PS5.07 Challenge test in pilot plant shows the inhibitory effect of organic acid against psychrotrophic Clostridium botulinum in low-salt meat products 439.00

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Abstract - For centuries, sodium chloride and sodium nitrite have been used to avoid growth of C. botulinum in meat products. Due to health aspects, an increasing interest has arisen to reduce the sodium content in meat products. The use of nitrite is also questioned by some consumers and authorities. This work shows the inhibitory effect of Na-lactate and Na-acetate in meat products with low salt content that were inoculated with C. botulinum, produced and packed in a pilot plant facility. The results show that addition of 2 % Na-lactate or 1 % Na-lactate + 0.25 % Na-acetate fails to inhibit growth of psychrotrophic C. botulinum in MA-packed meat products (pH 6.3 and 2 % NaCl/water) stored at 8 °C. However, the addition of 2 % Na-lactate or 1 % Na-lactate + 0.25 % Na-acetate to meat products stored at 5 °C inhibits the growth of C. botulinum for at least three weeks. After four weeks of storage at 5°C a slight increase in C. botulinum count and CO2 production in the inoculated samples were seen.

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Index Terms - challenge-test, Clostridium botulinum, lactate, meat products with low salt content, nitrite

I. INTRODUCTION

Psychrotrophic Clostridium botulinum is a spore forming, anaerobic pathogen capable of growth at temperatures above 3.0°C, salt concentrations below 5% (aqueous phase) and pH 5.0 [2]. The Food Standards Agency has elaborated guidance on production of raw and ready-to-eat vacuum packed or modified atmosphere (MA) packed chilled food to avoid growth and toxin formation of psychrotrophic C. botulinum during the shelf-life of the products. The guidance recommends heating to

90 °C for 10 minutes, pH <5, salt % >3.5 (aqueous phase) or another combination of heat treatment and preservative factors to prevent growth and toxin production by psychrotrophic C. botulinum [1]. For centuries, salt and nitrite have been used to avoid growth of C. botulinum in meat products. Due to health aspects, an increasing interest has arisen to reduce the sodium content in meat products. The use of nitrite is also questioned by some consumers and authorities. For this reason, it is necessary to document how NaCl can be reduced and nitrite omitted from meat products without affecting the safety in relation to growth of different pathogens. The aim of this work was to investigate the antimicrobial effect of sodium lactate and sodium acetate against growth of psychrotrophic C. botulinum strains in MA-packed meat products produced in a pilot plant facility thus mimicking real production conditions.

II. MATERIALS AND METHODS

A. Bacterial strains and spore suspensions The C. botulinum strains used were: C. botulinum DMRICC 3760, 3778, 3779 and 3780. 16S rDNA analyses have shown that the strains have 99.7-99.9 % homology to C. botulinum type B, E or F but all strains have shown to be toxin negative in the mouse assay. The strains were maintained as frozen stocks at -80°C. The frozen stock was transferred into Tryptone Peptone Glucose Yeast extracts. The inoculum was incubated at 30°C for 3 days, followed by spore production in a two-phase cooked meat model system as described in [3]. B. Meat products and inoculation The pork sausages were produced as previously described [4]. The pork batters had the following composition: pork shoulder, 70 % (w/w); breadcrumbs, 7 % (w/w); ice-water, 18.6 % (w/w); wheat flour, 2 % (w/w); caseinate EM6, 1.2 % (w/w); and sodium chloride, 1.2 % (w/w). To some batches of meat batters were added 1.67 % or 3.33 % (w/w) Purac SSP60 (Na-lactate content 58-61 %) (Purac, The Nederlands) or Na-acetat 0.25 % (w/w)

or Purac SSP60, 1.65 % and Na-acetat, 0.25 %. All additives were from SFK (Avedøre, Denmark). A four-strain cocktail of C. botulinum was used to inoculate the meat batter (9 ml per 3 kg) before it was stuffed in casings (diameter 92 mm) and pasteurized to a core temperature of 75 °C within 2.5 hours and cooled to 2 °C within 5.5 hours. Five different meat products containing different amounts of preserving factors were produced (Table 1).

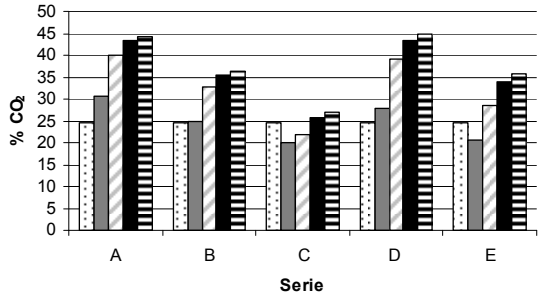
	Meat Sausage				
	A	B	C	D	E
pH	6.2	6.3	6.3	6.2	6.3
Salt %	1.3	1.3	1.3	1.3	1.3
Na-lactate %	0.5	1.5	2.4	0.5	1.4
Na-acetate %	0	0	0	0.12	0.15
Water %	66	67	67	66	67

C. Slicing, packaging and storage The meat sausages were sliced into 6 mm slices and packed in an atmosphere of 30 % CO2 and 70 % N2. The package material was NEP and had a OTR of 1.3 ml/m2 (24h, 23°C, 1 atm, 50% RH) and a CO2TR of approx 5.2 ml/m2 (24h, 23°C, 1 atm, 50% RH). The packages were stored at 5 °C or 8 °C for as long as four weeks. D. Gas and microbiological analyses The concentration of O2 and CO2 was measured before the packages were opened. A CheckMate 9900 CO2/O2 gas analyzer (PBI Dansensor, Denmark) was used for the analyses. Serial dilutions were made and the C. botulinum count was determined using Reinforced Clostridium Medium (RCM) (Merck 1.05410.0500) and modified Shahidi Ferguson Perfringens agar (mod. SFP-agar) consisting of Perfringens agar base (Oxoid CM0587) containing 5 % Oxoid Egg Yolk Emulsion (SR0047) but without antibiotics. The agar plates were incubated in an anaerobic bench at 30 °C for 3 days. Lactic acid bacteria were measured on APT agar (Merck 1.10453.0500) and incubated at 20 °C for 5 days to verify if the samples had been contaminated during packaging. During storage, the concentration of O2 and CO2 and the number of culturable C. botulinum and lactic acid bacteria were measured each week.

III. RESULTS AND DISCUSSION

Figure 1 shows the increasing CO2 concentration during storage of meat products with a low salt content.

8°C



5°C

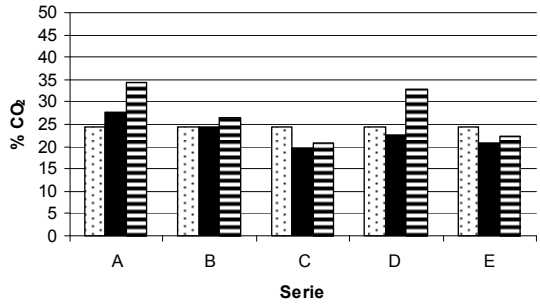


Figure 1. Changes in the CO_2 concentration in MA-packed (30% CO_2, 70% N_2) meat sausages (pH 6.2; 2% NaCl/water) inoculated with *Clostridium botulinum*. A) Control, B) 1% Na-lactate, C) 2% Na-lactate, D) 0.25% Na-acetat, E) 1% Na-lactate+0.25% Na-acetate. Each column is a mean of two packages.

day 0, day 7, day 14, day 21, day 28.

The increase is much more pronounced at 8 °C compared with 5 °C. The analyses also show that O2 remained low, approx. 0 %, throughout storage (results not shown) indicating no leakers among the packages. An increase in CO2 content indicates growth of either hetero fermentative lactic acid bacteria or growth of psychrotrophic C. botulinum. The microbiological analyses showed no growth of lactic acid bacteria in the packages stored at 5 °C and 8 °C (results not shown). Thus an increase in CO2 is most probably related to growth of the C. botulinum added. The gas analysis (figure 1) shows an increase in CO2 in all samples during four weeks of storage at 8 °C and the microbiological analysis (figure 2) shows an increase in the number of C. botulinum during storage. The increase in

CO2 is most pronounced in meat products containing only NaCl. The more Na-lactate the meat product contains, the less the increase in CO2 during storage. The data show that addition of 2 % Na-lactate or 1 % Na-lactate + 0.25 % Na-acetate fails to inhibit the growth of C. botulinum in a meat product with pH 6.3 and approx. 2 % NaCl in the aqueous phase when stored at 8°C. In samples stored at 5 °C, an increase in CO2 was found in products without organic acid (A) and in products containing 1 % Na-lactate (B) or 0.25 % Na-acetat (D) indicating growth in these samples. In products containing 2 % Na-lactate (C) or 1 % Na-lactate + 0.25 % Na-acetate (E), no increase in CO2 was observed after three weeks of storage, but after four weeks of storage there might be a slight increase in CO2. The microbiological analyses showed marginal growth, less than 0.5 log after four weeks (results not shown). In figure 2, the number of culturable C. botulinum during storage at 8 °C is shown. The results show that the number increases to a maximum and then it remains almost steady at that number during four weeks of storage for the meat sausages containing only NaCl (A). In the other products containing different concentrations of Na-lactate and Na-acetate (B, C, D, E), an increase in colony count is observed during the first one or two weeks of storage. Then there is a decrease in the number after three weeks of storage. At the same time, the CO2 concentration still increases (figure 1) showing microbial activity. These results illustrate how difficult it is to measure growth of C. botulinum using traditional microbial analysis and how important it is to make complementary tests on growth and metabolic activity.

IV. CONCLUSION

This work showed that the growth of psychrotrophic C. botulinum was affected by Na-lactate in a MA-packed meat product with pH 6.3 and a low salt content. However, the concentrations tested in this work were too low to avoid growth during storage at 8 °C. At the low temperature (5 °C) Na-lactate might be an alternative to nitrite, as growth was delayed for at least three weeks. However, more experiments are needed to verify these observations.

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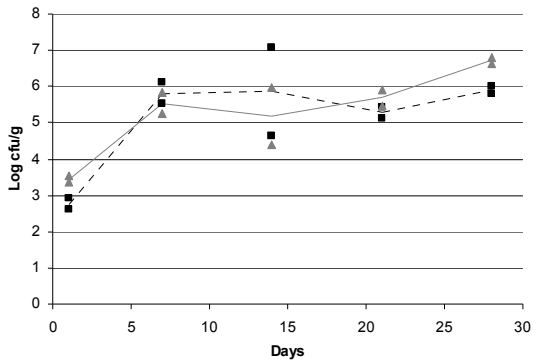
A)



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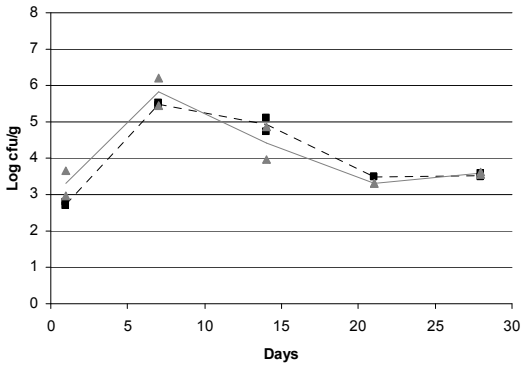

C)

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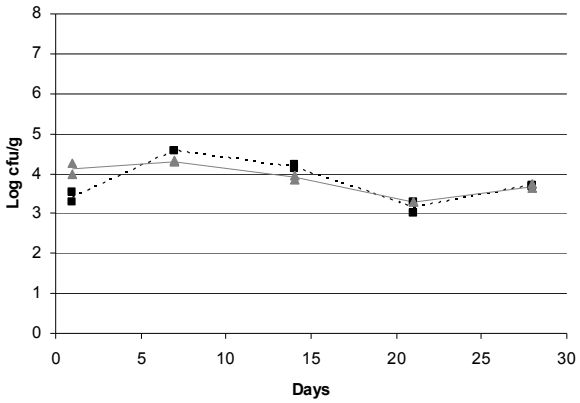

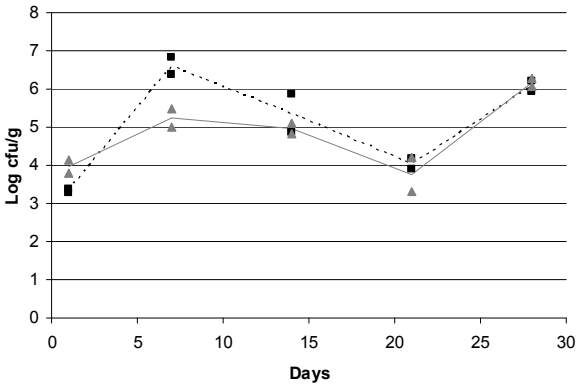

E)

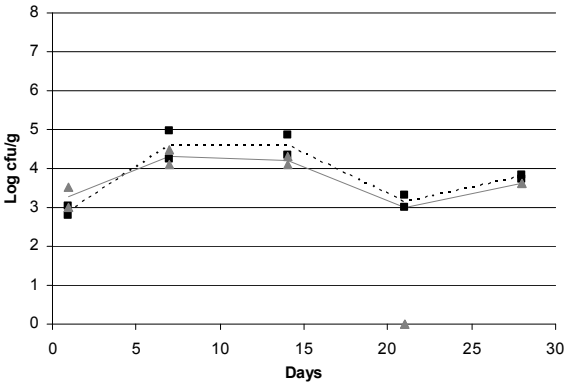

Figure 2. Growth of psychrotrophic *Clostridium botulinum* in meat sausages (pH 6.2; 2 % NaCl/water) stored in modified atmosphere (30 % CO_2, 70 % N_2) at 8°C. A) Control, B) 1 % Na-lactate, C) 2 % Na-lactate, D) 0.25 % Na-acetate, E) 1 % Na-lactate+0.25 % Na-acetate. ■) counts on RCM-agar, ▲) counts on modified SFP-agar. - - -) mean on RCM (n=2), ——) mean on modified SFP-agar (n=2).