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Short communication

Growth inhibition of heat-injured *Enterococcus faecium* by oligophosphates in a cured meat model

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Abstract

Cells of two heat-resistant strains of *Enterococcus faecium* were heated and incubated in meat suspensions containing curing ingredients. The concentrations of the curing ingredients were those frequently used for pasteurized ham-type products, except that the concentrations of the oligophosphates (triphosphate and diphosphate) varied. Heating tests at 69 °C were performed with inoculated meat suspensions in heat-sealed plastic pouches. Numbers of bacteria were counted immediately after heating and in parallel series of heated pouches incubated at 37 °C. Plating was performed in Tryptone Dextrose Yeast Meat Peptonised Milk Agar (TDYMP); in TDYMP Agar to which the curing ingredients were added; and in TDYMP Agar to which the curing ingredients except oligophosphates were added.

The inclusion of oligophosphates in the heating medium increased the heat-injury sustained by the *E. faecium* cells, and in combination with rather severe heat treatment even completely blocked the growth of surviving organisms in the meat suspension incubated at 37 °C.

The presence of oligophosphates in the culture medium TDYMP Agar severely reduced the counts of freshly heated cells; however, this effect disappeared after repair and growth of the surviving organisms in the meat suspension.

Keywords: *Enterococcus faecium*; Oligophosphates; Meat; Heat resistance; Recovery

1. Introduction

Enterococci are among the most heat-resistant of the non-sporulating bacteria (Houben, 1980a; Magnus et al., 1986; Ghazala et al., 1995; Incze et al., 1999). After surviving pasteurization, both *Enterococcus faecalis* and (more frequently) *E. faecium* can cause spoilage of cured meat products (Bell and De Lacy, 1984; Whiteley and D'Souza, 1989; Gordon and Ahmad, 1991; Ross et al., 1998). To prevent such spoilage, the initial contamination by heat-resistant organisms should be kept to a minimum, and adequate heating should be based on the *D*-values of the most heat-resistant enterococci isolated, preferably freshly, from pasteurized meat products (Houben, 1982; Gordon and Ahmad, 1991; Ghazala et al., 1995).

As reviewed by Cassens (1994), the major purpose of using polyphosphates in meat processing is to increase water-binding and to improve the product

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yield. They act primarily by increasing the pH, which in turn increases water-binding, resulting in a reduced jelly throw-out during cooking. Other possible mechanisms of action include increasing protein hydration by making more sites on the proteins available, by altering the structure, or by binding certain ions that might otherwise interfere with water-binding (Cassens, 1994). Phosphates may also function as preservatives because they retard the development of oxidative rancidity and may also slow microbial growth by binding heavy metal ions (Cassens, 1994). The polyphosphates most commonly used in the meat processing industry are the oligophosphates sodium tripolyphosphate ($Na_5P_3O_{10}$) and sodium acid pyrophosphate ($Na_2H_2P_2O_7$) (Cassens, 1994).

Few data are available on the inhibitory effects of oligophosphates on micro-organisms in processed meats. In many of the studies, commercial mixtures of polyphosphates and spore-forming micro-organisms were used (Wagner, 1986; Juneja et al., 1996). Di- and triphosphates were shown to inhibit, to varying degrees, the growth of non-sporulating bacteria, such as *Salmonella* spp. (Garibaldi et al., 1969), *Moraxella, Acinetobacter* (Firstenberg-Eden et al., 1981), *Salmonella typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus*, two lactic starters (Molins et al., 1984), *Escherichia coli* (Hughes and McDermott, 1989), *S. aureus* (Shelef et al., 1990), and *Aeromonas hydrophila* (Velázquez et al., 2001).

The triphosphate in most studies appeared to have a more pronounced effect than the diphosphate. Apart from a single report on the ability of pyrophosphate to decrease the heat resistance of *E. faecium* in broth at 55 °C (Kniewallner and Prändl, 1974), there is no information regarding the effects of oligophosphates on enterococci in meat products.

The present study was designed to test the effect of a mixture of triphosphate and diphosphate (weight ratio 10:1) on two heat-resistant *E. faecium* strains during and after heating in a meat suspension and in the culture (recovery-)medium Tryptone Dextrose Yeast Meat Peptonised Milk Agar (TDYMP-agar) used to assess numbers of Colony Forming Units (CFU). The ingredients for the TDYMP-agar and the other bacteriological media used in this study were purchased from Oxoid (Unipath, Basingstoke, England).

2. Materials and methods

2.1. Test strains

Two strains of *E. faecium* (E_1 and E_{20}) were selected from 67 isolates of enterococci from pasteurized hams and shoulders. Strain E_{20} appeared to be the most heat-resistant of the strains tested; strain E_1 displayed a weaker, though quite pronounced, thermal resistance (Houben, 1980a).

To maintain the viability of the strains, tubes with litmus milk (Oxoid CM 45) were heavily (10^7 CFU ml^{-1}) inoculated and stored at −40 °C. Cell suspensions were prepared by thawing a tube of litmus milk and incubating it for 24 h at 37 °C. A sample (1/10 ml) was then transferred to 100 ml of TDYMP broth. TDYMP is a rich medium (Mossel and Krugers Dagneaux, 1959) reported to encourage the growth of enterococci (Houben, 1980a,b). After a 48-h incubation at 37 °C, the cells were collected by centrifugation for 20 min at $2100 \times g$ with an IEC Centra-8R apparatus (International Equipment Company, Needham Heights, MA, USA). The cell crop was washed twice with 0.1% peptone physiological saline and finally suspended in 50 ml of this solution. The 0.1% peptone physiological saline contained (in % w/w): peptone (0.1); sodium chloride (0.85); distilled water (99.05). For E_{20}, two replicate suspensions were prepared from separately thawed tubes of litmus milk. All suspensions were kept under refrigeration (2 °C) and used for a maximum of 1 month. During this period, only minor changes in the numbers of cells and/or their thermal resistances were observed.

2.2. Preparations of meat suspensions

Lean pork ham (pH 5.7–6.0) was finely blended into a thick paste in a Stephanal TA-20 chopper (Stephan, Diessen am Ammersee, Germany). Thereafter, portions of 100 g were vacuum-packaged and stored at −40 °C. Before use, the packages were thawed in lukewarm water and then mixed with 200 ml distilled water in a Waring Blender (Waring Products Division, New Hartford, USA). After being hermetically sealed, the vessel was sterilised for 20 min at 112 °C. After chilling and renewed blending, a suspension was obtained which no longer coagulated

on heating. To this suspension, 18% w/w of an injection-type pasteurized ham brine was added. The brine contained (in % w/w): NaCl (13.22); $NaNO_2$ (0.08); $Na_2H_2P_2O_7$ (0.30); $Na_5P_3O_{10}$ (3.0); glucose (3.30); sodium-L-glutamate (0.60); ascorbic acid (0.30); distilled water (79.20). The brine was freshly prepared and filter-sterilised (0.45-μm syringe filter, Gelman Sciences, Ann Arbor, MI, USA). The pH of the meat suspension with the added brine (Meat Suspension Brine) was 6.2–6.3, the a_w (25 °C) was 0.97. pH was measured with a Schott pH meter type CG818 (Schott, Mainz, Germany) equipped with an Ingold electrode (Mettler-Toledo, Greifensee, Switzerland). Water activity was determined with a Lufft Durotherm Water Activity Meter Type 5802 (G.Lufft, Fellbach, Germany). The levels of ingredients in Meat Suspension Brine and its final pH roughly corresponded with those observed in the manufacture of pasteurized hams. In one experiment, the oligophosphates were omitted from the brine; the pH of this meat suspension was 5.86.

2.3. Further experimental details

The meat suspensions were inoculated with the cell suspensions, and after thorough mixing were pipetted in 5-ml portions into sterile polyethylene pouches (Whirl pak, 6 oz, Nasco, Ft. Atkinson, USA); thickness after filling 2 mm. These were heat-sealed and heated in triplicate for pre-determined times at 68.9 ± 0.1 °C (156 F) in a Colora precision water bath NB/DS 1056 (Colora, Frankenthal, Germany). The pouches were heated in a suspended position, completely immersed in the water. The internal temperature measured in blank pouches reached the water bath temperature in 30 s. For this measurement, copper-constantan thermocouples connected to a Honeywell Elektronik 15 recorder (Honeywell, Minneapolis, MN, USA) were used. After heating, the pouches were rapidly chilled in ice water. In one series of pouches, the surviving cells were counted immediately after heating. The two remaining series of pouches were incubated at 37 ± 0.1 °C for 3 and 7–9 days, respectively. Upon growth of enterococci in the meat suspensions, primarily lactic acid will be produced from glucose. The acidification of the suspensions during incubation was monitored by measuring pH. Numbers of CFU were obtained by plating decimal dilutions (full duplicates) in TDYMP-agar and parallel in two modified versions of TDYMP. The first was TDYMP + Brine agar, which contained 18% w/w of a freshly prepared and filter-sterilised brine (composition as above), added after partially cooling the medium, and the second was TDYMP+ (Brine, oligophosphates omitted) agar, which contained 18% w/w of a brine from which the oligophosphates were omitted. The numbers of CFU were counted after 3 days at 37 °C.

Survivor curves (TDYMP counts) were calculated by linear regression and Decimal Reduction Times (*D*-values) were recorded.

3. Results and discussion

The inclusion of oligophosphates in the plating medium (Table 1; strain E_{20} and Table 3; strain E_1) for strain E_{20} clearly had an inhibitory effect on unheated cells, but dramatically reduced the counts of both strains of bacteria that survived heating. The inhibitory effect disappeared almost completely after growth of the surviving populations in the meat suspensions. To assist in over viewing the presented data, the oligophosphate effect is visualized in Fig. 1. The bacteria that survived extended heating: strain E_{20} (3.06–3.50, log counts after 105–120 min) and; strain E_1 (2.52, log count after 45 min), appeared unable to grow in the meat suspensions at the optimum growth temperature of 37 °C. Based on previous experiments (Houben, 1980b), we may assume that the latter effect would probably have been even more pronounced at refrigerating temperatures, e.g. of 8 °C and below.

Parallel experiments were performed with TDYMP containing brines with the same weight percentages of diphosphate/triphosphate, but added as single oligophosphate, respectively. Very similar heat-injury phenomena were observed; however, triphosphate appeared slightly more effective in inhibiting growth than diphosphate (data not shown).

Comparison of Tables 1 and 2 (strain E_{20}) suggests that the omission of the oligophosphates from the heating medium diminished the thermal resistance of the cells; the *D*-values measured were 91 and 63 min for the cases with or without inclusion of the phosphates, respectively. The pH of the meat suspension was lower in the absence of the phos-

Table 1
Growth of *E. faecium* (strain E_{20}) heated and thereafter incubated at 37 °C in meat suspension brine (experiment fully duplicated)

Plating medium	Tryptone Dextrose Yeast Meat Peptonised Milk Agar (TDYMP)			TDYMP + Brine			TDYP + Brine, oligophosphates omitted		
Period of incubation (days at 37 °C)	0	3	8.5	0	3	8.5	0	3	8.5
Heating time (min 69 °C)									
0	4.65* (0.233)	7.77 (0.834)	5.00 (0.962)	3.69 (0.276)	7.76 (0.742)	4.68 (0.849)	4.69 (0.219)	7.91 (0.552)	5.17 (0.679)
15	4.31* (0.318)	8.13 (0.304)	6.84 (1.124)	2.54 (–)[a]	8.11 (0.417)	6.41 (1.626)	4.15 (0.191)	8.06 (0.453)	6.64 (1.351)
30	4.14* (0.205)	8.08 (0.431)	7.65 (0.049)	1.22 (–)	8.06 (0.530)	7.06 (0.665)	4.02 (0.191)	8.03 (0.566)	7.47 (0.099)
45	3.99 (0.233)*	8.45 (nd)[b]	7.42 (0.304)	–[c]	8.43 (nd)	7.01 (0.785)	3.87 (0.247)	8.45 (nd)	7.32 (0.339)
60	3.94* (0.285)	6.71 (nd)	7.16 (0.523)	–	6.54 (nd)	6.97 (0.891)	3.51 (0.396)	6.61 (nd)	7.06 (0.728)
75	3.73* (0.290)	7.05 (0.354)	7.53 (–)	–	6.96 (0.537)	7.54 (–)	3.31 (0.537)	7.06 (0.396)	7.53 (–)
90	3.69* (0.283)	7.18 (–)	6.85 (–)	–	7.30 (–)	6.90 (–)	2.88 (0.283)	7.27 (–)	6.85 (–)
105	3.50* (0.332)	–	–	–	–	–	2.50 (0.021)	–	–
120	3.06* (0.290)	–	–	–	–	–	–	–	–

Average log counts (CFU. ml^{-1}) presented. Numbers shown between parentheses are standard deviations.

* $D_{69} = 91$ (4.2) min.

[a] Duplicate result below limit of detection (no colonies observed in duplicate plates containing 1 ml Meat Suspension Brine).

[b] (nd): No duplicate result obtained.

[c] Both results below limit of detection.

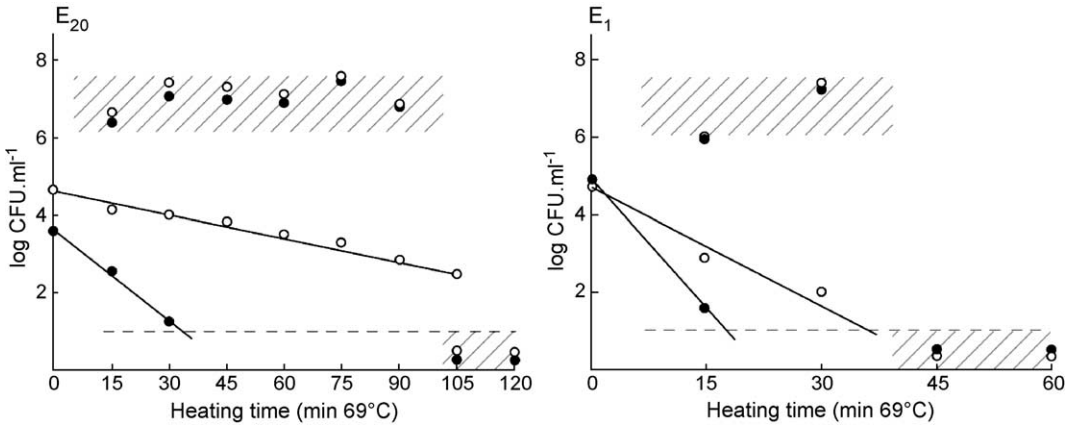

Fig. 1. Growth of *E. faecium* heated and thereafter incubated 9 days at 37 °C in Meat Suspension brine: schematic presentation of the observed oligophosphate effect. (Full circles: oligophosphates included in the plating medium TDYMP + Brine; open circles: the same plating medium without oligophosphates. Dashed line: limit of detection. Drawn lines: counts observed directly after heating. Hatched zones are covering the counts obtained after a period of incubation of 9 days at 37 °C).

phates: 5.86 compared to 6.2–6.3 in the other experiments, which probably negatively affected the heat resistance of the organisms.

In case of the absence of the oligophosphates in the heating medium (Table 2), the inclusion of these compounds in the plating medium did not show an inhibitory effect on unheated cells, but again dramatically reduced the counts of bacteria that survived heating. The inhibitory effect disappeared also here after growth of the surviving populations in the meat suspensions. Further, bacteria grew from fewer numbers of cells that survived (log counts of 2.77–2.95, observed after heating times up to 120 min), compared to the numbers of bacteria (log counts up to 3.50, obtained after 105 min heating) in suspensions containing oligophosphates.

Table 2
Growth of *E. faecium* (strain E_{20}) heated and thereafter incubated at 37 °C in meat suspension brine without oligophosphates

Plating medium	Tryptone Dextrose Yeast Meat Peptonised Milk Agar (TDYMP)			TDYMP + Brine			TDYP + Brine, oligophosphates omitted		
Period of incubation (days at 37 °C)	0	3	7	0	3	7	0	3	7
Heating (min 69 °C)									
0	4.73*	8.40	5.28	4.71	8.26	4.66	4.78	8.45	5.48
15	4.42*	8.38	5.78	2.84	8.34	5.00	4.58	8.40	7.04
30	4.38*	8.30	6.63	–[a]	8.26	6.18	4.00	8.34	6.64
45	4.08*	8.26	6.95	–	8.18	6.43	3.51	8.30	6.94
60	3.99*	8.18	(nr)[b]	–	8.08	(nr)	2.90	8.20	(nr)
75	3.72*	8.08	8.05	–	7.94	7.83	1.99	8.11	8.43
90	3.51*	7.99	(nr)	–	7.76	8.15	–	8.00	(nr)
105	2.95*	(nr)	7.95	–	7.57	7.26	–	(nr)	8.04
120	2.77*	7.72	7.72	–	7.51	6.48	–	7.90	7.75

Log counts (CFU. ml^{-1}) presented.

* $D_{69} = 63$ min.

[a] Below limit of detection (no colonies observed in duplicate plates containing 1 ml Meat Suspension Brine).

[b] (nr): No result.

Table 3
Growth of *E. faecium* (strain E_1) heated and thereafter incubated at 37 °C in meat suspension brine

Plating medium	Tryptone Dextrose Yeast Meat Peptonised Milk Agar (TDYMP)			TDYMP + Brine			TDYMP + Brine, oligophos-phates omitted		
Period of incubation (d at 37 °C)	0	3	9	0	3	9	0	3	9
Heating time (min 69 °C)									
0	4.84*	8.32	6.30	4.89	8.32	6.18	4.85	8.40	6.26
15	3.61*	8.20	6.20	1.60	8.30	6.00	2.71	8.32	6.00
30	3.30*	(nr)	7.52	–[a]	(nr)[b]	7.23	2.00	(nr)	7.40
45	2.52*	–	–	–	–	–	–	–	–
60	–	–	–	–	–	–	–	–	–
75	–	–	–	–	–	–	–	–	–

Log counts (CFU. ml^{-1}) presented.
*D_{69} = 21 min.
[a] See footnotes Table 2.
[b] See footnotes Table 2.

In actively growing cultures, the pH of the meat suspensions during incubation dropped to approximately 4.7 and 4.3 for the suspensions with and without oligophosphates, respectively.

Overall, these data (Tables 1–3) demonstrate that the inclusion of oligophosphates in the heating medium increased the heat-injury sustained by *E. faecium* cells, and in combination with rather severe heat treatment, even blocked the growth of surviving bacteria (number of about 3000 ml^{-1} for the most heat-resistant strain E_{20}) during the subsequent incubation at 37 °C. Shortly after heating, the growth of *E. faecium* was strongly reduced by the presence of oligophosphates in the culture medium; but, the inhibitory effect disappeared after growth of the surviving organisms upon incubation of the heating medium.

Polyphosphates perform three basic functions in foods: (a) they control pH by buffering, (b) they sequester metal ions, and (c) they act as polyanions to increase the ionic strength of solutions. Oligophosphates are very effective in sequestering heavy metal ions, such as iron and copper (Wagner, 1986). Probably, this effect is responsible for the inhibition of the recovery and growth of heat-injured cells observed here. The subsequent apparent loss of the inhibitory properties during incubation may be due to the gradual hydrolysis of oligophosphates (Li et al., 1993; Li et al., 2001), reducing their concentrations and liberating relatively inactive orthophosphate ions, thus making metal ions again available.

The observation that organisms surviving a relatively severe heating process in significant numbers (of thousands ml^{-1}) are unable to multiply in the heated system containing oligophosphates appears highly relevant to the manufacturing practice of pasteurized meat products. In conclusion, it is not always necessary to intensify the pasteurization processes with the aim of obtaining very low numbers of surviving enterococci. This may lead to improvement of the quality of product and significant energy savings. The results should be replicated under meat processing conditions.

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