Macromolecular acidic coating increases shelf life by inhibition of bacterial growth

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ABSTRACT

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

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

Specifically, we show that the coatings 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*.

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

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.

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

A.

B

sugars: β-1,4-linked D-mannuronic acid (M) and its 5-epimer α-L-guluronic acid (G) (Fig. 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 experiments with salmon 5 different fillets where purchased spread out over a 2 months period. Beef was obtained directly from freshly slaughtered cattle at a local slaughterhouse (Nortura SA, Malvik, Norway). Pork fillet, chicken fillet, shrimp and rice were obtained from a local food store. Fillets and meat samples were cut into pieces of 10 g (± 1 g) pieces. One fillet or cut of meat was used as the source of meat or fish pieces in each experiment.

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

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

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

2.2. Analytical methods

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

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

2.3. Bacterial strains

The following five bacteria were used in the tests: *Escherichia coli* (CCUG 17620), *Bacillus cereus* (NVH0075/95), *B. weihenstephanensis* (10394), *Listeria monocytogenes* serotype 1 (NVH738) and *Staphylococcus aureus* (50090). *B. weihenstephanensis* (strain 10,394) was used in experiments carried out at 4 °C since *B. cereus* does not grow below 8 °C. All strains were from stock cultures stored at −80 °C in 30% glycerol. Samples were streaked out onto blood agar plates (bovine) and grown at 30 °C overnight. One colony was then used for growth in 10 ml BHI medium (Oxoid, Basingstoke, UK) for 18 ± 1 h at 37 °C for *E. coli* and 30 °C for the four other strains. The cfu is then about 10^8/ml for *B. cereus*, *B. weihenstephanensis* and about 10^9/ml for *S. aureus*, *E. coli* and L. *monocytogenes*. Before use, all strains were diluted to about 10^5 or 10^7 cfu/ml in sterile peptone water (Oxoid, Basingstoke, UK).

2.4. Spores of B. cereus

B. cereus NVH 0075/95 was sporulated in a chemically defined sporulation medium (de Vries et al., 2004). In brief, a 1/10 dilution of a

four hours culture of brain heart infusion broth (BHI) (Becton, Dickinson & Co, Sparks, MD, USA) was resuspended in the chemically defined sporulation medium (30 °C, 250 rpm rotary shaking). After 2–5 days of sporulation spore batches, 95% free of germinated spores as observed by phase-contrast microscopy, were cleaned by repeated centrifugation (10 min, 6500 ×g, 4 °C, Sorvall RC-5B) and washing with 10 mM potassium phosphate buffer pH 7.2. The spores were stored in the buffer at 4 °C protected from light. To ensure stable spore crops, spores were stored for at least a fortnight after washing before used for experiments.

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 1 h overnight at 4 °C, and then left at room temperature for 1 h. Ten microliter 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 (10 g ± 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 min 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 min 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 h 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%. Analysis data are included in the Supplementary Information file.

3. Results

3.1. Coating formulation and acidification of food surfaces

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

3.1.1. Coating formulation and pH on salmon fillets

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

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

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

A distinct behaviour was observed in the presence of alginic acid, with a rapid increase in pH (1–1.5 pH units) during the first 24 h,
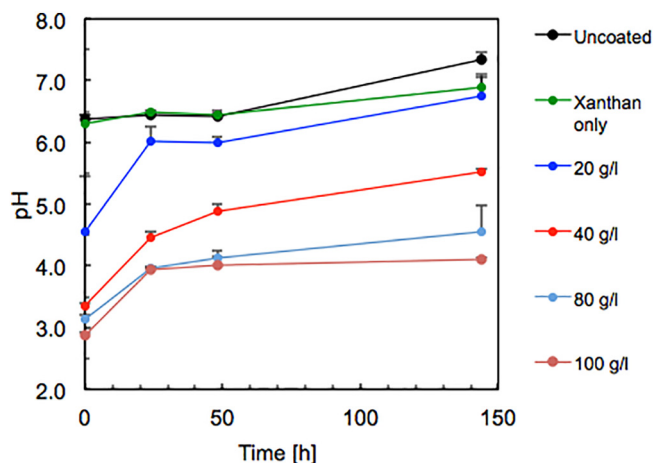
Fig. 2. Surface pH of salmon fillets dip-coated with xanthan (5 g/l) containing various amounts (0–100 g/l) of alginic acid.
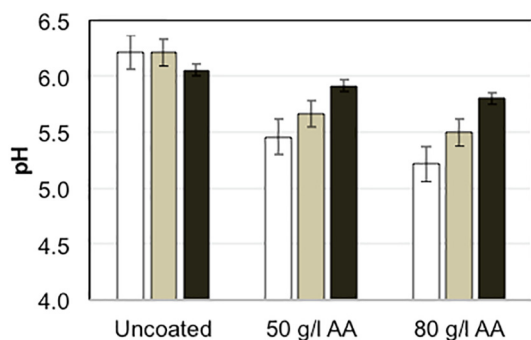
Fig. 3. pH profiles of chicken fillets dip-coated with 5 g/l xanthan containing various amounts of alginic acid. The pH was determined at the surface (white), 5 mm below the surface (grey), and in the middle (black) of the fillets after 96 h of incubation at 4 °C.

followed by a slower increase in pH. For 100 g/l alginic acid the pH stabilized in the range 4.0–4.1 even up to 150 h.

3.1.2. Coating formulation and pH on chicken fillets

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

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

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 h before pH was monitored after suspending 50 g of boiled rice in 100 ml 0.17 M KCl (Fig. 4).

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
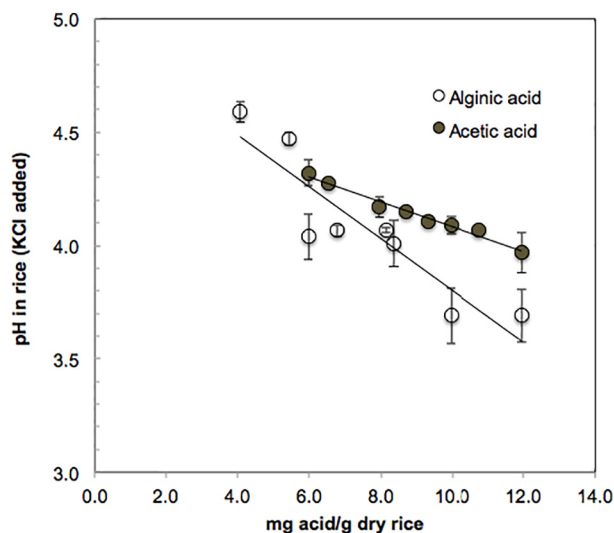
effect above 5 mg/g added. It may be noted there appeared to be negligible influence on the taste and texture of the rice up to ca. 10 mg alginic acid added.

3.2. Protection against external contamination of pathogens

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

3.3. Microbiology of coated foods

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

3.3.1. Microbiology of coated salmon fillets

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

Uncoated fillets reached 10^4 bacteria/cm² 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
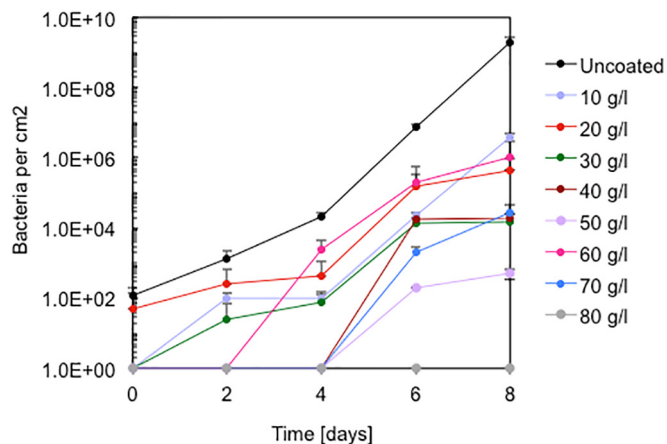

Fig. 4. pH in boiled sushi rice treated with alginic acid or acetic acid. Similar results were obtained for Jasmin rice.

Fig. 5. Total bacterial counts on salmon fillets dip-coated with 5 g/l xanthan containing various amounts of alginic acid. Incubation temperature: 4 °C.
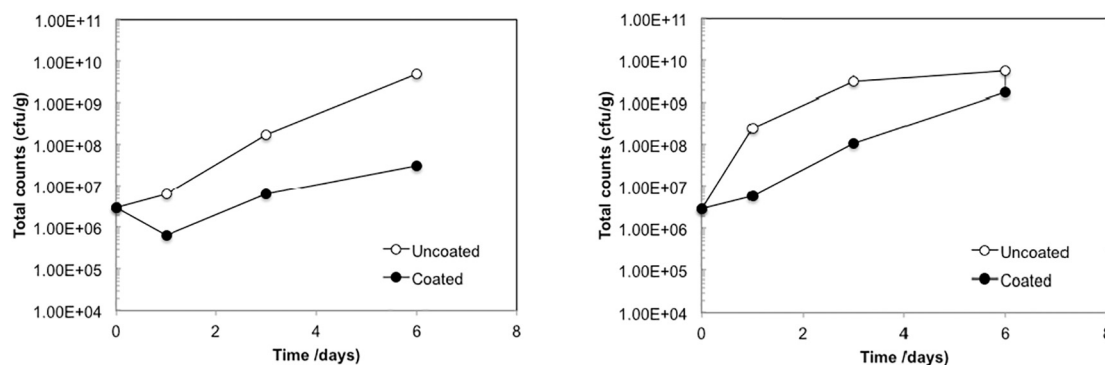
Fig. 6. Bacterial growth (natural flora) on coated and uncoated cod fillets incubated at 4 °C (A) and 12 °C (B). The coating contained 50 g/l alginic acid dispersed in 5 g/l xanthan. Note error bars are mostly too small to appear on the figure (log scale).

were in all cases below 10^6.

3.3.2. Microbiology of coated cod fillets

We continued with monitoring growth coated and uncoated cod fillets, using the natural flora (analogous to the salmon fillets). Samples were incubated at 4 and 12 °C, respectively (Fig. 6). The higher temperature was included to study the protective effect under typical 'temperature abuse' conditions. The fillets had a natural flora of about 3×10^6 CFU/g at the start of the experiments, increasing significantly to about 5×10^9 after 6 days incubation at both temperatures. The increase (growth rate) was however much slower initially at 4 °C, as expected. After coating and using an incubation temperature at 12 °C the development of the flora was close to that of 4 °C without coating, although a little slower after the first day of incubation. The coated cod stored at 4 °C had a decrease in bacterial number the first 24 h, and thereafter the bacterial count increased gradually to 3×10^7 after six days, ending up two orders of magnitude and significantly lower in bacterial count than the uncoated cod stored at the same temperature. The experiment at 4 °C was repeated using a cod fillet having lower bacterial content prior to coating (4×10^4). The effect of coating was similar to the previous case, i.e. a general decrease in bacterial counts of 1–1.5 orders of magnitude (data not shown).

3.3.3. Coated salmon fillets pre-incubated with pathogenic bacteria

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

The same experiments were conducted at 4 °C, but using 10^7 cfu/g initially (Fig. 7B), showing that apart from *L. monocytogenes* (and the natural flora) the added pathogens hardly grew at all. Moreover, cell

counts were in fact significantly reduced by about one order of magnitude after coating. Even for *L. monocytogenes* the number of bacteria was significantly reduced after coating at 4 °C.

3.3.4. Microbiology and pH of coated shrimp

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

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 stabilized 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 h of incubation for the respective coatings although the bacterial counts on coated shrimp were significantly lower than uncoated shrimp. By 168 h 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 Fig. 9. The high temperature of 12 °C was chosen to simulate conditions considered as 'temperature abuse' of foods.

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

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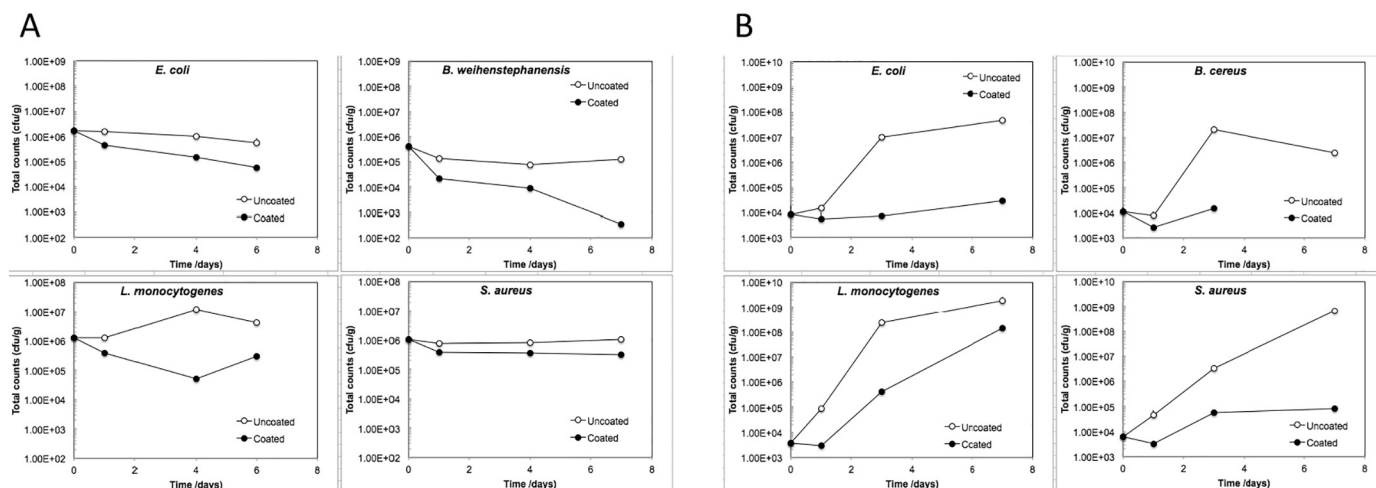
Fig. 7. Salmon fillets pre-incubated by pathogens and further incubated at 4 °C (A) and 12 °C (B). *B. weihensephanensis* was used instead of *B. cereus* at 4 °C since the latter does not grow at 4 °C. Note error bars are generally too small to appear on the figure (log scale).

consumption for > 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 Fig. S-1), but was also detected on meat, whereas the surface of coated shrimp was not visibly affected.
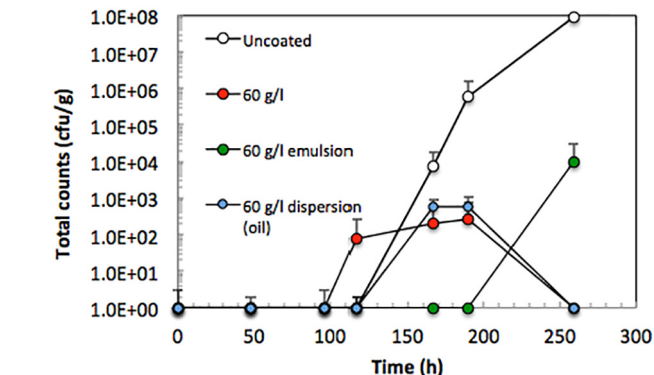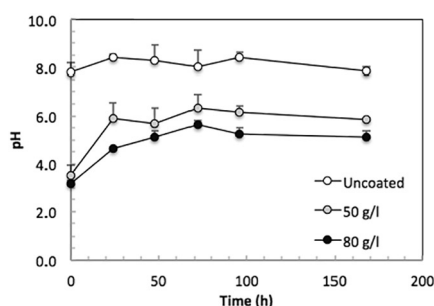

Fig. 9. Total bacterial counts on beef stored at 12 °C using four different formulations containing alginic acid: Uncoated, coating containing 60 g/l alginic acid (in 5 g/l xanthan), emulsion coating (10/90 o/w) containing 60 g/l alginic acid, and an oil dispersion containing 60 g/l alginic acid.

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.
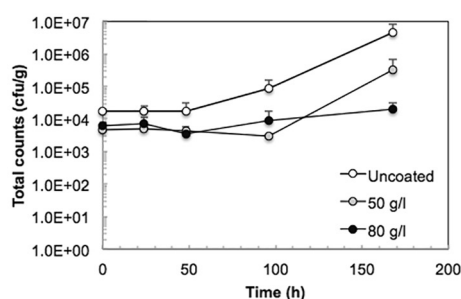

Fig. 8. Surface pH and bacterial counts of uncoated and alginic acid (50 and 80 g/l in 5 g/l xanthan) coated shrimp incubated at 4 °C.
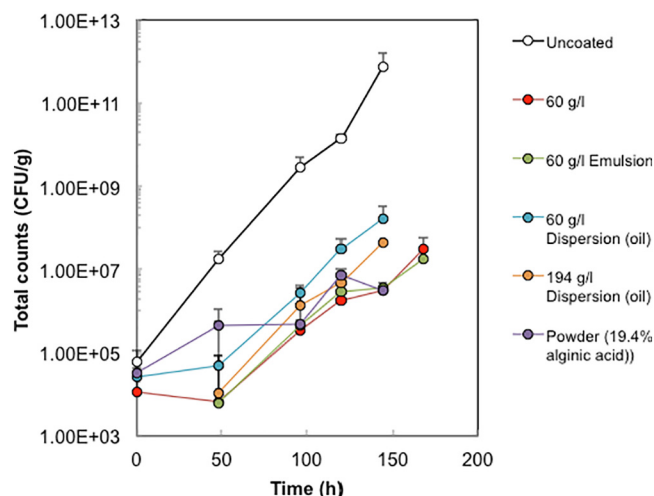
Fig. 10. Total bacterial counts on pork incubated at 12 °C using different formulations containing alginic acid: Uncoated, coating containing 60 g/l alginic acid (in 5 g/l xanthan), emulsion coating (10/90 oil/water) containing 60 g/l alginic acid, oil dispersions containing 60 or 194 g/l alginic acid, and a powder coating containing 19.4% alginic acid.

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 neutralised 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 h of incubation at 4 °C, whereas on chicken filled under the same conditions reaches a pH of 5.3 (Fig. 3), i.e. the neutralization is more rapid in the latter case. The neutralization may be due to the outwards diffusion of metabolites, but clearly also inwards diffusion of protons as evidenced by a detectable pH gradient in chicken fillet. Although the alginic acid due to its macromolecular size is not expected to diffuse into the food as would low molecular weight acids such as acetic acid, the Grotthus mechanism (Hassanali et al., 2013) allow faster migration of protons in aqueous media compared to simple salts, thereby contributing to neutralization of the surface. The increasing pH will also gradually solubilize the alginic acid (as alginate). However, the dispersion in xanthan ensures that even soluble alginate remains in the coating.

Food safety is usually not a large problem for fish, given it is heat

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

When we had shown that the bacteria were not able to grow on top of the coating we continued to coat fresh fish (cod) from a local supermarket to see how well the coating inhibited growth of the natural flora of the fish. As shown in Fig. 6 the natural flora decreased nearly one order of magnitude the first 24 h, and then gradually increased from about 6×10^5 to 3×10^7 over the following 5 days, at 4 °C. For the uncoated fish the number of bacteria increased continuously from a starting point of 3×10^6 to 5×10^9 over the 6 days of the experiments, showing that the shelf life of the fish probably would increase by 4–6 day with coating at 4 °C, an effect which otherwise only can be obtained by methods like super chilling or extensive salt treatment (Duun and Rustad, 2007). At 12 °C we see the same tendency, but not as clear as for 4 °C.

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

The effects on natural flora obtained for salmon and cod are to a large extent also observed and extend generally to the other systems studied here, namely shrimp, chicken, beef and pork: The lower the pH of the coating, the larger antibacterial effect. For peeled shrimp (Fig. 8), whose surface appearance is largely unaltered by the coating, the bacterial count remained essentially unaltered (ca. 10^4) for up to 6 days with 80 g/l alginic acid coating.

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

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

In boiled rice the presence of heat resistant spores of *B. cereus* poses

a serious risk if the rice is stored for longer periods (production of the toxin cereulide) without effective cooling (de Vries et al., 2004). Our results (Supplementary Information Table S-1) demonstrate that adding alginic acid before cooking matches acetic acid and completely inhibits bacterial growth where spores (10^4 spores/g) had been added, even after incubation at 22 °C for 6 days. Adding alginic acid after cooking resulted in growth at 22 °C, but not at 4 or 12 °C. The 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 (Fig. 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).

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

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2018.08.001.

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