International Journal of Food Microbiology 285 (2018) 136–143

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Macromolecular acidic coating increases shelf life by inhibition of bacterial growth

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ARTICLE INFO

Keywords:
Coating
Alginic acid
Xanthan
Shelf life
Antimicrobial

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

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https://doi.org/10.1016/j.ijfoodmicro.2018.08.001
Received 20 October 2017; Received in revised form 18 June 2018; Accepted 2 August 2018
Available online 16 August 2018
0168-1605/

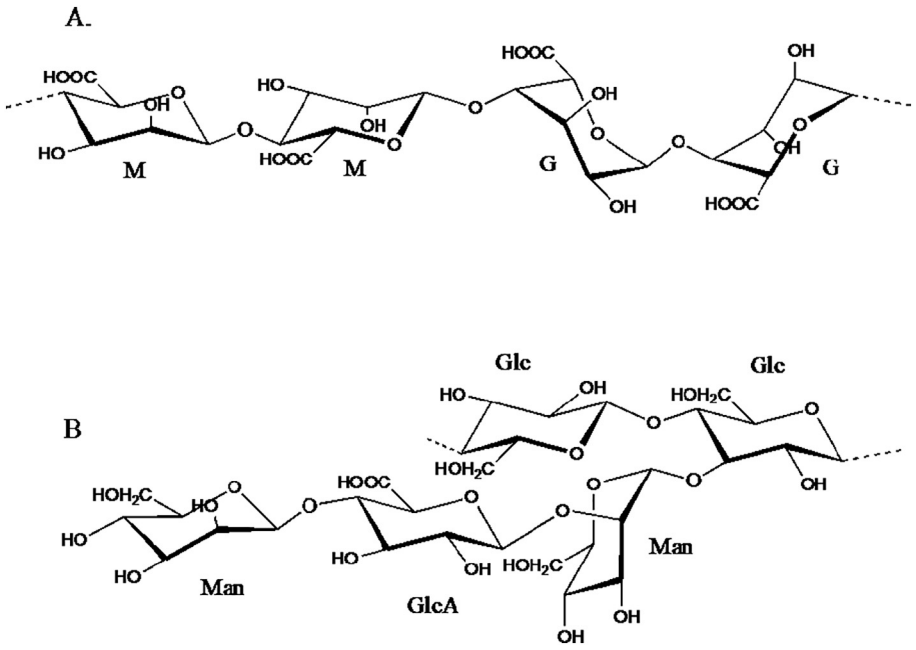

Fig. 1. Structure of alginic acid exemplified by an MMGG fragment (A) and xanthan (B). Abbreviations: M: β-D-mannuronic acid. G: α-L-guluronic acid. Glc: β-D-glucose. GlcA: β-D-glucuronic acid. Man: D-mannose (α for inner Man, β for terminal Man). Note that xanthans may contain various amounts of O-acetate 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.

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