Macromolecular acidic coating increases shelf life by inhibition of bacterial growth

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20 Highlights

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22 • Food coating formulations containing macromolecular alginic acid developed

23 • Provide low surface pH for extended periods without affecting interior parts

24 • Protect against external microbial contamination, including pathogens

25 • Increase shelf-life by reducing or suppressing natural microbial growth

26 • Applicable to a range of foods

27

28 Abstract

29 The sensitivity of microorganisms to low pH can be utilized in food protection by preparing
30 coatings based on macromolecular acids. Due to limited diffusivity of macromolecules low
31 pH occurs primarily at the surface, while the interior parts of the food remain unaffected.

32 This principle is demonstrated using food approved alginic acid in various types of coatings
33 (aqueous, emulsions, dispersions, dry coating) on a wide range of foods including meat, fish,
34 chicken, shrimp and boiled rice. Significant delay or inhibition of the natural flora is
35 generally demonstrated, particularly when exposed to 'temperature abuse'.

36 Specifically, we show that the coatings reduce or inhibit regrowth of pathogens (*Bacillus*
37 *cereus, B. weihenstephanensis, Listeria monocytogenes* serotype 1 and *Staphylococcus*
38 *aureus*). In special cases like boiled rice, alginic acid may largely replace acetic acid for
39 acidification and preservation, as demonstrated studying regrowth of added spores of *B.*
40 *cereus*.

41 Most formulations allow easy removal prior to further processing (cooking, frying).
42 Temporary side effects such as 'acid cooking' obtained for high acid concentrations on
43 sensitive surfaces (e.g. salmon) disappear during processing, recovering the normal taste
44 and texture. The coating is hence suitable for a large variety of foods.

45

1. Introduction

Preserving food has received new focus recently after the media and the public have discovered that we discard nearly half of the produced food (Gustavsson et al., 2011). To maintain food for longer than we do now, better infrastructure is necessary in many parts of the world, but also the ability to protect food from spoilage and growth of pathogenic bacteria. There are several ways of keeping foods safe by using different preserving methods. Antimicrobials are widely used (e.g. the E700 series approved by the European Union), but faces challenges related to the spread of microbial resistance. Cooling and freezing are very important in the developed part of the world, but also methods like salting, drying and fermentation are old but yet essential methods (Baird-Parker, 2000). In modern times acidification and the use of preservatives have helped us maintaining foods without cooling of many products since many pathogens do not grow at low pH (Lund and Eklund, 2000). Meat and especially fresh fish are difficult to keep for longer periods of time without extensive cooling, for fish usually on ice. Acidification by traditional organic acids such as acetic acid or citric acid (belonging to the E200 series of preservatives) have several disadvantages beyond the taste and odour associated with the acids. As small molecules diffuse rapidly into the food and cannot, if needed, readily be removed afterwards. In contrast, macromolecular acids may to a larger extent form an outer (acidic) layer and not diffuse into the food, allowing their removal if necessary. To our knowledge this type of food protection has been little described in the literature, with a possible exception of a report on antimicrobial effects of alginic acid coated polyethylene films (Karbassi et al., 2014), although the role of pH was not considered in this case.

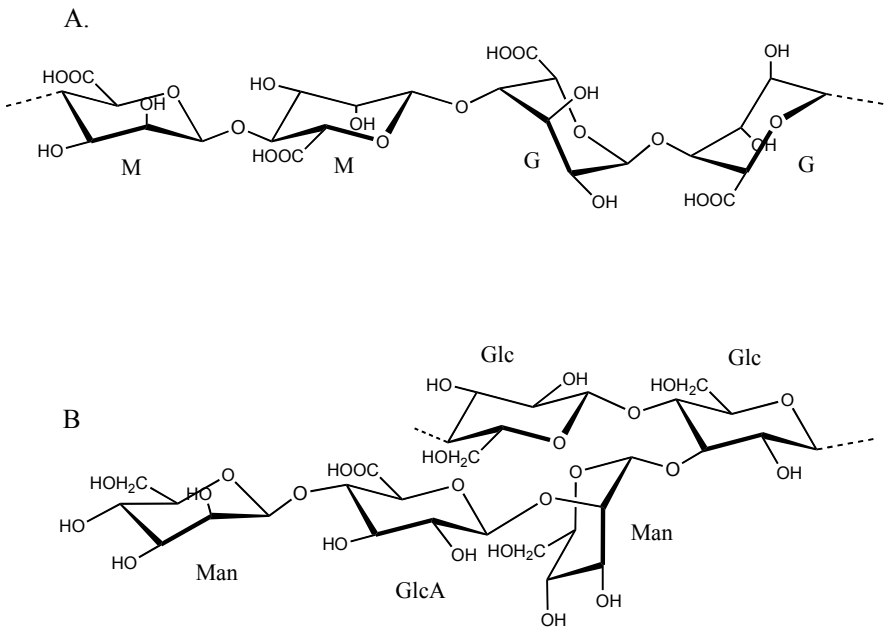

Figure 1. Structure of alginic acid exemplified by an MMGG fragment (A) and xanthan (B). Abbreviations: M: β-D-mannuronic acid. G: α-L-guluronic acid. Glc: β-D-glucose. GlcA: β-D-glucuronic acid. Man: D-mannose (α for inner Man, β for terminal Man). Note that xanthans may contain various amounts of O-acetate esterified at O6 of the inner Man, and pyruvate diketal linked to O4 and O6 of the terminal Man. The pyruvate contains an additional carboxylic acid.

Alginates are food-approved polysaccharides obtained from brown algae (Draget et al., 2006). Alginic acid (E400) refers to the acidic (H^+) form of alginate. They are unbranched polysaccharides containing two sugars: β-1,4-linked D-mannuronic acid (M) and its 5-epimer α-L-guluronic acid (G) (Figure 1.) The latter is introduced by processive C5 epimerases on the polymer level. Alginates may vary considerably in the content and intra-chain distribution of the two monomers. High-G alginates are often used due to their ability to form hydrogels with calcium salts. In the present context the type of alginate used for

preparing the alginic acid is of less importance, as the pK_a of the alginate is not very different for M and G (3.38 and 3.65, respectively) (Donati and Paoletti, 2009). Alginic acid is insoluble in water and therefore needs to be formulated in a manner suitable for the specific product. In the present work we explore the alginic acid dispersed in xanthan (Fig 1b), itself being a food approved, water-soluble polysaccharide (E415). It is able to form stable solutions also at low pH (without precipitation) at low concentrations. Dispersions and solutions are generally suitable for coating by either dipping and spraying. As alternative formulation we also explore alginic acid dispersed in vegetable oil or oil/water emulsions. In certain cases, like in boiled rice, the alginic acid may be added directly as a dry powder without dispersion agent.

Here we show that applying alginic acid based coatings effectively protects and reduces bacterial growth (natural flora) on fish (salmon, cod), meat (beef, pork, chicken), and shrimp. We further show they prevent external contamination, and specifically reduce or inhibit regrowth of pathogens (*Bacillus cereus, B. weihenstephanensis, Listeria monocytogenes* serotype 1 and *Staphylococcus aureus*). In special cases like boiled rice alginic acid may largely replace acetic acid for acidification and preservation, as demonstrated studying regrowth of added spores of *B. cereus*.

2. Materials and methods

2.1. Materials and foods

Salmon belly loin fillets ('Salma laks"), Salma, Norway (vacuum packed with a very good hygiene; usually ≤3000 cfu/g) and cod fillets were bought at a local supermarket. For

98 experiments with salmon 5 different fillets where purchased spread out over a 2 months
99 period. Beef was obtained directly from freshly slaughtered cattle at a local slaughterhouse
100 (Nortura SA, Malvik, Norway). Pork fillet, chicken fillet, shrimp and rice were obtained from
101 a local food store. Fillets and meat samples were cut into pieces of 10 g (+/- 1 g) pieces. One
102 fillet or cut of meat was used as the source of meat or fish pieces in each experiment.

103 Alginic acid (Protacid F120) and water-soluble sodium alginate (LF 10/60) were both
104 obtained from FMC Biopolymer AS, Norway. The sodium alginate was converted to water-
105 insoluble alginic acid by precipitation with dilute hydrochloric acid followed by washing in
106 pure water, and finally freeze-drying.

107 Xanthan was food grade Keltrol XCD obtained from CP Kelco, USA. Clear solutions were
108 prepared by dispersing in water followed by Ultra-Turrax T25 treatment (9500 rpm). The H^+
109 form of xanthan was obtained by sequential dialysis against 0.2 M HCl and then MQ water.

110 Rice (jasmine type) was obtained in a local food store.

111 2.2. Analytical methods

112 The surface pH of coated foodstuffs was determined using a PHC2441-8 combination pH
113 electrode obtained from Radiometer, allowing direct measurements without removing the
114 coatings.

115 The pH of boiled rice was determined using a conventional (calibrated) pH electrode
116 following dispersion of 50 g of rice in 100 ml of 0.17 M KCl.

117 2.3. Bacterial strains

118 The following five bacteria were used in the tests: *Escherichia coli* (CCUG 17620), *Bacillus*
119 *cereus* (NVH0075/95), *B. weihenstephanensis* (10394), *Listeria monocytogenes* serotype 1
120 (NVH738) and *Staphylococcus aureus* (50090). *B. weihenstephanensis* (strain 10394) was
121 used in experiments carried out at 4 °C since *B. cereus* does not grow below 8 °C. All strains
122 were from stock cultures stored at -80 °C in 30 % glycerol. Samples were streaked out onto
123 blood agar plates (bovine) and grown at 30 °C overnight. One colony was then used for
124 growth in 10 ml BHI medium (Oxoid, Basingstoke, UK) for 18±1 hour at 37 °C for *E. coli* and
125 30 °C for the four other strains. The cfu is then about 10^8/ml for *B. cereus*, *B.*
126 *weihenstephanensis* and about 10^9/ml for *S. aureus, E. coli and L. monocytogenes*. Before
127 use, all strains were diluted to about 10^5 or 10^7 cfu/ml in sterile peptone water (Oxoid,
128 Basingstoke, UK).

129 2.4. Spores of B. cereus

130 *B. cereus* NVH 0075/95 was sporulated in a chemically defined sporulation medium (de
131 Vries et al., 2004). In brief, a 1/10 dilution of a four hours culture of brain heart infusion
132 broth (BHI) (Becton, Dickinson & Co, Sparks, MD, USA) was resuspended in the chemically
133 defined sporulation medium (30 °C, 250 rpm rotary shaking). After 2-5 days of sporulation
134 spore batches, 95 % free of germinated spores as observed by phase-contrast microscopy,
135 were cleaned by repeated centrifugation (10 min, 6500 x *g*, 4 °C, Sorvall RC-5B) and washing
136 with 10 mM potassium phosphate buffer pH 7.2. The spores were stored in the buffer at 4
137 °C protected from light. To ensure stable spore crops, spores were stored for at least a
138 fortnight after washing before used for experiments.

139 2.5. Coating formulation

Aqueous coatings were prepared by first adding xanthan powder to Milli-Q water to a final concentration of 5 g/l. The viscous solution was further homogenized at room temperature with an Ultra-Turrax T25 operating at 9500 rpm. Alginic acid powder was then added and mixed into the solution by a second round of Ultra-Turrax treatment. The dispersions were kept at 4 °C until further use.

Oil/water emulsions containing alginic acid were prepared by adding 10% (v/v) vegetable oil to an alginic acid–xanthan dispersion, followed by Ultra-Turrax treatment to homogenise. The emulsions were stable for at least one week. Dispersions in vegetable oil were prepared by direct dispersion of dry alginic acid (6%) followed by homogenisation.

Powder coating was obtained by mixing dry alginic acid (19.6%) with wheat flour.

2.6. Coating of blood agar plates and incubation with bacteria

Blood agar plates were coated by pouring a solution (50 g/l alginic acid in 5 g/l xanthan) onto the plates, so that it just coved the plate (< 1 mm thickness). Before seeding of the bacteria on top of the coating material, the agar plates were incubated one hour overnight at 4 °C, and then left at room temperature for 1 hour. Ten µl of bacterial suspension containing either 10^7 or 10^8 cfu/ml was used.

2.7. Coating of fish/meat and incubation with bacteria

Pieces of fish or meat obtained from a single cut or fillet (10g +/- 1 g,) were first immersed in solutions (peptone water) containing either about 10^5 or 10^7 cfu of the different bacteria. The pieces were kept at room temperature for 45 minutes before coating by briefly immersing the pieces into the alginic acid/xanthan coating (three pieces for each inoculum), and let excess coating drip off before incubation.

2.8 Microbial analysis

Pieces of fish or meat were tested both with the natural flora and after inoculation with the different pathogens. In order to keep the number of bacteria as low as possible before inoculation the pathogens the pieces were incubated under UVC light for 3 minutes on each side. The surviving bacteria were then about 100 cfu/g, before the coating procedure was started.

Each piece of coated food was then incubated at 4, 12, 22 and 30 °C for up to 8 days. Positive controls were treated the same way but without coating. For some experiments the fish was coated containing its natural flora only. The pieces were serial diluted in peptone water and 0.1 ml seeded on to blood agar plates, or for *E. coli* VRB agar plates (Oxoid, Basingstoke, UK) (in duplicate). Plates were incubated for 24 hours at 30 and 37 °C before counting. All the pathogens apart from *E. coli* could be separated from the natural flora due to haemolysis and colony appearance.2.9. Statistical analysis

Plate counts were conducted using conventional dilution series with two parallels, each parallel being analysed in duplicate or triplicate. Standard deviations are included in the figures. A two-way analysis of variance (ANOVA) was conducted to compare the main effects on each food item of coating type and incubation time for the response of pH or natural logarithm transformed bacterial counts (CFU/g or CFU/cm^2)). The General Linear Model (GLM) procedure in Minitab version 18 was used included interaction effects. The criterion for significance was a two-tailed $P < 0.05$. Comparison between the main and interaction effects was made with the *post-hoc* Tukey test at a confidence interval of 95%.

184 3. Results

185 3.1. Coating formulation and acidification of food surfaces

186 We first assayed the ability of alginic acid to acidify and maintain a low surface pH on
187 salmon and chicken fillets when formulated as a viscous dispersion in xanthan. Chicken
188 fillets were in addition assayed for development of a pH gradient below the surface. Then the
189 pH of alginic acid treated boiled rice was determined and compared to acetic acid.

190 3.1.1. Coating formulation and pH on salmon fillets.

191 Alginic acid (0 – 100 g/l) was dispersed in aqueous xanthan (5 g/l) to form a viscous
192 dispersion suitable for dip-coating, spraying, etc. Xanthan was chosen among several other
193 food-approved polysaccharides as dispersing agent for insoluble alginic acid. The acidic form
194 of xanthan was used to avoid partial neutralization of the alginic acid when used at low
195 concentrations. The pH of the coating solutions was between 2.7 and 2.9, depending on the
196 amount of alginic acid. Salmon fillets were dip-coated and stored at 4°C, and the surface pH
197 was monitored at regular intervals (Figure 2)

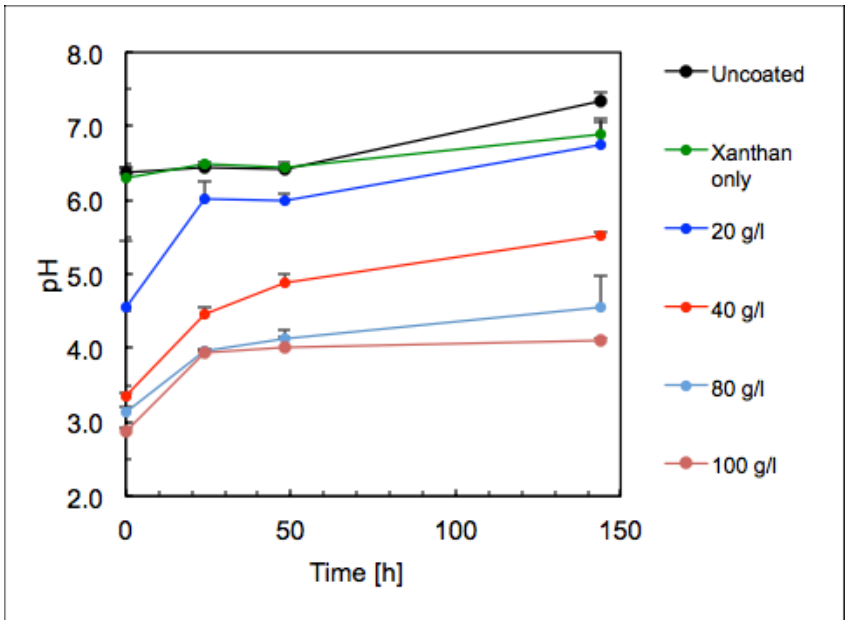

Figure 2. Surface pH of salmon fillets dip-coated with xanthan (5 g/l) containing various amounts (0 – 100 g/l) of alginic acid.

198 The surface pH measured immediately after coating depended strongly and was significantly
199 different depending on the alginic acid content, reaching as low as 2.8 for 100 g/l. An initial
200 pH below pH 3.5 was obtained using 40 g/l alginic acid or more.

201 The fillets coated only with xanthan behaved quite similar to uncoated fillets by having
202 stable and no significant difference in pH of 6.4-6.5 for up to 50 h. For longer incubation
203 times the pH increased slightly with a significantly higher pH for uncoated fillets by 144
204 hours.

205 A distinct behaviour was observed in the presence of alginic acid, with a rapid increase in pH
206 (1 – 1.5 pH units) during the first 24 hours, followed by a slower increase in pH. For 100 g/l
207 alginic acid the pH stabilized in the range 4.0 – 4.1 even up to 150 hours.

208 3.1.2. Coating formulation and pH on chicken fillets.

209 Chicken fillets were similarly coated with alginic acid (0, 50 and 80 g/l) dispersed in xanthan
210 and incubated at 4°C. The pH was determined after 96 hours at three different positions:
211 surface, 5 mm below surface and in the middle of the fillets (Figure 3)

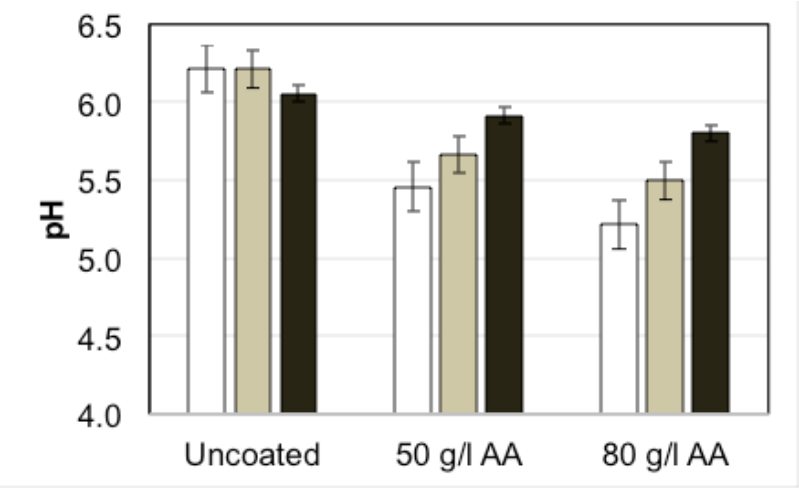

Figure 3. pH profiles of chicken fillets dip-coated with 5 g/l xanthan containing various amounts of alginic acid. The pH was determined at the surface (white), 5 mm below the surface (grey), and in the middle (black) of the fillets after 96 hours of incubation at 4°C.

212

213 As for salmon the coating is able to maintain a relatively low surface pH over a long time (pH
214 5.5 for 50 g/l and pH 5.3 for 80 g/l alginic acid). The decrease in pH was smaller but still
215 significant 5 mm below the surface, and even smaller but significant in the middle of the
216 fillet. However, compared to coated salmon fillets the chicken coatings were more
217 effectively neutralised.

218 3.1.3. Alginic acid powder added to boiled rice - pH

Two types of rice (sushi rice and Jasmin rice) were boiled for 20 min. Alginic acid (dry) or acetic acid (control) was added after cooling and mixed well into the rice. The samples were left to equilibrate for 16 hours before pH was monitored after suspending 50 grams of boiled rice in 100 ml 0.17 M KCl (Fig. 4).

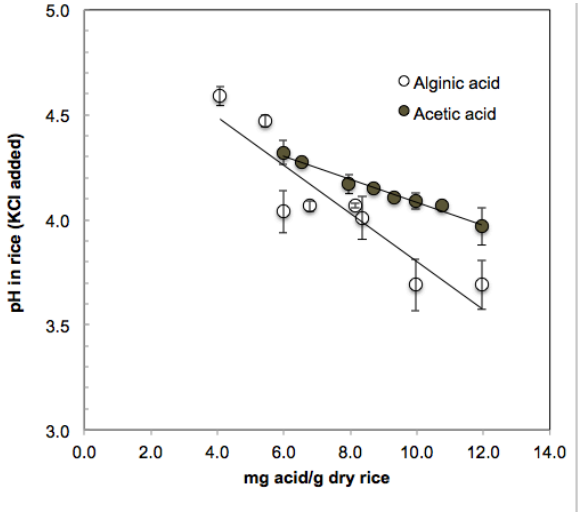

Fig. 4. pH in boiled sushi rice treated with alginic acid or acetic acid. Similar results were obtained for Jasmin rice.

Both acids demonstrate as expected decreasing pH with increasing amounts. Data for alginic acid seem to fluctuate more than for acetic acid, which is ascribed to the influence of the mixing process for a dry powder (alginic acid) to boiled rice. Nevertheless, alginic acid has, due to its lower pK_a (ca. 3.5 vs 4.76 for acetic acid) a stronger acidifying effect above 5 mg/g added. It may be noted there appeared to be negligible influence on the taste and texture of the rice up to ca. 10 mg alginic acid added.

3.2. Protection against external contamination of pathogens

232 Applying an external coating should in principle provide efficient protection against bacterial
233 growth due to external contamination. To demonstrate this effect it was investigated if
234 pathogenic bacteria (10^5 and 10^6 bacteria in 10 µl drops) could grow when applied on top of
235 blood agar plates coated with alginic acid (50 g/l) in xanthan. The plates were incubated at
236 4, 12, 22 °C, and visually inspected after 1 and 4 days, respectively. As expected, no growth
237 was observed on top of the plates, even after 4 days incubation at 22 °C.

238 3.3. Microbiology of coated foods

239 After demonstrating the ability of alginic acid coatings to acidify food surfaces, we
240 continued by monitoring the growth of the natural microbial flora in a range of different
241 foods following coating with alginic acid. Further, specific food pathogens, including heat
242 resistant bacterial spores, were added in a controlled way before assaying their growth
243 following coating. In some cases the range of coating formulation was expanded to include
244 dispersions and emulsions using vegetable oil.

245 3.3.1. Microbiology of coated salmon fillets

246 We first assayed the development of natural flora in salmon fillets under conditions
247 corresponding to the pH profiles described in Section 3.1.1. Salmon fillets containing coating
248 with 0 – 80 g/l alginic acid were thus assayed for development of the natural bacterial flora
249 following incubation at 4°C (Figure 5). These fillets have originally low bacterial counts (<
250 1000).

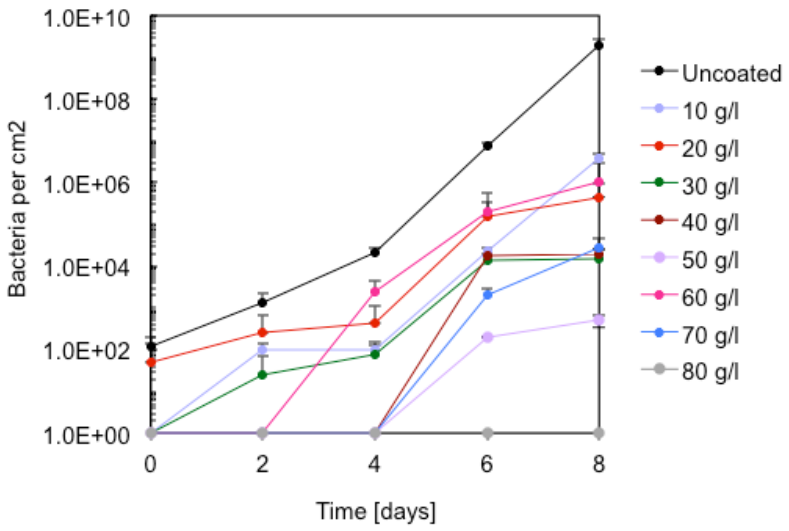

Figure 5. Total bacterial counts on salmon fillets dip-coated with 5 g/l xanthan containing various amounts of alginic acid. Incubation temperature: 4°C.

Uncoated fillets reached 10^4 bacteria/cm^2 after 4 days, before a more rapid growth was observed, reaching 10^9 after 8 days. The presence of an alginic acid coating generally significantly suppressed bacterial growth in a clear concentration-dependent manner. For 10 – 30 g/l the growth curves were essentially shifted downwards 3-4 orders of magnitude compared to uncoated filets. 20 g/l was sufficient to keep the bacterial counts under 10^6 even after 8 days where uncoated fillets are considered inedible. Concentrations above 30 g/l completely suppressed growth the first 2 days, increasing to 4 days for 40-70 g/l, whereas 80 g/l completely suppressed growth throughout the test period (8 days). Interestingly concentrations in the range 20-70 g/l resulted in a levelling off in bacterial counts for longer times, with marginal growth between 6 and 8 days. Further, the plateau values were in all cases below 10^6.

262 3.3.2. Microbiology of coated cod fillets

263 We continued with monitoring growth coated and uncoated cod fillets, using the natural
264 flora (analogous to the salmon fillets). Samples were incubated at 4 and 12 °C, respectively
265 (Figure 6). The higher temperature was included to study the protective effect under typical
266 'temperature abuse' conditions. The fillets had a natural flora of about $3x10^6$ CFU/g at the
267 start of the experiments, increasing significantly to about $5x10^9$ after 6 days incubation at
268 both temperatures. The increase (growth rate) was however much slower initially at 4 °C, as
269 expected. After coating and using an incubation temperature at 12 °C the development of
270 the flora was close to that of 4 °C without coating, although a little slower after the first day
271 of incubation. The coated cod stored at 4 °C had a decrease in bacterial number the first 24
272 hours, and thereafter the bacterial count increased gradually to $3x10^7$ after six days, ending
273 up two orders of magnitude and significantly lower in bacterial count than the uncoated cod
274 stored at the same temperature. The experiment at 4°C was repeated using a cod fillet
275 having lower bacterial content prior to coating ($4x10^4$). The effect of coating was similar to
276 the previous case, i.e. a general decrease in bacterial counts of 1-1.5 orders of magnitude
277 (data not shown).

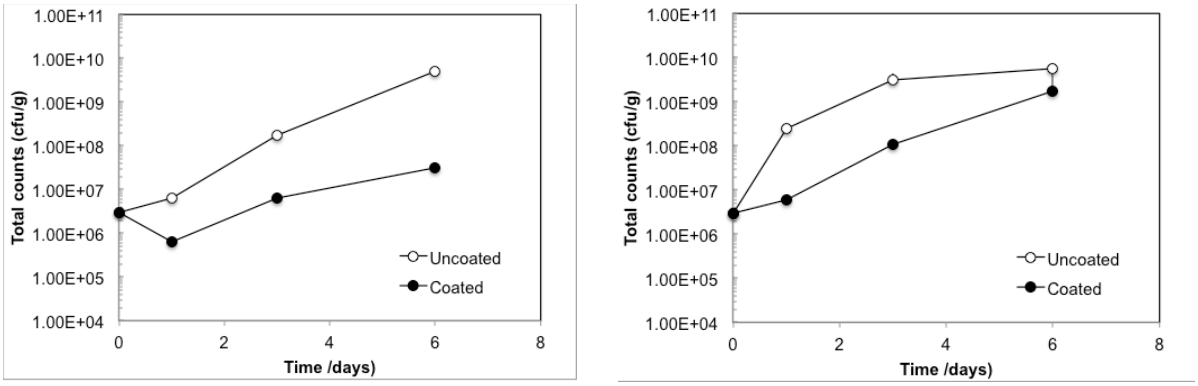

Figure 6. Bacterial growth (natural flora) on coated and uncoated cod fillets incubated at 4°C

(A) and 12°C (B). The coating contained 50 g/l alginic acid dispersed in 5 g/l xanthan. Note error bars are mostly too small to appear on the figure (log scale).

278

279 3.3.3. Coated salmon fillets pre-incubated with pathogenic bacteria

280 The ability to protect against specific pathogens present on fillets was assayed by using
281 salmon fillets which had been pre-coated with four pathogenic bacteria, i.e. prior to adding
282 the alginic acid/xanthan coating. The pathogens were *E. coli*, *B. cereus* (NVH0075/95), *B.*
283 *weihenstephanensis* (10394), *L. monocytogenes* serotype 1 (NVH738) and *S. aureus* (50090).
284 *B. cereus* was substituted with *B. weihenstephanensis* at 4 °C since *B. cereus* does not grow
285 at this temperature). The fillets were UVC treated and then pre-incubated by dipping into
286 pure cultures. Figure 7A shows the results of the growth experiments at 12 °C, with and
287 without coating. After UVC treatment all the fish pieces contained about 10^2 cfu/g of natural
288 flora, which increased gradually to at least 10^9 cfu/g after 7 days of storage without coating,
289 and to between 10^6 and 10^8 cfu/g (significantly less) with coating (the initial natural flora
290 may not be identical). However, the added pathogenic flora (about 10^4 cfu/g) grew to at
291 least 3 orders of magnitude higher values (significantly more) during the experiments
292 without coating. After coating *E. coli* and *B. cereus* hardly grew at all during the 7 days of
293 storage, while *S. aureus* grew to a little below 10^5. *L. monocytogenes* was less affected by
294 the coating, but even for this species the growth was inhibited well, both initially and
295 further up to 3 days of storage (three orders of magnitude fewer bacteria with coating after
296 3 days of storage).

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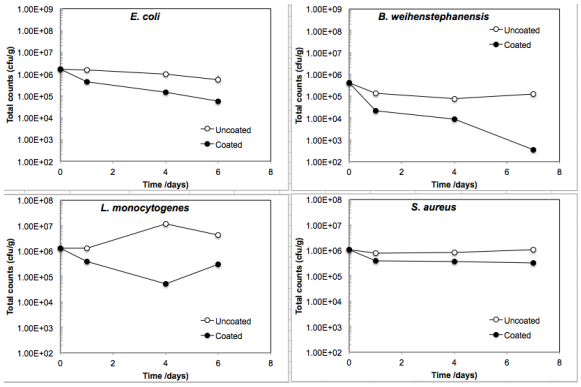
A



B

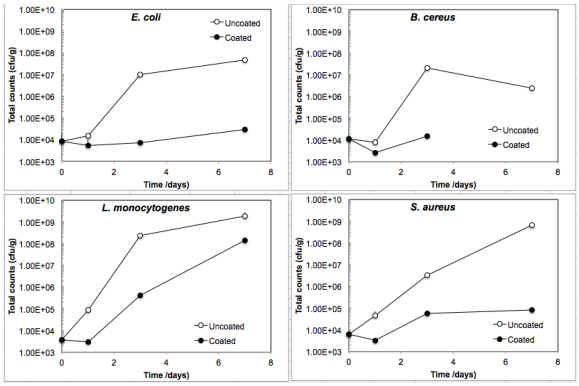


Figure 7. Salmon fillets pre-incubated by pathogens and further incubated at 4°C (A) and 12°C (B). *B. weihensephanensis* was used instead of *B. cereus* at 4°C since the latter does not grow at 4°C. Note error bars are generally too small to appear on the figure (log scale)

298

299 The same experiments were conducted at 4 °C, but using 10^7 cfu/g initially (Figure 7B),
300 showing that apart from *L. monocytogenes* (and the natural flora) the added pathogens
301 hardly grew at all. Moreover, cell counts were in fact significantly reduced by about one
302 order of magnitude after coating. Even for *L. monocytogenes* the number of bacteria was
303 significantly reduced after coating at 4 °C.

304 3.3.4. Microbiology and pH of coated shrimp.

305 Shrimp were peeled, coated with either 50 g/l or 80 g/l alginic acid in xanthan, and
306 incubated at 4°C. Surface pH and bacterial growth (natural flora) were monitored (Figure 8).

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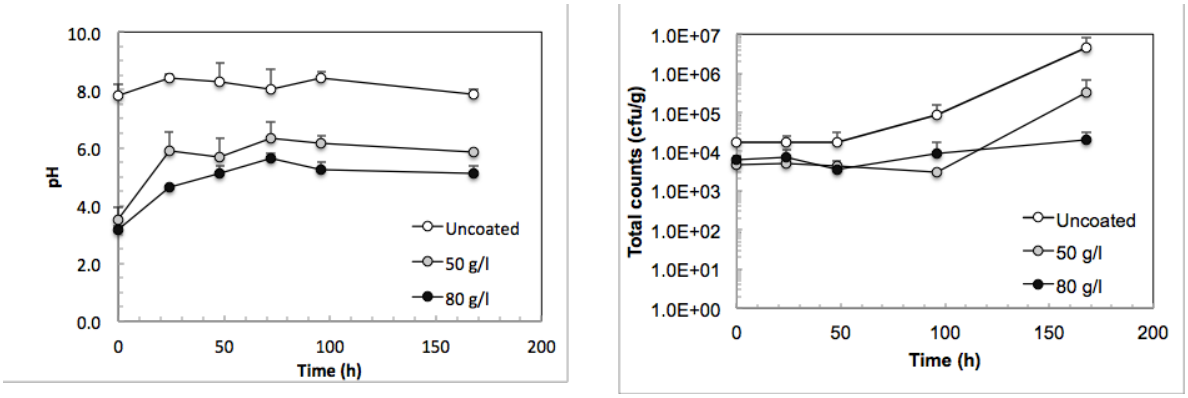

Fig. 8. Surface pH and bacterial counts of uncoated and alginic acid (50 and 80 g/l in 5 g/l xanthan) coated shrimp incubated at 4°C.

Uncoated shrimp had a stable surface pH of about 8. Bacterial counts were stable for 2 days before they significantly increased. The coating significantly reduced the initial pH to below 4, but it increased the first day and stabilised around 6.0 and 5.0 for 50 and 80 g/l, respectively but was still significantly lower than the uncoated shrimp. No significant changes in bacterial counts were observed up to 100 hours of incubation for the respective coatings although the bacterial counts on coated shrimp were significantly lower then uncoated shrimp. By 168 hours there were a significantly greater number of bacteria (bacterial growth) on all coatings and there was a significant and dose-dependent difference in bacterial counts for the three coatings tested.

3.3.5. Microbiology of coated beef and pork – alternative formulations

Beef from freshly slaughtered cattle was directly coated (no UV treatment) with 60 g/l alginic acid dispersed in either xanthan (as in preceding experiments), vegetable oil, or a 10% oil in water emulsion. Bacterial counts following incubation at 12°C are shown in Figure 9. The high temperature of 12°C was chosen to simulate conditions considered as 'temperature abuse' of foods.

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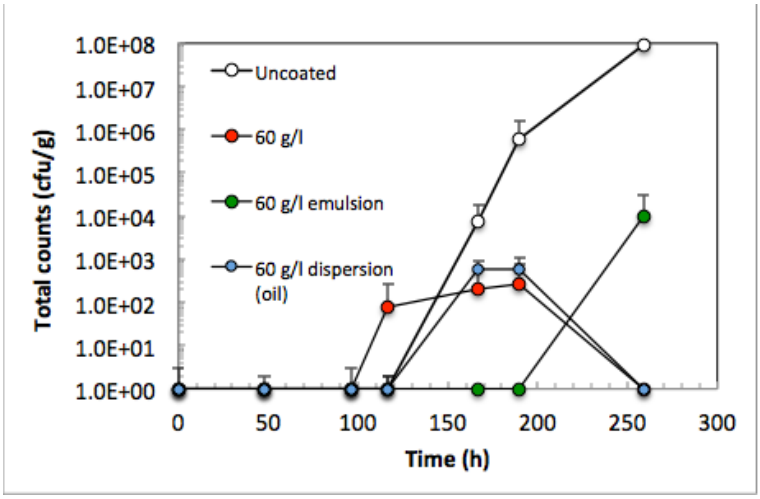

Figure 9. Total bacterial counts on beef stored at 12°C using four different formulations containing alginic acid: Uncoated, coating containing 60 g/l alginic acid (in 5 g/l xanthan), emulsion coating (10/90 o/w) containing 60 g/l alginic acid, and an oil dispersion containing 60 g/l alginic acid.

324 The uncoated beef had undetectable bacterial counts for up to 100 h, reflecting the hygiene
325 adapted in the slaughtering process. However, rapid and essentially exponential growth was
326 then observed, reaching counts of 10^8 after 260 hours. Also coated beef had detectable
327 growth after 100-120 hours, but did not reach counts above 10^4 even after 260 hours, i.e.
328 four orders of magnitude lower than uncoated beef. A peculiar behaviour was observed for
329 alginic acid dispersed in xanthan or in pure oil as demonstrated by a transient emergence of
330 culturable bacteria, although in relatively low numbers (maximum 1000 CFU) between 100
331 and 200 hours, but no detectable growth after 200 hours. The o/w emulsion containing

alginic acid was effective up to 200 hours, but rapid growth similar to uncoated beef was then observed.

Pork fillet was also coated as described above, but widening the range of formulations to include alginic acid powder coating and an additional oil dispersion containing 19.4% alginic acid. Again, samples were incubated at 12°C to simulate conditions considered as 'temperature abuse'. Results are given in Figure 10.

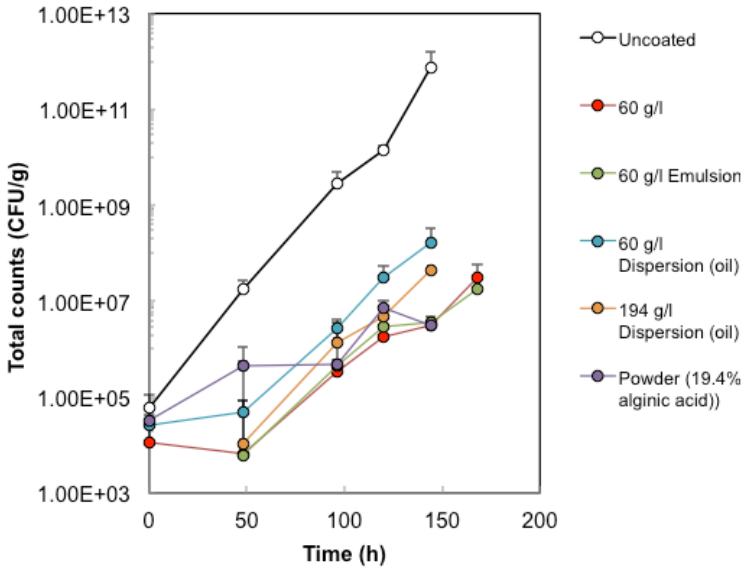

Figure 10. Total bacterial counts on pork incubated at 12°C using different formulations containing alginic acid: Uncoated, coating containing 60 g/l alginic acid (in 5 g/l xanthan), emulsion coating (10/90 oil/water) containing 60 g/l alginic acid, oil dispersions containing 60 or 194 g/l alginic acid, and a powder coating containing 19.4% alginic acid.

Whereas uncoated pork showed a rapid and essentially exponential increase in bacterial counts, reaching hygienically unacceptable values after just a few days, all coatings had significant stabilizing effects, resulting in decrease in bacterial counts between 1.5 to two orders of magnitude, rendering the coated pork fillets in principle acceptable for consumption for more than 4 days. Interestingly, increasing the amount of alginic acid from 60 to 194 g/l seemed to have no additional stabilizing effect.

3.3.6. Regrowth of B. cereus spores in rice treated with alginic acid

Spores of *B. cereus* were added to rice (10^4 spores/g) before cooking. Alginic acid (final pH of 4.0) was added either before or after cooking. Acetic acid was included for comparison. Portions of the boiled rice were then incubated aerobically or anaerobically at 4, 12 and 22°C, and bacterial counts determined. Results (bacterial counts) are given in the Supplementary Information (Table SI-1). At 4°C significant growth was only observed for the control sample containing added spores incubated aerobically for 6 days. At 12°C extensive growth was observed from day 3 for control samples with added spores, both for aerobic and anaerobic incubation. Samples containing spores and alginic acid or acetic acid did not exhibit growth. Incubation at 22°C resulted in even more extensive growth in the control samples, including the one without added spores, showing that external contamination had taken place. Addition of alginic acid before cooking or acetic acid (after cooking) eliminated bacterial growth, whereas significant growth was observed when alginic acid was added after cooking.

3.4 Discoloration, texture and 'acid cooking'

Different foods respond quite differently to the presence of an acidic coating. In general, the process of ‘acid cooking’, referring to the whitening of the surface attributed to protein denaturation, and which is well known for traditional acids, did indeed occur. It was most prominent on salmon, where visible whitening developed upon acidification (Supplementary Information Figure S-1), but was also detected on meat, whereas the surface of coated shrimp was not visibly affected.

3.5. Practical aspects of coating: cooking and frying, edibility, colour.

Pieces from fillets from beef, pork and chicken, as well as peeled shrimp, were dip-coated with 20 and 80 g/l alginic acid dispersed in xanthan (5 g/l). They were subsequently boiled in salt water or fried in vegetable oil for a few minutes (until uncoated pieces were edible). Cooking removed the coating in seconds. Frying also seemed to remove or conceal the coating. In all cases there were no differences in colour, texture, or taste between coated and uncoated samples.

4. Discussion

Alginic acid formulated as dispersions suitable for dip-coating works effectively as a means to protect foods from microbial decay. Firstly, once coated, the coating prevents further contamination from external, for example airborne, sources. The results obtained using coated blood agar plates showed that subsequently added pathogens were effectively neutralized and did not show regrowth. Secondly, bacteria already present before coating i.e. the natural microbial flora, or specifically added pathogens, exhibit a clear pH-dependent delayed regrowth on a wide range of different foods. This also applies to regrowth of heat-resistant spores (*B. cereus*). In general, a prolonged shelf life is obtained, even at higher temperatures where uncoated materials rapidly become inedible.

Of practical importance is the fact the coatings contain only food-approved ingredients. Equally important are the properties of the coatings during processing (e.g. cooking or frying). The coating may easily be washed away in tap water, or it may simply be present in during process where it normally disintegrates and leave no detectable trace related to texture, taste and appearance for a wide range of tested foods. In addition to aqueous dispersions the alginic acid may be easily formulated by dispersion in vegetable oil, as o/w emulsions, or simply as added powder, depending on the specific system.

The antimicrobial properties of the coatings are strongly related to the pH, which is determined by the amount of added alginic acid and the rate of neutralization. The latter differs between different foods. For example, a coating containing 80 g/l alginic acid (pH 2.8) reaches a surface pH of 4.3 on salmon fillet (Fig. 2) after 96 hours of incubation at 4°C, whereas on chicken filled under the same conditions reaches a pH of 5.3 (Fig. 3), i.e. the

395 neutralization is more rapid in the latter case. The neutralization may be due to the
396 outwards diffusion of metabolites, but clearly also inwards diffusion of protons as evidenced
397 by a detectable pH gradient in chicken fillet. Although the alginic acid due to its
398 macromolecular size is not expected to diffuse into the food as would low molecular weight
399 acids such as acetic acid, the Grotthus mechanism (Hassanali et al., 2013) allow faster
400 migration of protons in aqueous media compared to simple salts, thereby contributing to
401 neutralisation of the surface. The increasing pH will also gradually solubilize the alginic acid
402 (as alginate). However, the dispersion in xanthan ensures that even soluble alginate remains
403 in the coating.

404 Food safety is usually not a large problem for fish, given it is heat treated before
405 consumption. However, the quality of fish (shelf life) is a considerable challenge because of
406 transport and usually several sales teams on its way to the consumer. We therefore wanted
407 to test an edible acid coating to possibly prolong the shelf life. Our first test was to see if our
408 coating completely inhibited five selected bacterial species from growing on top of the
409 coating. The test was carried out by applying a thin layer of coating (< 1mm) on the surface
410 of blood agar plates. Even as much as 10^6 bacteria (in a 10 µl droplet) did not grow on
411 surface of the coating when incubated at 4, 12, 22 or 30 °C after as much as 4 days. At least
412 three of the species we used in our tests will grow at pH down to 4.0-4.3, but the double
413 effect of even lower pH (2.7-2.9) and the physical barrier preventing bacterial transport to
414 the underlying blood agar prevented growth completely.

415 When we had shown that the bacteria were not able to grow on top of the coating we
416 continued to coat fresh fish (cod) from a local supermarket to see how well the coating
417 inhibited growth of the natural flora of the fish. As shown in Figure 6 the natural flora

418 decreased nearly one order of magnitude the first 24 hours, and then gradually increased
419 from about 6 x 10^5 to 3 x 10^7 over the following 5 days, at 4 °C. For the uncoated fish the
420 number of bacteria increased continuously from a starting point of 3 x 10^6 to 5 x 10^9 over
421 the 6 days of the experiments, showing that the shelf life of the fish probably would
422 increase by 4-6 day with coating at 4 °C, an effect which otherwise only can be obtained by
423 methods like super chilling or extensive salt treatment (Duun and Rustad, 2007). At 12 °C we
424 see the same tendency, but not as clear as for 4 °C.

425 We then continued to investigate the influence of coating on possible pathogenic bacteria:
426 *E. coli*, *B. cereus* (substituted with close relative *B. weihenstephanensis* at 4 °C since *B.*
427 *cereus* does not grow at that temperature) *L. monocytogenes* and *S. aureus*. We wanted to
428 use bacteria that can contaminate fish through handling and that could grow at relatively
429 low pH (4.0-4.8). As shown in Figure 7A all the pathogens grew well at 12 °C without
430 coating. In the presence of coating only *L. monocytogenes* grew relatively fast, but even
431 here the growth was significantly retarded the first 3 days. At 4 °C (Fig 7B) all species were
432 maintained at the initial numbers, except for *L. monocytogenes*, which grew from 10^6/g to
433 10^7/g. In contrast, the presence of coating showed in all cases a steady decrease in cell
434 counts. It should be emphasized this occurred even without competition from the natural
435 flora (that was reduced to about 100/g with UVC light). These experiments show that our
436 edible coating has a very good potential to stop growth of pathogens at 4 °C, and reduce the
437 growth at higher temperatures. Even the natural flora is strongly inhibited by our coating at
438 both 4 °C, and show slower growth at 12 °C.

439 The effects on natural flora obtained for salmon and cod are to a large extent are also
440 observed and extend generally to the other systems studied here, namely shrimp, chicken,

441 beef and pork: The lower the pH of the coating, the larger antibacterial effect. For peeled
442 shrimp (Fig. 8), whose surface appearance is largely unaltered by the coating, the bacterial
443 count remained essentially unaltered (ca. 10^4) for up to 6 days with 80 g/l alginic acid
444 coating.

445 For beef and pork we chose to incubate at 12°C to simulate 'temperature abuse' conditions,
446 and generally increase bacterial growth rates. Remarkably, several formulations (dispersions
447 in, emulsions or simply powder) had roughly the same effect by delaying growth for about 2
448 days compared to the uncoated pork. Hence, these coatings are particularly effective in
449 cases were 'temperature abuse' may be a challenge.

450 Alginic acid powder could easily be dispersed in boiled rice to provide the desired pH.
451 Adding alginic acid before boiling gave the same result. Compared to acetic acid, the normal
452 acidifier used e.g. in sushi rice, a lower pH was obtained due to the lower pK_a of alginic acid.
453 It is evident that alginic acid/acetic acid mixtures can be tailored to obtain both desired pH
454 and a range of tastes. The taste of alginic acid itself becomes detectable for the highest
455 concentrations used here.

456 In boiled rice the presence of heat resistant spores of *B. cereus* poses a serious risk if the
457 rice is stored for longer periods (production of the toxin cereulide) without effective cooling
458 (de Vries et al., 2004). Our results (Supplementary Information Table S-1) demonstrate that
459 adding alginic acid before cooking matches acetic acid and completely inhibits bacterial
460 growth where spores (10^4 spores/g) had been added, even after incubation at 22°C for 6
461 days. Adding alginic acid after cooking resulted in growth at 22°C, but not at 4 or 12°C. The
462 reason for this behaviour is presently unclear. A tentative explanation could be uneven

distribution of alginic acid due to inadequate mixing, but since addition before cooking should be trivial, this approach is recommended.

In the present work alginic acid was used as the sole macromolecular acid. Besides being food approved it is also commercially available, or can easily be prepared by precipitation of the more common sodium alginate with dilute hydrochloric acid (or any suitable acid). However, other polysaccharides rich in acidic groups may in principle be used. This includes common food hydrocolloids like pectins (especially those high in un-esterified galacturonic acid) or carboxymethyl cellulose (high DS), in both cases after conversion to the acidic form. Xanthan itself, here used mainly as a dispersion stabilizer, can also function as a macromolecular acid. The disadvantage is a relatively low content of carboxylic acid (in the glucuronic acid and the pyruvate (Figure 1B)) compared to alginic acid or CMC, resulting in the need for more concentrated coatings to obtain a predetermined pH. The acidic form of xanthan is, in contrast to alginic acid, soluble in water (Christensen and Smidsrød, 1991; Zhang et al., 1987), allowing more transparent coatings. Sulphated polysaccharides (hydrocolloids) such as the carrageenans (E407) are much used as food ingredients, but are normally not manufactured on the acidic form, and are further less suited as acidic coatings due to their higher susceptibility towards acid hydrolysis (Hjerde et al., 1998).

6. Conclusions

The acidifying properties of alginic acid form an excellent basis for preparing antimicrobial food coatings solely based on acidification. In contrast to biologically active ingredients such as antibacterial peptides, development of antimicrobial resistance seems less probable. Alginic acid is insoluble in water unless neutralised, and can easily be dispersed in both

485 aqueous and non-aqueous coatings, or simply mixed in (as in boiled rice) or added directly
486 as a powder. Alginic acid coatings prevent external contamination, inhibit outgrowth of *B.*
487 *cereus* spores, and further inhibit the growth of the naturally occurring bacteria for a range
488 of different foods. The shelf life is hence increased for up to several days, even at elevated
489 temperatures. The low surface pH may in some cases change the surface structure due to
490 'acid cooking', but this effect disappears upon further treatment (cooking, frying). Long-
491 term effects of the coatings are restricted by the rate of neutralisation of the coatings,
492 which depends on the type of food used.

493 In future work it could be useful to investigate hurdle technology were acidic coatings are
494 combined with other common preservation methods such as modified atmosphere
495 packaging.

496

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501

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535

536

537 **SUPPLEMENTARY INFORMATION**

538

539

540

541 **Table S-1.**

542 Total bacterial counts in boiled rice containing spores of *B. cereus*, incubated at 3 different
543 temperatures.

544

	Sample	Incubation time					
		Day 0		Day 3		Day 6	
		Aerob	Anaerob	Aerob	Anaerob	Aerob	Anaerob
4 °C	S1	<100	<100	<100	<100	100	<100
	$S1_s$	<100	<100	<100	<100	$1x10^3$	<100
	S2			<100	<100	<100	<100
	$S2_s$			<100	<100	800	100
	S3			<100	<100	100	<100
	$S3_s$			<100	<100	100	<100
	S4			<100	<100	<100	<100
	$S4_s$			<100	<100	<100	<100
12 °C	S1	<100	<100	-	-	-	-
	$S1_s$	<100	<100	$^{*}1,8{\cdot}10^3$	$1,6 \cdot 10^3$	$^{*}2,2{\cdot}10^5$	$> 10^4$
	$S2_s$			<100	<100	<100	<100
	$S3_s$			<100	<100	<100	<100
	$S4_s$			<100	<100	<100	<100
22 °C	S1	<100	<100	$^{*}2{\cdot}10^5$		$2,4{\cdot}10^5$	
	$S1_s$	<100	<100	$^{*}1,6{\cdot}10^5$ $^{**}2{\cdot}10^6$		$^{*}3{\cdot}10^8$	
	$S2_s$			<100	<100	<100	<100
	$S3_s$			$^{*}1,3{\cdot}10^5$	$^{*}1,1 \cdot 10^5$	$^{*}1,3{\cdot}10^5$	
	$S4_s$			< 100	<100	<100	<100

Table 1. Total bacterial counts in boiled rice containing spores of *B. cereus*, incubated at 3 different temperatures. Abbreviations: S1: Control (no spores added). S2: Alginic acid added before cooking. S3: Alginic acid added after cooking. S4: Acetic acid added after cooking. $S1_s$-$S4_s$: Spores added.

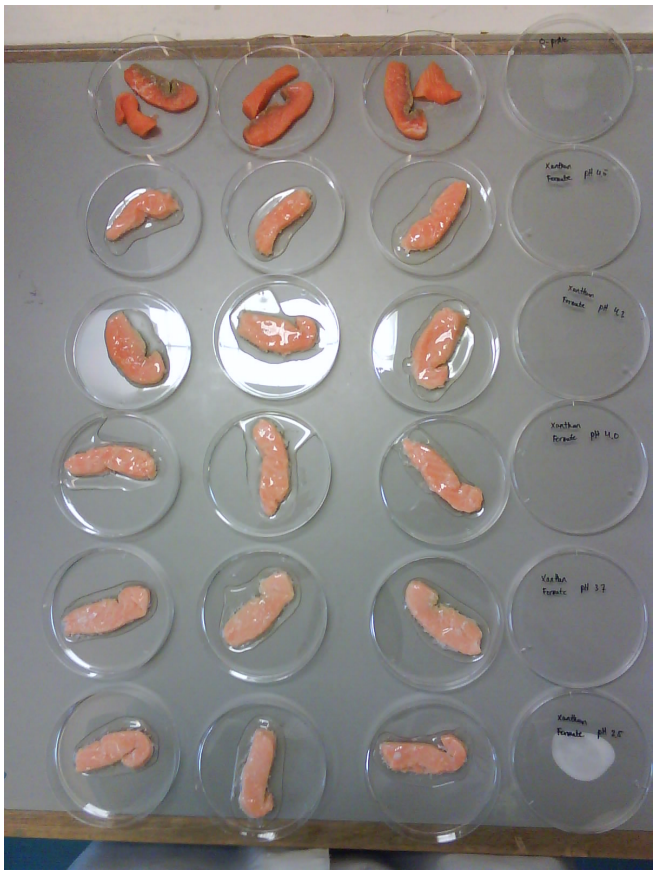
*Identified as *B. cereus*. **Species other than *B. cereus*.

545

546

Figure S-1.

Discoloration of raw salmon fillet



Salmon fillets were coated with varying amounts of alginic acid dispersed in 5 g/l xanthan, incubated at 4°C, and observed at regular intervals. Photo shows fillets after 24 hours. Top to bottom: Uncoated, pH 4.5, pH 4.3, pH 4.0, pH 3.7, pH 3.5.

Figure 2.

General Linear Model: pH versus Time; Coating

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 24; 48; 144
Coating	Fixed	6	0.5% xanthan; 0.5% xanthan/100mg/mL alginic acid; 0.5% xanthan/20mg/mL alginic acid; 0.5% xanthan/40mg/mL alginic acid; 0.5% xanthan/80mg/mL alginic acid; Without coating

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	18,838	6,2794	116,11	0,000
Coating	5	100,521	20,1043	371,76	0,000
Time*Coating	15	5,132	0,3421	6,33	0,000
Error	48	2,596	0,0541		
Total	71	127,087			

Comment SBA: It is the P-values that we need to look at in the analysis of variance table for each analysis. The interaction effect is the most important to consider. All p are less than 0.05 for all tests. Perhaps just add a sentence on this in the text.

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,232549	97,96%	96,98%	95,40%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	5,2067	0,0274	189,98	0,000	
Time					
0	−0,7772	0,0475	−16,37	0,000	1,50
24	0,0122	0,0475	0,26	0,798	1,50
48	0,1089	0,0475	2,29	0,026	1,50
Coating					
0.5% xanthan	1,3217	0,0613	21,57	0,000	1,67
0.5% xanthan/100mg/mL alginic acid	−1,4742	0,0613	−24,06	0,000	1,67
0.5% xanthan/20mg/mL alginic acid	0,6192	0,0613	10,10	0,000	1,67
0.5% xanthan/40mg/mL alginic acid	−0,6442	0,0613	−10,51	0,000	1,67
0.5% xanthan/80mg/mL alginic acid	−1,2608	0,0613	−20,57	0,000	1,67
Time∗Coating					
0 0.5% xanthan	0,546	0,106	5,14	0,000	2,50
0 0.5% xanthan/100mg/mL alginic acid	−0,082	0,106	−0,77	0,444	2,50
0 0.5% xanthan/20mg/mL alginic acid	−0,505	0,106	−4,76	0,000	2,50
0 0.5% xanthan/40mg/mL alginic acid	−0,425	0,106	−4,01	0,000	2,50
0 0.5% xanthan/80mg/mL alginic acid	−0,042	0,106	−0,40	0,694	2,50

24	0.5% xanthan	−0,047	0,106	−0,44	0,658	2,50
24	0.5% xanthan/100mg/mL alginic acid	0,195	0,106	1,84	0,072	2,50
24	0.5% xanthan/20mg/mL alginic acid	0,175	0,106	1,65	0,105	2,50
24	0.5% xanthan/40mg/mL alginic acid	−0,105	0,106	−0,99	0,329	2,50
24	0.5% xanthan/80mg/mL alginic acid	0,002	0,106	0,02	0,985	2,50
48	0.5% xanthan	−0,204	0,106	−1,92	0,061	2,50
48	0.5% xanthan/100mg/mL alginic acid	0,169	0,106	1,59	0,119	2,50
48	0.5% xanthan/20mg/mL alginic acid	0,065	0,106	0,61	0,541	2,50
48	0.5% xanthan/40mg/mL alginic acid	0,225	0,106	2,12	0,039	2,50
48	0.5% xanthan/80mg/mL alginic acid	0,082	0,106	0,77	0,444	2,50

566 ## Regression Equation

```
pH = 5,2067 − 0,7772 Time_0 + 0,0122 Time_24 + 0,1089 Time_48
     + 0,6561 Time_144
     + 1,3217 Coating_0.5% xanthan  − 1,4742 Coating_0.5%
     xanthan/100mg/mL alginic acid
     + 0,6192 Coating_0.5% xanthan/20mg/mL alginic acid −
      0,6442 Coating_0.5% xanthan/40mg/mL
     alginic acid − 1,2608 Coating_0.5% xanthan/80mg/mL alginic acid
     + 1,4383 Coating_Without
     coating + 0,546 Time∗Coating_0 0.5% xanthan  − 0,082 Time∗Coating_0
     0.5%
     xanthan/100mg/mL alginic acid − 0,505 Time∗Coating_0 0.5%
     xanthan/20mg/mL alginic acid
     − 0,425 Time∗Coating_0 0.5% xanthan/40mg/mL alginic acid −
      0,042 Time∗Coating_0 0.5%
     xanthan/80mg/mL alginic acid + 0,509 Time∗Coating_0 Without coating
     − 0,047 Time∗Coating_24 0.5% xanthan  + 0,195 Time∗Coating_24 0.5%
     xanthan/100mg/mL
     alginic acid + 0,175 Time∗Coating_24 0.5% xanthan/20mg/mL alginic
     acid
     − 0,105 Time∗Coating_24 0.5% xanthan/40mg/mL alginic acid
```

```
+ 0,002 Time*Coating_24 0.5%
xanthan/80mg/mL alginic acid - 0,221 Time*Coating_24 Without coating
- 0,204 Time*Coating_48 0.5% xanthan   + 0,169 Time*Coating_48 0.5%
xanthan/100mg/mL
alginic acid + 0,065 Time*Coating_48 0.5% xanthan/20mg/mL alginic
acid
+ 0,225 Time*Coating_48 0.5% xanthan/40mg/mL alginic acid
+ 0,082 Time*Coating_48 0.5%
xanthan/80mg/mL alginic acid - 0,337 Time*Coating_48 Without coating
- 0,294 Time*Coating_144 0.5% xanthan   - 0,282 Time*Coating_144 0.5%
xanthan/100mg/mL
alginic acid + 0,265 Time*Coating_144 0.5% xanthan/20mg/mL alginic
acid
+ 0,305 Time*Coating_144 0.5% xanthan/40mg/mL alginic acid -
 0,042 Time*Coating_144 0.5%
xanthan/80mg/mL alginic acid + 0,049 Time*Coating_144 Without coating
```

Fits and Diagnostics for Unusual Observations

Obs	pH	Fit	Resid	Std Resid	
7	5,580	4,543	1,037	5,46	R
8	4,010	4,543	-0,533	-2,81	R
9	4,040	4,543	-0,503	-2,65	R
67	5,040	4,560	0,480	2,53	R

R Large residual

Residual Plots for pH

Comparisons for pH

Tukey Pairwise Comparisons: Time

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping		
144	18	5,86278	A		
48	18	5,31556		B	
24	18	5,21889		B	
0	18	4,42944			C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Coating

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
Without coating	12	6,64500	A
0.5% xanthan	12	6,52833	A
0.5% xanthan/20mg/mL alginic acid	12	5,82583	B
0.5% xanthan/40mg/mL alginic acid	12	4,56250	C
0.5% xanthan/80mg/mL alginic acid	12	3,94583	D
0.5% xanthan/100mg/mL alginic acid	12	3,73250	D

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Time*Coating

Grouping Information Using the Tukey Method and 95% Confidence

Time*Coating	N	Mean	Grouping
144 Without coating	3	7,35000	A
144 0.5% xanthan	3	6,89000	A B
144 0.5% xanthan/20mg/mL alginic acid	3	6,74667	A B
24 0.5% xanthan	3	6,49333	B C
24 Without coating	3	6,43667	B C
48 0.5% xanthan	3	6,43333	B C
48 Without coating	3	6,41667	B C
0 Without coating	3	6,37667	B C
0 0.5% xanthan	3	6,29667	B C
24 0.5% xanthan/20mg/mL alginic acid	3	6,01333	C D
48 0.5% xanthan/20mg/mL alginic acid	3	6,00000	C D
144 0.5% xanthan/40mg/mL alginic acid	3	5,52333	D E

48 0.5% xanthan/40mg/mL alginic acid	**3**	**4,89667**			**E**	**F**		
144 0.5% xanthan/80mg/mL alginic acid	**3**	**4,56000**				**F**	**G**	
0 0.5% xanthan/20mg/mL alginic acid	**3**	**4,54333**				**F**	**G**	
24 0.5% xanthan/40mg/mL alginic acid	**3**	**4,47000**				**F**	**G**	
48 0.5% xanthan/80mg/mL alginic acid	**3**	**4,13667**					**G**	
144 0.5% xanthan/100mg/mL alginic acid	**3**	**4,10667**					**G**	
48 0.5% xanthan/100mg/mL alginic acid	**3**	**4,01000**					**G**	**H**
24 0.5% xanthan/80mg/mL alginic acid	**3**	**3,96000**					**G**	**H**
24 0.5% xanthan/100mg/mL alginic acid	**3**	**3,94000**					**G**	**H**
0 0.5% xanthan/40mg/mL alginic acid	**3**	**3,36000**						**H** **I**
0 0.5% xanthan/80mg/mL alginic acid	**3**	**3,12667**						**I**
0 0.5% xanthan/100mg/mL alginic acid	**3**	**2,87333**						**I**

Means that do not share a letter are significantly different.

This table above shows which treatment and time is different from which. If it is allowed I would put the ANOVA table and this table in the supplemental data. Just upload this word file (minus the intro and my comments☺). If you want to say something specific in the text about a difference between treatments and time then you can just refer to the supplemental data.

Figure 3

General Linear Model: pH versus Coating; Location

Method

Factor coding (−1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Coating	Fixed	3	5%; 8%; uncoated
Location	Fixed	3	5 mm below surface; Middle; Surface

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Coating	2	5,1266	2,56331	234,78	0,000
Location	2	0,8831	0,44156	40,44	0,000
Coating*Location	4	1,1119	0,27798	25,46	0,000
Error	58	0,6333	0,01092		
Total	66	7,8930			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,104490	91,98%	90,87%	89,28%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	5,7793	0,0129	448,09	0,000	
Coating					
5%	−0,1027	0,0185	−5,55	0,000	1,35
8%	−0,2772	0,0179	−15,51	0,000	1,34
Location					

5 mm below surface	0,0093	0,0175	0,53	0,597	1,28
Middle	0,1427	0,0185	7,71	0,000	1,28
Coating*Location					
5% 5 mm below surface	−0,0234	0,0252	−0,93	0,356	1,74
5% Middle	0,0924	0,0268	3,44	0,001	1,71
8% 5 mm below surface	−0,0180	0,0244	−0,74	0,462	1,69
8% Middle	0,1553	0,0254	6,10	0,000	1,66

Regression Equation

```
p  =  5,7793 − 0,1027 Coating_5% − 0,2772 Coating_8%
H     + 0,3799 Coating_uncoated
      + 0,0093 Location_5 mm below surface + 0,1427 Location_Middle −
       0,1520 Location_Surface
      − 0,0234 Coating*Location_5% 5 mm below surface
      + 0,0924 Coating*Location_5% Middle
      − 0,0689 Coating*Location_5% Surface − 0,0180 Coating*Location_8% 5
      mm below surface
      + 0,1553 Coating*Location_8% Middle − 0,1372 Coating*Location_8%
      Surface
      + 0,0415 Coating*Location_uncoated 5 mm below surface −
       0,2476 Coating*Location_uncoated
      Middle + 0,2061 Coating*Location_uncoated Surface
```

Fits and Diagnostics for Unusual Observations

Obs	pH	Fit	Resid	Std Resid	
7	5,2200	5,4557	−0,2357	−2,44	R
12	5,7900	5,4557	0,3343	3,46	R
14	5,4100	5,2129	0,1971	2,04	R
16	4,9900	5,2129	−0,2229	−2,30	R
34	5,8600	5,6625	0,1975	2,02	R
43	5,2400	5,4933	−0,2533	−2,57	R

R Large residual

Residual Plots for pH

Comparisons for pH

Tukey Pairwise Comparisons: Coating

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
uncoated	22	6,15921	A
5%	21	5,67663	B
8%	24	5,50206	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Location

Grouping Information Using the Tukey Method and 95% Confidence

Location	N	Mean	Grouping
Middle	21	5,92198	A
5 mm below surface	26	5,78861	B
Surface	20	5,62730	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Coating*Location

Grouping Information Using the Tukey Method and 95% Confidence

Coating*Location	N	Mean	Grouping
uncoated Surface	6	6,21333	A
uncoated 5 mm below surface	9	6,21000	A
uncoated Middle	7	6,05429	A B
5% Middle	6	5,91167	B C
8% Middle	8	5,80000	C D
5% 5 mm below surface	8	5,66250	D
8% 5 mm below surface	9	5,49333	E
5% Surface	7	5,45571	E

8%	**Surface**	**7**	**5,21286**	**F**

615 ***Means that do not share a letter are significantly different.***

616

617 Figure 4 - I don’t have the necessary raw data

618

Figure 5. Experiments conducted on five different occasions with different fillets for controls and at different concentrations of alginic acid

General Linear Model: Log CFU per cm2 0510 versus ... ; Coating 0510

Method

Factor coding (−1; 0; +1)

Rows unused 2

Factor Information

Factor	Type	Levels	Values
Day	Fixed	5	0; 2; 4; 6; 8
Coating 0510	Fixed	2	20 mg/ml 0510; No coating 0510

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Day	4	738,49	184,623	43,89	0,000
Coating 0510	1	122,97	122,968	29,24	0,000
Day*Coating 0510	4	34,07	8,517	2,02	0,134
Error	18	75,71	4,206		
Total	27	1052,97			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2,05089	92,81%	89,21%	83,33%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	9,555	0,393	24,33	0,000	
Day					
0	−7,165	0,758	−9,45	0,000	1,50
2	−3,357	0,825	−4,07	0,001	1,62

4	−1,182	0,758	−1,56	0,137	1,50
6	4,637	0,758	6,12	0,000	1,50
Coating 0510					
20 mg/ml 0510	−2,123	0,393	−5,41	0,000	1,03
Day∗Coating 0510					
0 20 mg/ml 0510	1,404	0,758	1,85	0,081	1,50
2 20 mg/ml 0510	0,096	0,825	0,12	0,909	1,59
4 20 mg/ml 0510	0,548	0,758	0,72	0,479	1,50
6 20 mg/ml 0510	0,024	0,758	0,03	0,975	1,50

Regression Equation

```
Log CFU per cm2  =  9,555 − 7,165 Day_0 − 3,357 Day_2 − 1,182 Day_4
0510                + 4,637 Day_6
                    + 7,067 Day_8 − 2,123 Coating 0510_20 mg/ml 0510
                    + 2,123 Coating 0510_No coating 0510
                    + 1,404 Day∗Coating 0510_0 20
                    mg/ml 0510 − 1,404 Day∗Coating 0510_0 No coating 0510
                    + 0,096 Day∗Coating 0510_2 20 mg/ml 0510 −
                     0,096 Day∗Coating 0510_2 No
                    coating 0510 + 0,548 Day∗Coating 0510_4 20 mg/ml 0510
                    − 0,548 Day∗Coating 0510_4 No coating 0510
                    + 0,024 Day∗Coating 0510_6
                    20 mg/ml 0510 − 0,024 Day∗Coating 0510_6 No coating
                    0510
                    − 2,072 Day∗Coating 0510_8 20 mg/ml 0510
                    + 2,072 Day∗Coating 0510_8 No
                    coating 0510
```

Fits and Diagnostics for Unusual Observations

Obs	Log CFU per cm2 0510	Fit	Resid	Std Resid	
21	0,00	4,17	−4,17	−2,49	R

R Large residual

Comparisons for Log CFU per cm2 0510

Tukey Pairwise Comparisons: Day

Grouping Information Using the Tukey Method and 95% Confidence

Day	N	Mean	Grouping
8	5	16,6216	A
6	6	14,1919	A
4	6	8,3734	B
2	5	6,1982	B
0	6	2,3898	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Coating 0510

Grouping Information Using the Tukey Method and 95% Confidence

Coating 0510	N	Mean	Grouping
No coating 0510	14	11,6784	A
20 mg/ml 0510	14	7,4316	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Day*Coating 0510

Grouping Information Using the Tukey Method and 95% Confidence

Day*Coating 0510	N	Mean	Grouping		
8 No coating 0510	3	20,8165	A		
6 No coating 0510	3	16,2917	A	B	
8 20 mg/ml 0510	2	12,4267		B	C
6 20 mg/ml 0510	3	12,0922		B	C
4 No coating 0510	3	9,9486			C D
2 No coating 0510	2	8,2258			C D E
4 20 mg/ml 0510	3	6,7983			C D E
2 20 mg/ml 0510	3	4,1706			D E
0 No coating 0510	3	3,1094			E

0 20 mg/ml 0510	3	1,6702		E

Means that do not share a letter are significantly different.

General Linear Model: Log CFU per cm2 1610 versus ... ; Coating 1610

Method

Factor coding (−1; 0; +1)

Rows unused 1

Factor Information

Factor	Type	Levels	Values
Day	Fixed	5	0; 2; 4; 6; 8
Coating 1610	Fixed	2	60 mg/ml 1610; No coating 1610

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Day	4	893,78	223,444	116,74	0,000
Coating 1610	1	180,32	180,317	94,21	0,000
Day*Coating 1610	4	31,93	7,982	4,17	0,014
Error	19	36,37	1,914		
Total	28	1182,22			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1,38349	96,92%	95,47%	92,99%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	8,792	0,259	33,97	0,000	
Day					
0	−7,237	0,508	−14,24	0,000	1,62

2	−4,790	0,553	−8,65	0,000	1,75
4	−0,119	0,508	−0,23	0,817	1,62
6	4,548	0,508	8,95	0,000	1,62
Coating 1610					
60 mg/ml 1610	−2,512	0,259	−9,71	0,000	1,01
Day*Coating 1610					
0 60 mg/ml 1610	0,958	0,508	1,88	0,075	1,62
2 60 mg/ml 1610	−1,490	0,553	−2,69	0,014	1,75
4 60 mg/ml 1610	1,261	0,508	2,48	0,023	1,62
6 60 mg/ml 1610	0,264	0,508	0,52	0,609	1,62

Regression Equation

```
Log CFU per cm2 1610 = 8,792 − 7,237 Day_0 − 4,790 Day_2 − 0,119 Day_4
                       + 4,548 Day_6
                       + 7,598 Day_8 − 2,512 Coating 1610_60 mg/ml 1610
                       + 2,512 Coating 1610_No coating 1610
                       + 0,958 Day*Coating 1610_0 60
                       mg/ml 1610 − 0,958 Day*Coating 1610_0 No coating 1610
                       − 1,490 Day*Coating 1610_2 60 mg/ml 1610
                       + 1,490 Day*Coating 1610_2 No
                       coating 1610 + 1,261 Day*Coating 1610_4 60 mg/ml 1610
                       − 1,261 Day*Coating 1610_4 No coating 1610
                       + 0,264 Day*Coating 1610_6
                       60 mg/ml 1610 − 0,264 Day*Coating 1610_6 No coating
                       1610
                       − 0,993 Day*Coating 1610_8 60 mg/ml 1610
                       + 0,993 Day*Coating 1610_8 No
                       coating 1610
```

Fits and Diagnostics for Unusual Observations

Obs	Log CFU per cm2 1610	Fit	Resid	Std Resid	
2	0,000	3,109	−3,109	−2,75	R

R Large residual

Comparisons for Log CFU per cm2 1610

Tukey Pairwise Comparisons: Day

Grouping Information Using the Tukey Method and 95% Confidence

Day	N	Mean	Grouping
8	6	16,3895	A
6	6	13,3403	B
4	6	8,6727	C
2	5	4,0024	D
0	6	1,5547	D

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Coating 1610

Grouping Information Using the Tukey Method and 95% Confidence

Coating 1610	N	Mean	Grouping
No coating 1610	14	11,3041	A
60 mg/ml 1610	15	6,2797	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Day*Coating 1610

Grouping Information Using the Tukey Method and 95% Confidence

Day*Coating 1610	N	Mean	Grouping
8 No coating 1610	3	19,8946	A
6 No coating 1610	3	15,5883	B
8 60 mg/ml 1610	3	12,8844	B C
6 60 mg/ml 1610	3	11,0922	C D
4 No coating 1610	3	9,9235	C D
2 No coating 1610	2	8,0047	D
4 60 mg/ml 1610	3	7,4220	D
0 No coating 1610	3	3,1094	E

0	60 mg/ml 1610	3	0,0000	E
2	60 mg/ml 1610	3	0,0000	E

Means that do not share a letter are significantly different.

General Linear Model: Log CFU per cm2 0511 versus ... ; Coating 0511

Method

Factor coding (−1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Days	Fixed	5	0; 2; 4; 6; 8
Coating 0511	Fixed	3	30 mg/ml 0511; 70 mg/ml 0511; No coating 0511

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Days	4	949,02	237,256	169,47	0,000
Coating 0511	2	325,07	162,534	116,10	0,000
Days*Coating 0511	8	70,33	8,792	6,28	0,000
Error	30	42,00	1,400		
Total	44	1386,42			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1,18320	96,97%	95,56%	93,18%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6,202	0,176	35,16	0,000	
Days					
0	−4,486	0,353	−12,72	0,000	1,60

2	−3,750	0,353	−10,63	0,000	1,60
4	−2,727	0,353	−7,73	0,000	1,60
6	4,116	0,353	11,67	0,000	1,60
Coating 0511					
30 mg/ml 0511	−1,374	0,249	−5,51	0,000	1,33
70 mg/ml 0511	−2,383	0,249	−9,55	0,000	1,33
Days∗Coating 0511					
0 30 mg/ml 0511	−0,342	0,499	−0,69	0,498	2,13
0 70 mg/ml 0511	0,667	0,499	1,34	0,192	2,13
2 30 mg/ml 0511	0,361	0,499	0,72	0,475	2,13
2 70 mg/ml 0511	−0,069	0,499	−0,14	0,890	2,13
4 30 mg/ml 0511	1,008	0,499	2,02	0,052	2,13
4 70 mg/ml 0511	−1,093	0,499	−2,19	0,036	2,13
6 30 mg/ml 0511	1,127	0,499	2,26	0,031	2,13
6 70 mg/ml 0511	1,122	0,499	2,25	0,032	2,13

676 ## Regression Equation

```
Log CFU per cm2  =  6,202 − 4,486 Days_0 − 3,750 Days_2 − 2,727 Days_4
0511                + 4,116 Days_6
                    + 6,847 Days_8 − 1,374 Coating 0511_30 mg/ml 0511
                    − 2,383 Coating 0511_70 mg/ml 0511
                    + 3,756 Coating 0511_No coating
                    0511 − 0,342 Days∗Coating 0511_0 30 mg/ml 0511
                    + 0,667 Days∗Coating 0511_0 70 mg/ml 0511 −
                     0,324 Days∗Coating 0511_0
                    No coating 0511 + 0,361 Days∗Coating 0511_2 30 mg/ml
                    0511
                    − 0,069 Days∗Coating 0511_2 70 mg/ml 0511 −
                     0,291 Days∗Coating 0511_2
                    No coating 0511 + 1,008 Days∗Coating 0511_4 30 mg/ml
                    0511
                    − 1,093 Days∗Coating 0511_4 70 mg/ml 0511
                    + 0,085 Days∗Coating 0511_4
                    No coating 0511 + 1,127 Days∗Coating 0511_6 30 mg/ml
                    0511
                    + 1,122 Days∗Coating 0511_6 70 mg/ml 0511 −
                     2,249 Days∗Coating 0511_6
```

```
No coating 0511 - 2,153 Days*Coating 0511_8 30 mg/ml
0511
- 0,626 Days*Coating 0511_8 70 mg/ml 0511
+ 2,780 Days*Coating 0511_8
No coating 0511
```

Fits and Diagnostics for Unusual Observations

Obs	Log CFU per cm2 0511	Fit	Resid	Std Resid	
19	4,317	1,439	2,878	2,98	R
24	0,000	3,109	-3,109	-3,22	R

R Large residual

Comparisons for Log CFU per cm2 0511

Tukey Pairwise Comparisons: Days

Grouping Information Using the Tukey Method and 95% Confidence

Days	N	Mean	Grouping		
8	9	13,0491	A		
6	9	10,3179		B	
4	9	3,4751			C
2	9	2,4520			C D
0	9	1,7160			D

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Coating 0511

Grouping Information Using the Tukey Method and 95% Confidence

Coating 0511	N	Mean	Grouping		
No coating 0511	15	9,95811	A		
30 mg/ml 0511	15	4,82845		B	
70 mg/ml 0511	15	3,81951		B	

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Days*Coating 0511

Grouping Information Using the Tukey Method and 95% Confidence

Days*Coating 0511	N	Mean	Grouping
8 No coating 0511	3	19,5848	A
6 No coating 0511	3	11,8250	B
6 30 mg/ml 0511	3	10,0713	B C
8 70 mg/ml 0511	3	10,0402	B C
8 30 mg/ml 0511	3	9,5224	B C
6 70 mg/ml 0511	3	9,0574	B C D
4 No coating 0511	3	7,3159	C D E
2 No coating 0511	3	5,9168	D E F
0 No coating 0511	3	5,1480	E F
4 30 mg/ml 0511	3	3,1094	F G
2 30 mg/ml 0511	3	1,4392	G
0 70 mg/ml 0511	3	0,0000	G
2 70 mg/ml 0511	3	0,0000	G
4 70 mg/ml 0511	3	0,0000	G
0 30 mg/ml 0511	3	-0,0000	G

Means that do not share a letter are significantly different.

General Linear Model: Log CFU per cm2 1311 versus ... ; Coating 1311

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Days	Fixed	5	0; 2; 4; 6; 8
Coating 1311	Fixed	3	10 mg/ml 1311; 50 mg/ml 1311; No coating 1311

698 ## Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Days	4	895,93	223,984	333,48	0,000
Coating 1311	2	430,75	215,376	320,66	0,000
Days*Coating 1311	8	97,24	12,155	18,10	0,000
Error	30	20,15	0,672		
Total	44	1444,07			

699 ## Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,819549	98,60%	97,95%	96,86%

700 ## Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6,257	0,122	51,22	0,000	
Days					
0	-5,221	0,244	-21,37	0,000	1,60
2	-2,846	0,244	-11,65	0,000	1,60
4	-2,337	0,244	-9,56	0,000	1,60
6	3,684	0,244	15,08	0,000	1,60
Coating 1311					
10 mg/ml 1311	0,582	0,173	3,37	0,002	1,33
50 mg/ml 1311	-4,046	0,173	-23,42	0,000	1,33
Days*Coating 1311					
0 10 mg/ml 1311	-1,618	0,346	-4,68	0,000	2,13

0	50 mg/ml 1311	3,010	0,346	8,71	0,000	2,13
2	10 mg/ml 1311	0,555	0,346	1,61	0,118	2,13
2	50 mg/ml 1311	0,635	0,346	1,84	0,076	2,13
4	10 mg/ml 1311	0,047	0,346	0,14	0,893	2,13
4	50 mg/ml 1311	0,126	0,346	0,37	0,718	2,13
6	10 mg/ml 1311	−0,507	0,346	−1,47	0,153	2,13
6	50 mg/ml 1311	−0,652	0,346	−1,89	0,069	2,13

701 ## Regression Equation

```
Log CFU per cm2   =  6,257 − 5,221 Days_0 − 2,846 Days_2 − 2,337 Days_4
1311                 + 3,684 Days_6
                     + 6,720 Days_8 + 0,582 Coating 1311_10 mg/ml 1311
                     − 4,046 Coating 1311_50 mg/ml 1311
                     + 3,465 Coating 1311_No coating
                     1311 − 1,618 Days*Coating 1311_0 10 mg/ml 1311
                     + 3,010 Days*Coating 1311_0 50 mg/ml 1311 −
                      1,392 Days*Coating 1311_0
                     No coating 1311 + 0,555 Days*Coating 1311_2 10 mg/ml
                     1311
                     + 0,635 Days*Coating 1311_2 50 mg/ml 1311 −
                      1,190 Days*Coating 1311_2
                     No coating 1311 + 0,047 Days*Coating 1311_4 10 mg/ml
                     1311
                     + 0,126 Days*Coating 1311_4 50 mg/ml 1311 −
                      0,173 Days*Coating 1311_4
                     No coating 1311 − 0,507 Days*Coating 1311_6 10 mg/ml
                     1311
                     − 0,652 Days*Coating 1311_6 50 mg/ml 1311
                     + 1,159 Days*Coating 1311_6
                     No coating 1311 + 1,523 Days*Coating 1311_8 10 mg/ml
                     1311
                     − 3,119 Days*Coating 1311_8 50 mg/ml 1311
                     + 1,596 Days*Coating 1311_8
                     No coating 1311
```

702 ## Fits and Diagnostics for Unusual Observations

Obs	Log CFU per cm2 1311	Fit	Resid	Std Resid	
2	0,000	3,109	−3,109	−4,65	R
3	5,011	3,109	1,901	2,84	R

45	4,317	5,812	−1,494	−2,23	R

R Large residual

Residual Plots for Log CFU per cm2 1311

Comparisons for Log CFU per cm2 1311

Tukey Pairwise Comparisons: Days

Grouping Information Using the Tukey Method and 95% Confidence

Days	N	Mean	Grouping
8	9	12,9772	A
6	9	9,9415	B
4	9	3,9202	C
2	9	3,4114	C
0	9	1,0365	D

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Coating 1311

Grouping Information Using the Tukey Method and 95% Confidence

Coating 1311	N	Mean	Grouping
No coating 1311	15	9,72217	A
10 mg/ml 1311	15	6,83900	B
50 mg/ml 1311	15	2,21093	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Days*Coating 1311

Grouping Information Using the Tukey Method and 95% Confidence

Days*Coating 1311	N	Mean	Grouping
8 No coating 1311	3	18,0380	A

8	10 mg/ml 1311	3	15,0817		B		
6	No coating 1311	3	14,5655		B		
6	10 mg/ml 1311	3	10,0163			C	
4	No coating 1311	3	7,2122				D
8	50 mg/ml 1311	3	5,8119				D E
2	No coating 1311	3	5,6857				D E
6	50 mg/ml 1311	3	5,2428				D E F
2	10 mg/ml 1311	3	4,5485				E F
4	10 mg/ml 1311	3	4,5485				E F
0	No coating 1311	3	3,1094				F
0	50 mg/ml 1311	3	0,0000				G
2	50 mg/ml 1311	3	0,0000				G
4	50 mg/ml 1311	3	0,0000				G
0	10 mg/ml 1311	3	-0,0000				G

Means that do not share a letter are significantly different.

General Linear Model: log CFU cm2 1911 versus Days; Coating 1911

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Days	Fixed	5	0; 2; 4; 6; 8
Coating 1911	Fixed	3	40 mg/ml 1911; 80 mg/ml 1911; No coating 1911

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value

Days	4	481,66	120,414	1352,22	0,000
Coating 1911	2	909,15	454,576	5104,80	0,000
Days*Coating 1911	8	277,85	34,731	390,03	0,000
Error	30	2,67	0,089		
Total	44	1671,33			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,298411	99,84%	99,77%	99,64%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	4,9378	0,0445	111,00	0,000	
Days					
0	-3,2991	0,0890	-37,08	0,000	1,60
2	-3,0261	0,0890	-34,01	0,000	1,60
4	-1,4759	0,0890	-16,59	0,000	1,60
6	3,2408	0,0890	36,43	0,000	1,60
Coating 1911					
40 mg/ml 1911	-0,9979	0,0629	-15,86	0,000	1,33
80 mg/ml 1911	-4,9378	0,0629	-78,49	0,000	1,33
Days*Coating 1911					
0 40 mg/ml 1911	-0,641	0,126	-5,09	0,000	2,13
0 80 mg/ml 1911	3,299	0,126	26,22	0,000	2,13
2 40 mg/ml 1911	-0,914	0,126	-7,26	0,000	2,13
2 80 mg/ml 1911	3,026	0,126	24,05	0,000	2,13
4 40 mg/ml 1911	-2,464	0,126	-19,58	0,000	2,13
4 80 mg/ml 1911	1,476	0,126	11,73	0,000	2,13

6	40 mg/ml 1911	2,630	0,126	20,90	0,000	2,13
6	80 mg/ml 1911	−3,241	0,126	−25,76	0,000	2,13

Regression Equation

```
log CFU cm2    =  4,9378 − 3,2991 Days_0 − 3,0261 Days_2 − 1,4759 Days_4
1911              + 3,2408 Days_6
                  + 4,5602 Days_8 − 0,9979 Coating 1911_40 mg/ml 1911
                  − 4,9378 Coating 1911_80 mg/ml 1911
                  + 5,9357 Coating 1911_No coating 1911
                  − 0,641 Days*Coating 1911_0 40 mg/ml 1911
                  + 3,299 Days*Coating 1911_0 80
                  mg/ml 1911 − 2,658 Days*Coating 1911_0 No coating 1911
                  − 0,914 Days*Coating 1911_2 40 mg/ml 1911
                  + 3,026 Days*Coating 1911_2 80
                  mg/ml 1911 − 2,112 Days*Coating 1911_2 No coating 1911
                  − 2,464 Days*Coating 1911_4 40 mg/ml 1911
                  + 1,476 Days*Coating 1911_4 80
                  mg/ml 1911 + 0,988 Days*Coating 1911_4 No coating 1911
                  + 2,630 Days*Coating 1911_6 40 mg/ml 1911 −
                   3,241 Days*Coating 1911_6 80
                  mg/ml 1911 + 0,611 Days*Coating 1911_6 No coating 1911
                  + 1,389 Days*Coating 1911_8 40 mg/ml 1911 −
                   4,560 Days*Coating 1911_8 80
                  mg/ml 1911 + 3,172 Days*Coating 1911_8 No coating 1911
```

Fits and Diagnostics for Unusual Observations

Obs	log CFU cm2 1911	Fit	Resid	Std Resid	
1	5,421	4,916	0,504	2,07	R
3	4,317	4,916	−0,599	−2,46	R
5	6,265	5,735	0,530	2,18	R
6	5,011	5,735	−0,725	−2,97	R
14	18,030	18,605	−0,575	−2,36	R
15	19,388	18,605	0,783	3,21	R

R Large residual

Residual Plots for log CFU cm2 1911

Comparisons for log CFU cm2 1911

Tukey Pairwise Comparisons: Days

Grouping Information Using the Tukey Method and 95% Confidence

Days	N	Mean	Grouping
8	9	9,49801	A
6	9	8,17857	B
4	9	3,46194	C
2	9	1,91173	D
0	9	1,63874	D

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Coating 1911

Grouping Information Using the Tukey Method and 95% Confidence

Coating 1911	N	Mean	Grouping
No coating 1911	15	10,8735	A
40 mg/ml 1911	15	3,9399	B
80 mg/ml 1911	15	0,0000	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Days*Coating 1911

Grouping Information Using the Tukey Method and 95% Confidence

Days*Coating 1911	N	Mean	Grouping
8 No coating 1911	3	18,6054	A
6 No coating 1911	3	14,7248	B
4 No coating 1911	3	10,3858	C
8 40 mg/ml 1911	3	9,8886	C
6 40 mg/ml 1911	3	9,8109	C
2 No coating 1911	3	5,7352	D
0 No coating 1911	3	4,9162	D

0	**80 mg/ml**	**1911**	**3**	**0,0000**	**E**
2	**80 mg/ml**	**1911**	**3**	**0,0000**	**E**
4	**80 mg/ml**	**1911**	**3**	**0,0000**	**E**
8	**80 mg/ml**	**1911**	**3**	**0,0000**	**E**
6	**80 mg/ml**	**1911**	**3**	**0,0000**	**E**
2	**40 mg/ml**	**1911**	**3**	**-0,0000**	**E**
4	**40 mg/ml**	**1911**	**3**	**-0,0000**	**E**
0	**40 mg/ml**	**1911**	**3**	**-0,0000**	**E**

Means that do not share a letter are significantly different.

Figure 6 A

General Linear Model: Log N versus Treatment; Dag

Method

Factor coding (−1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Treatment	Fixed	2	With coating 4 C cod; Without coating 4 C cod
Dag	Fixed	4	0; 1; 3; 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	47,644	47,6436	303,83	0,000
Dag	3	125,378	41,7925	266,51	0,000
Treatment*Dag	3	17,827	5,9424	37,90	0,000
Error	20	3,136	0,1568		
Total	27	214,403			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,395994	98,54%	98,03%	97,36%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	16,5108	0,0783	210,96	0,000	
Treatment					
With coating 4 C cod	−1,3642	0,0783	−17,43	0,000	1,09
Dag					
0	−1,612	0,160	−10,05	0,000	1,88

	1	−2,061	0,126	−16,33	0,000	1,63
	3	0,501	0,126	3,97	0,001	1,62
Treatment*Dag						
	With coating 4 C cod 0	1,364	0,160	8,51	0,000	1,97
	With coating 4 C cod 1	0,238	0,126	1,89	0,074	1,63
	With coating 4 C cod 3	−0,589	0,126	−4,66	0,000	1,63

Regression Equation

```
Log N = 16,5108 − 1,3642 Treatment_With coating 4 C cod
        + 1,3642 Treatment_Without coating 4 C cod − 1,612 Dag_0 − 2,061 Dag_1 + 0,501 Dag_3 + 3,172 Dag_6
        + 1,364 Treatment*Dag_With coating 4 C cod 0
        + 0,238 Treatment*Dag_With coating 4 C cod 1 − 0,589 Treatment*Dag_With coating 4 C cod 3 −
        1,014 Treatment*Dag_With coating 4 C cod 6 − 1,364 Treatment*Dag_Without coating 4 C cod 0
        − 0,238 Treatment*Dag_Without coating 4 C cod 1 + 0,589 Treatment*Dag_Without coating 4 C cod 3
        + 1,014 Treatment*Dag_Without coating 4 C cod 6
```

Fits and Diagnostics for Unusual Observations

Obs	Log N	Fit	Resid	Std Resid	
8	15,944	15,059	0,885	2,58	R
10	13,816	15,059	−1,243	−3,63	R

R Large residual

Residual Plots for Log N

Comparisons for Log N

Tukey Pairwise Comparisons: Treatment

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping
Without coating 4 C cod	14	18,1044	A
With coating 4 C cod	14	15,2181	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Dag

Grouping Information Using the Tukey Method and 95% Confidence

Dag	N	Mean	Grouping
6	8	19,8271	A
3	8	17,1323	B
0	4	14,8993	C
1	8	14,4949	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatment*Dag

Grouping Information Using the Tukey Method and 95% Confidence

Treatment*Dag	N	Mean	Grouping
Without coating 4 C cod 6	4	22,0626	A
Without coating 4 C cod 3	4	18,9648	B
With coating 4 C cod 6	4	17,3052	C
Without coating 4 C cod 1	4	15,5777	D
With coating 4 C cod 3	4	15,0787	D
With coating 4 C cod 0	2	14,8993	D
Without coating 4 C cod 0	2	14,8993	D
With coating 4 C cod 1	4	13,3243	E

Means that do not share a letter are significantly different.

Figure 6B

General Linear Model: logN versus Treatment; Dag

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Treatment	Fixed	2	With coating torsk 12C; Without coating torsk 12C
Dag	Fixed	4	0; 1; 3; 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	27,917	27,9170	770,40	0,000
Dag	3	159,507	53,1690	1467,25	0,000
Treatment*Dag	3	16,338	5,4461	150,29	0,000
Error	18	0,652	0,0362		
Total	25	213,275			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,190361	99,69%	99,58%	99,37%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	18,5831	0,0389	478,24	0,000	
Treatment					
With coating torsk 12C	-1,0785	0,0389	-27,76	0,000	1,08
Dag					
0	-3,6841	0,0777	-47,41	0,000	1,90

1	−1,1406	0,0614	−18,57	0,000	1,67
3	1,5560	0,0673	23,12	0,000	1,73
Treatment∗Dag					
With coating torsk 12C 0	1,0785	0,0777	13,88	0,000	2,00
With coating torsk 12C 1	−0,9056	0,0614	−14,74	0,000	1,67
With coating torsk 12C 3	−0,7165	0,0673	−10,65	0,000	1,75

Regression Equation

```
log  =  18,5831 − 1,0785 Treatment_With coating torsk 12C
N       + 1,0785 Treatment_Without coating
        torsk 12C − 3,6841 Dag_0 − 1,1406 Dag_1 + 1,5560 Dag_3
        + 3,2688 Dag_6
        + 1,0785 Treatment∗Dag_With coating torsk 12C 0 −
         0,9056 Treatment∗Dag_With coating
        torsk 12C 1 − 0,7165 Treatment∗Dag_With coating torsk 12C 3
        + 0,5436 Treatment∗Dag_With coating torsk 12C 6 −
         1,0785 Treatment∗Dag_Without coating
        torsk 12C 0 + 0,9056 Treatment∗Dag_Without coating torsk 12C 1
        + 0,7165 Treatment∗Dag_Without coating torsk 12C 3 −
         0,5436 Treatment∗Dag_Without
        coating torsk 12C 6
```

Residual Plots for logN

Figure 7A E. coli

General Linear Model: LogN versus Treatment; Dag

Method

Factor coding (–1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Treatment	Fixed	2	E. Coli 12 C with coating; E. Coli 12 C without coating
Dag	Fixed	4	0; 1; 3; 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	78,972	78,9724	586,64	0,000
Dag	3	92,292	30,7640	228,53	0,000
Treatment*Dag	3	57,942	19,3141	143,47	0,000
Error	15	2,019	0,1346		
Total	22	226,624			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,366903	99,11%	98,69%	97,70%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	11,1716	0,0805	138,72	0,000	
Treatment					
E. Coli 12 C with coating	–1,9506	0,0805	–24,22	0,000	1,11
Dag					

0	−2,144	0,153	−14,04	0,000	1,39
1	−1,981	0,122	−16,23	0,000	1,25
3	1,376	0,128	10,77	0,000	1,28
Treatment*Dag					
E. Coli 12 C with coating 0	1,951	0,153	12,78	0,000	1,39
E. Coli 12 C with coating 1	1,332	0,122	10,91	0,000	1,33
E. Coli 12 C with coating 3	−1,562	0,128	−12,24	0,000	1,33

Regression Equation

```
Log  =  11,1716 − 1,9506 Treatment_E. Coli 12 C with coating
N       + 1,9506 Treatment_E. Coli 12 C
        without coating − 2,144 Dag_0 − 1,981 Dag_1 + 1,376 Dag_3
        + 2,749 Dag_7
        + 1,951 Treatment*Dag_E. Coli 12 C with coating 0
        + 1,332 Treatment*Dag_E. Coli 12 C
        with coating 1 − 1,562 Treatment*Dag_E. Coli 12 C with coating 3
        − 1,720 Treatment*Dag_E. Coli 12 C with coating 7 −
         1,951 Treatment*Dag_E. Coli 12 C
        without coating 0 − 1,332 Treatment*Dag_E. Coli 12 C without
        coating 1
        + 1,562 Treatment*Dag_E. Coli 12 C without coating 3
        + 1,720 Treatment*Dag_E. Coli 12
        C without coating 7
```

Fits and Diagnostics for Unusual Observations

Obs	LogN	Fit	Resid	Std Resid	
17	10,463	9,809	0,654	2,06	R

R Large residual

Comparisons for LogN

Tukey Pairwise Comparisons: Treatment

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping
E. Coli 12 C without coating	11	13,1221	A
E. Coli 12 C with coating	12	9,2210	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Dag

Grouping Information Using the Tukey Method and 95% Confidence

Dag	N	Mean	Grouping
7	4	13,9207	A
3	7	12,5473	B
1	8	9,1904	C
0	4	9,0278	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatment*Dag

Grouping Information Using the Tukey Method and 95% Confidence

Treatment*Dag	N	Mean	Grouping
E. Coli 12 C without coating 7	2	17,5914	A
E. Coli 12 C without coating 3	3	16,0600	B
E. Coli 12 C with coating 7	2	10,2501	C
E. Coli 12 C without coating 1	4	9,8093	C D
E. Coli 12 C with coating 3	4	9,0345	D E
E. Coli 12 C with coating 0	2	9,0278	C D E
E. Coli 12 C without coating 0	2	9,0278	C D E
E. Coli 12 C with coating 1	4	8,5716	E

Means that do not share a letter are significantly different.

Figure 7 L.monocytogenes

General Linear Model: LogN versus Treatment; Dag

Method

Factor coding (–1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Treatment	Fixed	2	L. monocytogenes with coating; L. monocytogenes without coating
Dag	Fixed	4	0; 1; 3; 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	47,048	47,048	869,11	0,000
Dag	3	487,942	162,647	3004,53	0,000
Treatment*Dag	3	20,700	6,900	127,46	0,000
Error	14	0,758	0,054		
Total	21	628,401			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,232667	99,88%	99,82%	99,77%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	13,5250	0,0524	257,96	0,000	
Treatment					
L. monocytogenes with coating	–1,5457	0,0524	–29,48	0,000	1,11
Dag					

0	−5,2955	0,0975	−54,29	0,000	1,73
1	−3,8726	0,0783	−49,45	0,000	1,57
3	2,5841	0,0975	26,49	0,000	1,73
Treatment*Dag					
L. monocytogenes with coating 0	1,5457	0,0975	15,85	0,000	1,73
L. monocytogenes with coating 1	−0,0997	0,0783	−1,27	0,224	1,57
L. monocytogenes with coating 3	−1,6469	0,0975	−16,88	0,000	1,73

Regression Equation

```
Log  =  13,5250 − 1,5457 Treatment_L. monocytogenes with coating
N       + 1,5457 Treatment_L.
        monocytogenes without coating − 5,2955 Dag_0 − 3,8726 Dag_1
        + 2,5841 Dag_3
        + 6,5840 Dag_7 + 1,5457 Treatment*Dag_L. monocytogenes with coating
        0
        − 0,0997 Treatment*Dag_L. monocytogenes with coating 1 −
         1,6469 Treatment*Dag_L.
        monocytogenes with coating 3 + 0,2010 Treatment*Dag_L.
        monocytogenes with coating 7
        − 1,5457 Treatment*Dag_L. monocytogenes without coating 0
        + 0,0997 Treatment*Dag_L.
        monocytogenes without coating 1 + 1,6469 Treatment*Dag_L.
        monocytogenes without
        coating 3 − 0,2010 Treatment*Dag_L. monocytogenes without coating 7
```

Fits and Diagnostics for Unusual Observations

Obs	LogN	Fit	Resid	Std Resid	
5	7,601	8,007	−0,406	−2,02	R
6	8,517	8,007	0,510	2,53	R

R Large residual

Comparisons for LogN

Tukey Pairwise Comparisons: Treatment

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping
L. monocytogenes without coating	12	15,0707	A

L. monocytogenes with coating	10	11,9793	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Dag

Grouping Information Using the Tukey Method and 95% Confidence

Dag	N	Mean	Grouping
7	6	20,1090	A
3	4	16,1090	B
1	8	9,6524	C
0	4	8,2294	D

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatment*Dag

Grouping Information Using the Tukey Method and 95% Confidence

Treatment*Dag	N	Mean	Grouping
L. monocytogenes without coating 7	4	21,4538	A
L. monocytogenes without coating 3	2	19,3017	B
L. monocytogenes with coating 7	2	18,7643	B
L. monocytogenes with coating 3	2	12,9164	C
L. monocytogenes without coating 1	4	11,2978	D
L. monocytogenes without coating 0	2	8,2294	E
L. monocytogenes with coating 0	2	8,2294	E
L. monocytogenes with coating 1	4	8,0070	E

Means that do not share a letter are significantly different.

Figure 7A B.cereus

General Linear Model: Log N versus Treatment; Dag

Method

Factor coding (−1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Treatment	Fixed	2	B. cereus 12 C with coating; B. cereus 12 C without coating
Dag	Fixed	4	0; 1; 3; 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	69,225	69,2250	313,60	0,000
Dag	3	55,323	18,4410	83,54	0,000
Treatment*Dag	3	48,728	16,2425	73,58	0,000
Error	10	2,207	0,2207		
Total	17	167,189			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,469830	98,68%	97,76%	95,54%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	10,421	0,114	91,63	0,000	
Treatment					
B. cereus 12 C with coating	−2,014	0,114	−17,71	0,000	1,04
Dag					
0	−1,119	0,201	−5,56	0,000	1,47
1	−1,930	0,183	−10,53	0,000	1,49
3	2,761	0,201	13,72	0,000	1,47
Treatment*Dag					

B. cereus 12 C with coating	0	2,014	0,201	10,00	0,000	1,47
B. cereus 12 C with coating	1	1,359	0,183	7,41	0,000	1,49
B. cereus 12 C with coating	3	−1,587	0,201	−7,88	0,000	1,47

Regression Equation

```
Log N = 10,421 − 2,014 Treatment_B. cereus 12 C with coating  + 2,014 Treatment_B. cereus 12 C without coating  − 1,119 Dag_0 − 1,930 Dag_1 + 2,761 Dag_3 + 0,287 Dag_7 + 2,014 Treatment*Dag_B. cereus 12 C with coating  0 + 1,359 Treatment*Dag_B. cereus 12 C with coating  1 − 1,587 Treatment*Dag_B. cereus 12 C with coating  3 − 1,786 Treatment*Dag_B. cereus 12 C with coating  7 − 2,014 Treatment*Dag_B. cereus 12 C without coating  0 − 1,359 Treatment*Dag_B. cereus 12 C without coating  1 + 1,587 Treatment*Dag_B. cereus 12 C without coating  3 + 1,786 Treatment*Dag_B. cereus 12 C without coating  7
```

Fits and Diagnostics for Unusual Observations

Obs	Log N	Fit	Resid	Std Resid	
17	15,202	14,509	0,693	2,09	R
18	13,816	14,509	−0,693	−2,09	R

R Large residual

Comparisons for Log N

Tukey Pairwise Comparisons: Treatment

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping
B. cereus 12 C without coating	10	12,4348	A
B. cereus 12 C with coating	8	8,4068	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Dag

Grouping Information Using the Tukey Method and 95% Confidence

Dag	N	Mean	Grouping
3	4	13,1821	A
7	4	10,7082	B
0	4	9,3023	C
1	6	8,4906	C

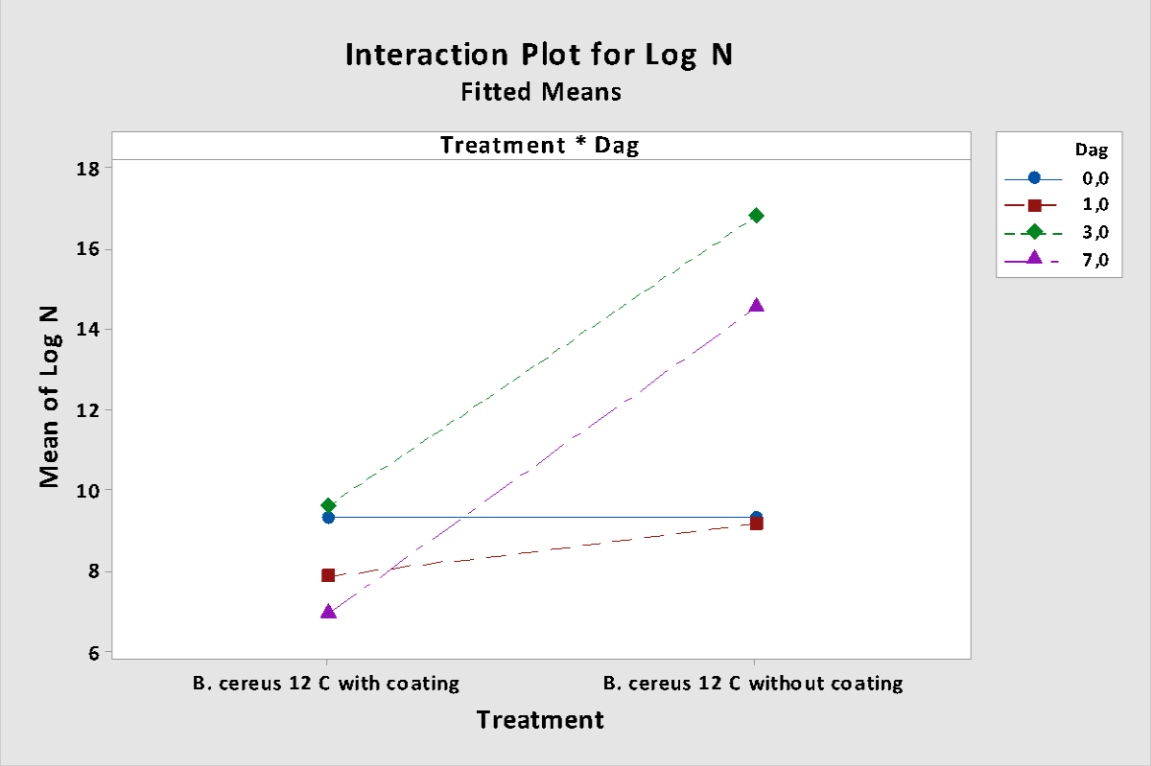
Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatment*Dag

Grouping Information Using the Tukey Method and 95% Confidence

Treatment*Dag	N	Mean	Grouping
B. cereus 12 C without coating 3	2	16,7830	A
B. cereus 12 C without coating 7	2	14,5087	B
B. cereus 12 C with coating 3	2	9,5813	C
B. cereus 12 C with coating 0	2	9,3023	C
B. cereus 12 C without coating 0	2	9,3023	C
B. cereus 12 C without coating 1	4	9,1453	C
B. cereus 12 C with coating 1	2	7,8359	C D
B. cereus 12 C with coating 7	2	6,9078	D

Means that do not share a letter are significantly different.



859

860

861 Just for future reference – this is another common way of viewing the data. In our case, the
862 graphs in figure 7 are clear enough so it is not necessary.

863

864

Figure 7 A S.aureus

General Linear Model: LogN versus Treatment; Dag

Method

Factor coding (–1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Treatment	Fixed	2	S. aureus 12 C with coating; S. aureus 12 C without coating
Dag	Fixed	4	0; 1; 3; 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	77,934	77,9337	934,36	0,000
Dag	3	153,056	51,0186	611,67	0,000
Treatment*Dag	3	47,183	15,7276	188,56	0,000
Error	15	1,251	0,0834		
Total	22	275,641			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,288806	99,55%	99,33%	98,58%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	11,6745	0,0634	184,17	0,000	
Treatment					
S. aureus 12 C with coating	–1,9377	0,0634	–30,57	0,000	1,11
Dag					

0	−2,989	0,120	−24,87	0,000	1,39
1	−2,361	0,101	−23,49	0,000	1,28
3	1,2956	0,0961	13,48	0,000	1,25

Treatment*Dag

S. aureus 12 C with coating	0	1,938	0,120	16,12	0,000	1,39
S. aureus 12 C with coating	1	0,790	0,101	7,86	0,000	1,33
S. aureus 12 C with coating	3	−0,0816	0,0961	−0,85	0,409	1,33

Regression Equation

```
Log  =  11,6745 − 1,9377 Treatment_S. aureus 12 C with
N       coating  + 1,9377 Treatment_S. aureus
        12 C without coating  − 2,989 Dag_0 − 2,361 Dag_1 + 1,2956 Dag_3
        + 4,055 Dag_7
        + 1,938 Treatment*Dag_S. aureus 12 C with coating  0
        + 0,790 Treatment*Dag_S. aureus
        12 C with coating  1 − 0,0816 Treatment*Dag_S. aureus 12 C with
        coating  3
        − 2,646 Treatment*Dag_S. aureus 12 C with coating  7 −
         1,938 Treatment*Dag_S. aureus
        12 C without coating  0 − 0,790 Treatment*Dag_S. aureus 12 C
        without coating  1
        + 0,0816 Treatment*Dag_S. aureus 12 C without coating  3
        + 2,646 Treatment*Dag_S.
        aureus 12 C without coating  7
```

Fits and Diagnostics for Unusual Observations

Obs	LogN	Fit	Resid	Std Resid	
10	11,695	11,146	0,549	2,69	R
11	10,597	11,146	−0,549	−2,69	R

R Large residual

Comparisons for LogN

Tukey Pairwise Comparisons: Treatment

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping

S. aureus 12 C without coating	12	13,6122	A
S. aureus 12 C with coating	11	9,7368	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Dag

Grouping Information Using the Tukey Method and 95% Confidence

Dag	N	Mean	Grouping
7	4	15,7293	A
3	8	12,9701	B
1	7	9,3135	C
0	4	8,6851	D

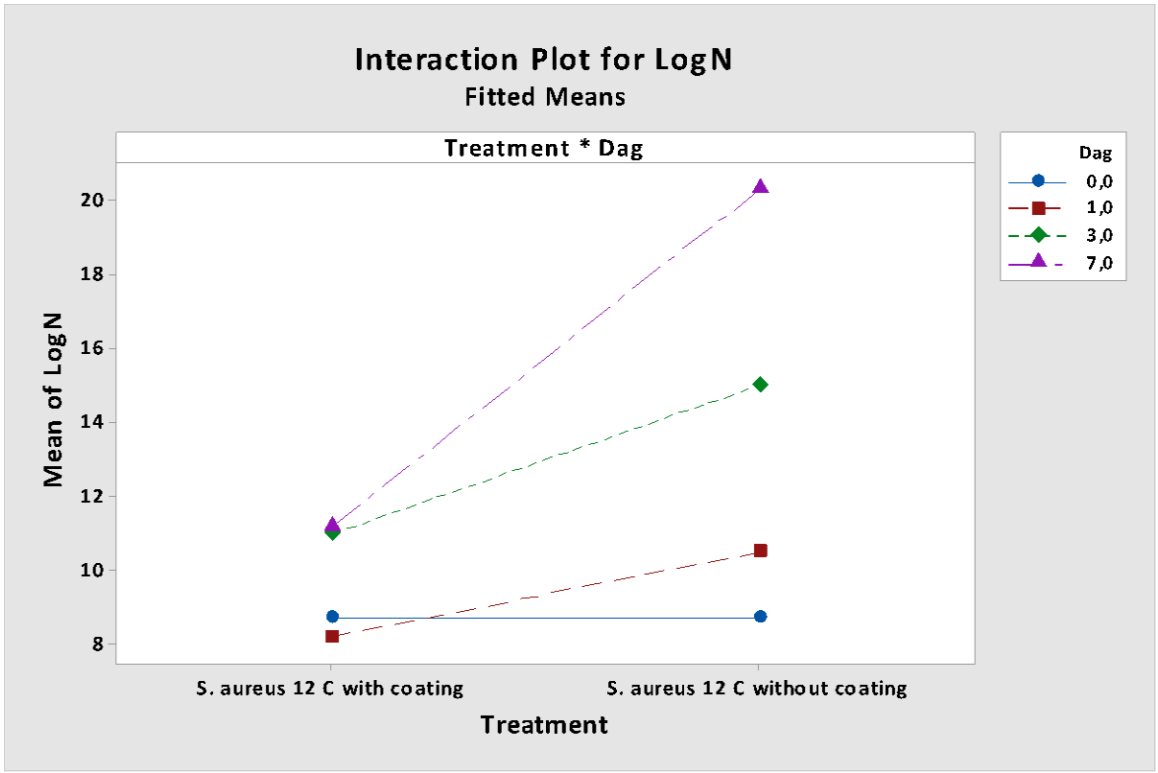
Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatment*Dag

Grouping Information Using the Tukey Method and 95% Confidence

Treatment*Dag		N	Mean	Grouping
S. aureus 12 C without coating	7	2	20,3127	A
S. aureus 12 C without coating	3	4	14,9895	B
S. aureus 12 C with coating	7	2	11,1459	C
S. aureus 12 C with coating	3	4	10,9508	C
S. aureus 12 C without coating	1	4	10,4616	C
S. aureus 12 C with coating	0	2	8,6851	D
S. aureus 12 C without coating	0	2	8,6851	D
S. aureus 12 C with coating	1	3	8,1655	D

Means that do not share a letter are significantly different.


Interaction Plot for LogN
Fitted Means
Treatment * Dag
Mean of LogN
20
18
16
14
12
10
8
S. aureus 12 C with coating
S. aureus 12 C without coating
Treatment
Dag
0,0
1,0
3,0
7,0

890

891

Figure 7B E.coli

General Linear Model: Log N versus Treatment; Dag

Method

Factor coding (−1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Treatment	Fixed	2	E.coli 4C with coating; E.coli 4C without coating
Dag	Fixed	4	0; 1; 4; 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	11,852	11,8518	153,88	0,000
Dag	3	12,050	4,0167	52,15	0,000
Treatment*Dag	3	4,148	1,3826	17,95	0,000
Error	14	1,078	0,0770		
Total	21	36,630			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,277529	97,06%	95,58%	92,18%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	13,2668	0,0617	214,90	0,000	
Treatment					
E.coli 4C with coating	−0,7658	0,0617	−12,40	0,000	1,08
Dag					
0	1,003	0,116	8,65	0,000	1,71

1	0,3019	0,0971	3,11	0,008	1,59
4	−0,131	0,109	−1,20	0,250	1,68
Treatment*Dag					
E.coli 4C with coating 0	0,766	0,116	6,61	0,000	1,71
E.coli 4C with coating 1	0,0638	0,0971	0,66	0,522	1,59
E.coli 4C with coating 4	−0,511	0,109	−4,70	0,000	1,63

900 ## Regression Equation

```
Log N = 13,2668 − 0,7658 Treatment_E.coli 4C with coating
        + 0,7658 Treatment_E.coli 4C
        without coating + 1,003 Dag_0 + 0,3019 Dag_1 − 0,131 Dag_4 −
         1,174 Dag_6
        + 0,766 Treatment*Dag_E.coli 4C with coating 0
        + 0,0638 Treatment*Dag_E.coli 4C with
        coating 1 − 0,511 Treatment*Dag_E.coli 4C with coating 4 −
         0,319 Treatment*Dag_E.coli
        4C with coating 6 − 0,766 Treatment*Dag_E.coli 4C without coating
        0
        − 0,0638 Treatment*Dag_E.coli 4C without coating 1
        + 0,511 Treatment*Dag_E.coli 4C
        without coating 4 + 0,319 Treatment*Dag_E.coli 4C without coating
        6
```

901 ## Fits and Diagnostics for Unusual Observations

Obs	Log N	Fit	Resid	Std Resid	
19	14,914	14,413	0,501	2,21	R
20	13,816	14,413	−0,597	−2,64	R

902 *R Large residual*

903 ### Residual Plots for Log N

904

905 # Comparisons for Log N

906 ## Tukey Pairwise Comparisons: Treatment

907 ### Grouping Information Using the Tukey Method and
908 ### 95% Confidence

Treatment	N	Mean	Grouping

E.coli 4C without coating	10	14,0326	A
E.coli 4C with coating	12	12,5010	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Dag

Grouping Information Using the Tukey Method and 95% Confidence

Dag	N	Mean	Grouping
0	4	14,2696	A
1	7	13,5686	B
4	5	13,1361	B
6	6	12,0927	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatment*Dag

Grouping Information Using the Tukey Method and 95% Confidence

Treatment*Dag	N	Mean	Grouping
E.coli 4C without coating 4	3	14,4128	A
E.coli 4C without coating 1	3	14,2706	A
E.coli 4C with coating 0	2	14,2696	A
E.coli 4C without coating 0	2	14,2696	A
E.coli 4C without coating 6	2	13,1772	B
E.coli 4C with coating 1	4	12,8667	B
E.coli 4C with coating 4	2	11,8595	C
E.coli 4C with coating 6	4	11,0082	D

Means that do not share a letter are significantly different.

Figure 7B **B. weihnstephanensis**

General Linear Model: LogN versus Treatment; Dag

Method

Factor coding (−1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Treatment	Fixed	2	B. weihnstephanensis 4 C with coating; B. weihnstephanensis 4 C without coating
Dag	Fixed	4	0; 1; 4; 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	32,084	32,0842	161,38	0,000
Dag	3	40,501	13,5003	67,90	0,000
Treatment*Dag	3	26,195	8,7318	43,92	0,000
Error	18	3,579	0,1988		
Total	25	89,143			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,445888	95,99%	94,42%	91,77%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	10,8173	0,0924	117,03	0,000	
Treatment					
B. weihnstephanensis 4 C with coating	−1,1741	0,0924	−12,70	0,000	1,11
Dag					
0	2,128	0,183	11,65	0,000	1,6

					5
1	0,206	0,145	1,42	0,172	1,46
4	−0,321	0,145	−2,22	0,040	1,46
Treatment*Dag					
B. weihnstephanensis 4 C with coating 0	1,174	0,183	6,43	0,000	1,65
B. weihnstephanensis 4 C with coating 1	0,359	0,145	2,48	0,023	1,46
B. weihnstephanensis 4 C with coating 4	0,341	0,145	2,35	0,030	1,46

926 ## Regression Equation

```
Log N = 10,8173 − 1,1741 Treatment_B. weihnstephanensis 4 C with coating
        + 1,1741 Treatment_B.
        weihnstephanensis 4 C without coating + 2,128 Dag_0 + 0,206 Dag_1 −
         0,321 Dag_4
        − 2,013 Dag_7 + 1,174 Treatment*Dag_B. weihnstephanensis 4 C with
        coating 0
        + 0,359 Treatment*Dag_B. weihnstephanensis 4 C with coating 1
        + 0,341 Treatment*Dag_B.
        weihnstephanensis 4 C with coating 4 − 1,874 Treatment*Dag_B.
        weihnstephanensis 4 C
        with coating 7 − 1,174 Treatment*Dag_B. weihnstephanensis 4 C
        without coating 0
        − 0,359 Treatment*Dag_B. weihnstephanensis 4 C without coating 1
        − 0,341 Treatment*Dag_B. weihnstephanensis 4 C without coating 4
        + 1,874 Treatment*Dag_B. weihnstephanensis 4 C without coating 7
```

927 ## Fits and Diagnostics for Unusual Observations

Obs	LogN	Fit	Resid	Std Resid	
10	11,002	9,663	1,339	3,47	R

928 *R Large residual*

929 # Comparisons for LogN

930 ## Tukey Pairwise Comparisons: Treatment

931 ## Grouping Information Using the Tukey Method and
932 ## 95% Confidence

Treatment	N	Mean	Grouping
B. weihnstephanensis 4 C without coating	14	11,9914	A
B. weihnstephanensis 4 C with coating	12	9,6431	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Dag

Grouping Information Using the Tukey Method and 95% Confidence

Dag	N	Mean	Grouping
0	4	12,9455	A
1	8	11,0233	B
4	8	10,4960	B
7	6	8,8044	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatment*Dag

Grouping Information Using the Tukey Method and 95% Confidence

Treatment*Dag	N	Mean	Grouping
B. weihnstephanensis 4 C without coating 0	2	12,9455	A
B. weihnstephanensis 4 C with coating 0	2	12,9455	A
B. weihnstephanensis 4 C without coating 7	4	11,8523	A B
B. weihnstephanensis 4 C without coating 1	4	11,8386	A B
B. weihnstephanensis 4 C without coating 4	4	11,3294	B
B. weihnstephanensis 4 C with coating 1	4	10,2080	C
B. weihnstephanensis 4 C with coating 4	4	9,6626	C
B. weihnstephanensis 4 C with coating 7	2	5,7565	D

Means that do not share a letter are significantly different.

Figure 7 B L.monocytogenes

General Linear Model: LogN versus Treatment; Dag

Method

Factor coding (–1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Treatment	Fixed	2	L. monocytogenes 4 C with coating; L. monocytogenes 4 C without coating
Dag	Fixed	4	0; 1; 4; 6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	22,1504	22,1504	544,59	0,000
Dag	3	0,8768	0,2923	7,19	0,004
Treatment*Dag	3	12,2689	4,0896	100,55	0,000
Error	13	0,5288	0,0407		
Total	20	36,5901			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,201677	98,55%	97,78%	*

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	13,9083	0,0483	288,12	0,000	
Treatment					
L. monocytogenes 4 C with coating	–	0,0483	–23,34	0,000	1,2

		1,1265				0
Dag						
	0	0,1843	0,0861	2,14	0,052	1,93
	1	-0,3271	0,0728	-4,49	0,001	1,82
	4	0,0805	0,0998	0,81	0,435	2,26
Treatment*Dag						
	L. monocytogenes 4 C with coating	1,1265	0,0861	13,08	0,000	2,00
	L. monocytogenes 4 C with coating	0,5348	0,0728	7,35	0,000	1,80
	L. monocytogenes 4 C with coating	-1,3494	0,0998	-13,52	0,000	2,40

951 ## Regression Equation

```
Log  =  13,9083 - 1,1265 Treatment_L. monocytogenes 4 C with coating
N       + 1,1265 Treatment_L.
        monocytogenes 4 C without coating + 0,1843 Dag_0 - 0,3271 Dag_1
        + 0,0805 Dag_4
        + 0,0623 Dag_6 + 1,1265 Treatment*Dag_L. monocytogenes 4 C with
        coating 0
        + 0,5348 Treatment*Dag_L. monocytogenes 4 C with coating 1 -
         1,3494 Treatment*Dag_L.
        monocytogenes 4 C with coating 4 - 0,3120 Treatment*Dag_L.
        monocytogenes 4 C with
        coating 6 - 1,1265 Treatment*Dag_L. monocytogenes 4 C without
        coating 0
        - 0,5348 Treatment*Dag_L. monocytogenes 4 C without coating 1
        + 1,3494 Treatment*Dag_L. monocytogenes 4 C without coating 4
        + 0,3120 Treatment*Dag_L. monocytogenes 4 C without coating 6
```

952 ## Fits and Diagnostics for Unusual Observations

Obs	LogN	Fit	Resid	Std Resid	
6	11,513	11,513	0,000	*	X
17	16,811	16,465	0,347	2,43	R
18	16,118	16,465	-0,347	-2,43	R

R Large residual
X Unusual X

Comparisons for LogN

Tukey Pairwise Comparisons: Treatment

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping
L. monocytogenes 4 C without coating	11	15,0349	A
L. monocytogenes 4 C with coating	10	12,7818	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Dag

Grouping Information Using the Tukey Method and 95% Confidence

Dag	N	Mean	Grouping
0	4	14,0927	A
4	3	13,9888	A B
6	7	13,9706	A
1	7	13,5812	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatment*Dag

Grouping Information Using the Tukey Method and 95% Confidence

Treatment*Dag	N	Mean	Grouping
L. monocytogenes 4 C without coating 4	2	16,4647	A
L. monocytogenes 4 C without coating 6	3	15,4091	B
L. monocytogenes 4 C without coating 1	4	14,1730	C
L. monocytogenes 4 C with coating 0	2	14,0927	C
L. monocytogenes 4 C without coating 0	2	14,0927	C
L. monocytogenes 4 C with coating 1	3	12,9895	D

L. monocytogenes 4 C with coating 6	**4**	**12,5322**		**D**
L. monocytogenes 4 C with coating 4	**1**	**11,5129**		**E**

Means that do not share a letter are significantly different.

Figure 7B S.aureus

General Linear Model: LogN versus Treatment; Dag

Method

Factor coding (−1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Treatment	Fixed	2	S. aureus 4 C with coating; S. aureus 4 C without coating
Dag	Fixed	4	0; 1; 4; 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	1	2,7278	2,72783	142,25	0,000
Dag	3	1,7388	0,57959	30,22	0,000
Treatment*Dag	3	1,0021	0,33404	17,42	0,000
Error	20	0,3835	0,01918		
Total	27	7,0213			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,138478	94,54%	92,63%	89,97%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	13,3893	0,0274	489,21	0,000	
Treatment					
S. aureus 4 C with coating	−0,3264	0,0274	−11,93	0,000	1,09
Dag					

0	0,5157	0,0561	9,19	0,000	1,88
1	−0,2636	0,0441	−5,97	0,000	1,63
4	−0,0936	0,0441	−2,12	0,047	1,62

Treatment*Dag

S. aureus 4 C with coating 0	0,3264	0,0561	5,82	0,000	1,97
S. aureus 4 C with coating 1	−0,0465	0,0441	−1,05	0,304	1,63
S. aureus 4 C with coating 4	−0,0016	0,0441	−0,04	0,971	1,63

Regression Equation

Log N = 13,3893 − 0,3264 Treatment_S. aureus 4 C with coating + 0,3264 Treatment_S. aureus 4 C without coating + 0,5157 Dag_0 − 0,2636 Dag_1 − 0,0936 Dag_4 − 0,1584 Dag_7 + 0,3264 Treatment*Dag_S. aureus 4 C with coating 0 − 0,0465 Treatment*Dag_S. aureus 4 C with coating 1 − 0,0016 Treatment*Dag_S. aureus 4 C with coating 4 − 0,2783 Treatment*Dag_S. aureus 4 C with coating 7 − 0,3264 Treatment*Dag_S. aureus 4 C without coating 0 + 0,0465 Treatment*Dag_S. aureus 4 C without coating 1 + 0,0016 Treatment*Dag_S. aureus 4 C without coating 4 + 0,2783 Treatment*Dag_S. aureus 4 C without coating 7

Fits and Diagnostics for Unusual Observations

Obs	LogN	Fit	Resid	Std Resid	
4	12,4607	12,7527	−0,2920	−2,43	R

R Large residual

Comparisons for LogN

Tukey Pairwise Comparisons: Treatment

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping
S. aureus 4 C without coating	14	13,7157	A

S. aureus 4 C with coating	14	13,0629	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Dag

Grouping Information Using the Tukey Method and 95% Confidence

Dag	N	Mean	Grouping
0	4	13,9050	A
4	8	13,2957	B
7	8	13,2309	B
1	8	13,1257	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatment*Dag

Grouping Information Using the Tukey Method and 95% Confidence

Treatment*Dag	N	Mean	Grouping		
S. aureus 4 C with coating 0	2	13,9050	A		
S. aureus 4 C without coating 0	2	13,9050	A		
S. aureus 4 C without coating 7	4	13,8355	A		
S. aureus 4 C without coating 4	4	13,6238	A	B	
S. aureus 4 C without coating 1	4	13,4986		B	
S. aureus 4 C with coating 4	4	12,9676			C
S. aureus 4 C with coating 1	4	12,7527			C D
S. aureus 4 C with coating 7	4	12,6262			D

Means that do not share a letter are significantly different.

Figure 8A

General Linear Model: pH versus Treatment; hours

Method

Factor coding (−1; 0; +1)

Rows unused 2

Factor Information

Factor	Type	Levels	Values
Treatment	Fixed	3	5%AA; 8%AA; uncoated
hours	Fixed	6	0; 24; 48; 72; 96; 168

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	2	75,228	37,6138	247,01	0,000
hours	5	15,073	3,0146	19,80	0,000
Treatment*hours	10	5,865	0,5865	3,85	0,006
Error	19	2,893	0,1523		
Total	36	96,804			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,390229	97,01%	94,34%	88,57%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6,1727	0,0644	95,80	0,000	
Treatment					
5%AA	−0,6067	0,0916	−6,63	0,000	1,37
8%AA	−1,3542	0,0916	−14,79	0,000	1,37
hours					
0	−1,344	0,139	−9,70	0,000	1,63
24	0,132	0,145	0,91	0,374	1,66
48	0,192	0,145	1,32	0,201	1,66

72	0,485	0,145	3,34	0,003	1,66
96	0,430	0,145	2,96	0,008	1,66
Treatment*hours					
5%AA 0	−0,697	0,201	−3,47	0,003	2,38
5%AA 24	0,180	0,205	0,88	0,391	2,22
5%AA 48	−0,086	0,205	−0,42	0,681	2,22
5%AA 72	0,263	0,205	1,28	0,216	2,22
5%AA 96	0,170	0,205	0,83	0,418	2,22
8%AA 0	−0,307	0,201	−1,53	0,143	2,38
8%AA 24	−0,306	0,205	−1,49	0,153	2,22
8%AA 48	0,114	0,205	0,56	0,585	2,22
8%AA 72	0,318	0,205	1,55	0,138	2,22
8%AA 96	−0,021	0,205	−0,10	0,919	2,22

1002 ## Regression Equation

```
p   =  6,1727 − 0,6067 Treatment_5%AA − 1,3542 Treatment_8%AA
H      + 1,9609 Treatment_uncoated
       − 1,344 hours_0 + 0,132 hours_24 + 0,192 hours_48 + 0,485 hours_72
       + 0,430 hours_96
       + 0,104 hours_168 − 0,697 Treatment*hours_5%AA 0
       + 0,180 Treatment*hours_5%AA 24
       − 0,086 Treatment*hours_5%AA 48 + 0,263 Treatment*hours_5%AA 72
       + 0,170 Treatment*hours_5%AA 96 + 0,170 Treatment*hours_5%AA 168
       − 0,307 Treatment*hours_8%AA 0 − 0,306 Treatment*hours_8%AA 24
       + 0,114 Treatment*hours_8%AA 48 + 0,318 Treatment*hours_8%AA 72
       − 0,021 Treatment*hours_8%AA 96 + 0,202 Treatment*hours_8%AA 168
       + 1,005 Treatment*hours_uncoated 0 + 0,125 Treatment*hours_uncoated
       24
       − 0,028 Treatment*hours_uncoated 48 − 0,581 Treatment*hours_uncoated
       72
       − 0,149 Treatment*hours_uncoated 96 − 0,372 Treatment*hours_uncoated
       168
```

1003 ## Residual Plots for pH

1004 # Comparisons for pH

1005 ## Tukey Pairwise Comparisons: Treatment

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping
uncoated	13	8,13366	A
5%AA	12	5,56604	B
8%AA	12	4,81854	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: hours

Grouping Information Using the Tukey Method and 95% Confidence

hours	N	Mean	Grouping
72	6	6,65750	A
96	6	6,60292	A
48	6	6,36500	A
24	6	6,30500	A
168	6	6,27708	A
0	7	4,82898	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatment*hours

Grouping Information Using the Tukey Method and 95% Confidence

Treatment*hours	N	Mean	Grouping
uncoated 96	2	8,41500	A
uncoated 24	2	8,39125	A
uncoated 48	2	8,29750	A
uncoated 72	2	8,03750	A
uncoated 168	2	7,86625	A B
uncoated 0	3	7,79444	A

5%AA 72	**2**	**6,31375**	**B**	**C**	
5%AA 96	**2**	**6,16625**		**C**	**D**
5%AA 24	**2**	**5,87875**		**C**	**D**
5%AA 168	**2**	**5,84000**		**C**	**D**
5%AA 48	**2**	**5,67250**		**C**	**D**
8%AA 72	**2**	**5,62125**		**C**	**D**
8%AA 96	**2**	**5,22750**		**C**	**D**
8%AA 48	**2**	**5,12500**		**C**	**D**
8%AA 168	**2**	**5,12500**		**C**	**D**
8%AA 24	**2**	**4,64500**			**D** **E**
5%AA 0	**2**	**3,52500**			**E**
8%AA 0	**2**	**3,16750**			**E**

Means that do not share a letter are significantly different.

Figure 8B

General Linear Model: Natural log versus treatment; hours

Method

Factor coding (−1; 0; +1)

Factor Information

Factor	Type	Levels	Values
treatment	Fixed	3	5%AA; 8%AA; uncoated
hours	Fixed	5	0; 24; 48; 96; 168

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
treatment	2	31,41	15,7068	22,36	0,000
hours	4	53,93	13,4821	19,19	0,000
treatment*hours	8	16,10	2,0125	2,86	0,038
Error	15	10,54	0,7026		
Total	29	111,98			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,838184	90,59%	81,81%	62,36%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	9,633	0,153	62,95	0,000	
treatment					
5%AA	−0,615	0,216	−2,84	0,012	1,33
8%AA	−0,827	0,216	−3,82	0,002	1,33
hours					

0	−0,697	0,306	−2,28	0,038	1,60
24	−0,634	0,306	−2,07	0,056	1,60
48	−0,959	0,306	−3,13	0,007	1,60
96	−0,365	0,306	−1,19	0,252	1,60
treatment*hours					
5%AA 0	0,020	0,433	0,05	0,964	2,13
5%AA 24	0,131	0,433	0,30	0,767	2,13
5%AA 48	0,306	0,433	0,71	0,490	2,13
5%AA 96	−0,682	0,433	−1,58	0,136	2,13
8%AA 0	0,632	0,433	1,46	0,165	2,13
8%AA 24	0,602	0,433	1,39	0,185	2,13
8%AA 48	0,296	0,433	0,68	0,504	2,13
8%AA 96	0,152	0,433	0,35	0,731	2,13

1026 ## Regression Equation

```
Natural log = 9,633 − 0,615 treatment_5%AA − 0,827 treatment_8%AA
              + 1,442 treatment_uncoated
              − 0,697 hours_0 − 0,634 hours_24 − 0,959 hours_48 −
               0,365 hours_96
              + 2,655 hours_168 + 0,020 treatment*hours_5%AA 0
              + 0,131 treatment*hours_5%AA
              24 + 0,306 treatment*hours_5%AA 48 −
               0,682 treatment*hours_5%AA 96
              + 0,225 treatment*hours_5%AA 168 + 0,632 treatment*hours_8%AA
              0
              + 0,602 treatment*hours_8%AA 24 + 0,296 treatment*hours_8%AA
              48
              + 0,152 treatment*hours_8%AA 96 − 1,681 treatment*hours_8%AA
              168
              − 0,652 treatment*hours_uncoated  0 −
               0,732 treatment*hours_uncoated  24
              − 0,602 treatment*hours_uncoated  48
              + 0,531 treatment*hours_uncoated  96
              + 1,456 treatment*hours_uncoated  168
```

1027 ## Fits and Diagnostics for Unusual Observations

Obs	Natural log	Fit	Resid	Std Resid

19	13,324	11,898	1,426	2,41	R
20	10,471	11,898	-1,426	-2,41	R

R Large residual

Residual Plots for Natural log

Comparisons for Natural log

Tukey Pairwise Comparisons: treatment

Grouping Information Using the Tukey Method and 95% Confidence

treatment	N	Mean	Grouping
uncoated	10	11,0747	A
5%AA	10	9,0175	B
8%AA	10	8,8060	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: hours

Grouping Information Using the Tukey Method and 95% Confidence

hours	N	Mean	Grouping
168	6	12,2875	A
96	6	9,2677	B
24	6	8,9990	B
0	6	8,9355	B
48	6	8,6740	B

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: treatment*hours

Grouping Information Using the Tukey Method and 95% Confidence

treatment*hours	N	Mean	Grouping
uncoated 168	2	15,1852	A

5%AA 168		**2**	**11,8977**	**A**	**B**	
uncoated	**96**	**2**	**11,2403**		**B**	**C**
8%AA 168		**2**	**9,7797**		**B**	**C**
uncoated	**0**	**2**	**9,7259**		**B**	**C**
uncoated	**24**	**2**	**9,7085**		**B**	**C**
uncoated	**48**	**2**	**9,5137**		**B**	**C**
8%AA 24		**2**	**8,7740**		**B**	**C**
8%AA 0		**2**	**8,7403**		**B**	**C**
8%AA 96		**2**	**8,5927**		**B**	**C**
5%AA 24		**2**	**8,5144**			**C**
5%AA 48		**2**	**8,3649**			**C**
5%AA 0		**2**	**8,3403**			**C**
8%AA 48		**2**	**8,1435**			**C**
5%AA 96		**2**	**7,9702**			**C**

Means that do not share a letter are significantly different.

1044

1045