mM | | 0.8mM | |
| | D _{100°C} (min) | SD | D _{100°C} (min) | SD |
| No fatty acid added to media | 6.90 ^(a) | 0.23 | 6.90 ^(a) | 0.23 |
| Fatty acid added to heating media | 3.48 ^(b) | 0.18 | 4.32 ^(b) | 0.26 |
| Fatty acid added to recovery media | 2.04 ^(c) | 0.15 | 0.98 ^(c) | 0.14 |
| Fatty acid added to both heating and recovery media | 1.77 | 0.16 | 0.66 | 0.06 |
| “Theoretical” or predicted value taking account D values (a), (b) and (c) | 1.07 | | 0.61 | |
| “Theoretical” or predicted value taking account D values from Tables 1 and z’ _{FFA} values from table 4 | 1.45 | | 0.75 | |

430

431 Table 5

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