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THE RELATION BETWEEN WATER ACTIVITY AND MICROBIAL SPOILAGE IN MEAT PRODUCT MODELS

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SUMMARY

Inhibition caused by potassium chloride was similar to that of sodium chloride at otherwise optimal conditions. Divalent metal chlorides ,might,however,show a stronger inhibition, and so might potassium chloride at suboptimal temperature or pH.The results indicated that substituting an organoleptically acceptable part of NaCl with KCl in meat products would be possible. This would be necessary if one cannot maintain a low temperature throughout the storage period of vacuum-packed,cooked,cured meat products. The results showed that at low temperature the organoleptic quality of a Bologna-type sausage was slightly influenced by a flora shift depending on salt concentration.At higher temperature (5 and 10^{o}C),however, rapid growth of especially Gram negative bacteria may result in a fast deterioration of the product. Thus, temperature control is essential.
The minimum water activity of a number of bacteria was shown to be dependent on the type of chloride salt.Divalent cations inhibited at much higher water activity (a_w) values than monovalent cations. The minimum a_w controlled with KCl was generally similar to NaCl.

INTRODUCTION

The objective of the study was to determine the influence of salts (metal chlorides) on bacterial development.Previous studies were done by replacing part or all of NaCl in model systems i.e. broth culture and meat emulsion experiments (Nielsen and Zeuthen 1987) and in Bologna-type sausage produced under commercial conditions (Nielsen and Zeuthen 1986).The model studies were carried out at fixed temperature and pH, and at a concentration resembling the amount of NaCl used in commercial products. In this study a few organisms were examined at several pH values and temperatures. Further, the minimum a_w for growth at optimal temperature as influenced by type of chloride salt was examined.
For several years there has been a demand for meat products produced with reduced amounts of salt. As NaCl cannot totally be replaced with other chloride salts , primarily KCl , studies must be done on products with reduced NaCl concentration without substitution. Thus, microbial development in three batches of Bologna-type sausage with different salt levels was examined.

MATERIALS AND METHODS

Microbial development of Staphylococcus aureus,Brochothrix thermosphacta, Salmonella enteritidis and Bacillus cereus was followed using OD measurements at 450 nm of broth cultures. The broth media consisted of 1 g tryptone and 0.6 g yeast extract per 100 g water. The levels of salts were (mol/kg water), NaCl 0.68 , KCL 0.70 or $MgCl_2$ 0.45. The a_w values of these models were 0.974-0.975, as determined on Novasina Equipment (Novasina AG,Switzerland). The instrument was calibrated using saturated salt solutions. pH was controlled with 1 N HCl. Growth at minimum a_w was followed using the same medium with addition of NaCl, KCl, $MgCl_2$ or $CaCl_2$ (g/100g water). pH was controlled with 1N HCl and NaOH. Water activities were measured using the Novasina equipment.Broth cultures were inoculated with typically 50μl in 50 ml broth,using an organism in logaritmic growth.Concentrations c. 10^4-5 x 10^4 /ml. Cultures were incubated at temperatures appropriate for the organism.Growt was determined visually , and by spreading on plat count agar. Bacteria used were B.thermosphacta,Ser ratia liquefaciens,B.cereus, S.aureus, Yersinia ent rocolitica and Salmonella typhimurium.
The experiment with Bologna-type sausage was done using three batches of sausage with carefully controlled addition of water and sodium chloride. The intended salt concentrations in the water phase wer 4 , 5 and 6 %. A standard recipe was used in producing the sausages (with an added amount of sodium nitrite of 60 ppm).
Sausages were pasteurized in a steam cabinet to a center temperature of 70^{o}C. After cooling, the sausa ges were sliced at a commercial manufacturer and vacuum-packed using a film with a O_2 permeability of 52 cm^3 m^{-2} 24hr 1 atm at 75% RH and 25^{o}C.Packages wer stored at 2, 5 and 10^{o}C, and examined for total coun on plate count agar ; lactics on nitrite actidione polymycin agar (Davidson and Cronin 1973); B.thermosphacta on streptomycin sulphate thallous acetate actidione agar (Gardner 1966); Gram negative bacteri on desoxycholate hydrogen sulfide lactose agar (Merc and yeast on all purpose medium with tween (Difco) with 100 ppm oxytetracycline added. The content of the packages were also examined organoleptically, by a taste panel evaluating odour and overall acceptabi lity.Moisture (Anon. 1955) and chloride content (Ano 1974)was determined on fresh sausages.

RESULTS AND DISCUSSION

Staphylococcus aureus grew equally well with NaCl,KCl and $MgCl_2$ at pH 6.0 and 5.5 at 37^{o}C (Fig.1) . Reducing the incubation temperature had little influence on growth, but at 10^{o}C, a temperature more appropriate for refrigerated meat products, growth was somewhat inhibited with KCl and even more with $MgCl_2$.
B.thermosphacta grew irrespective of chloride salt at pH 6.0 (and 6.3,not shown) at 2^{o}C.However, reducing pH to 5.5, inhibited growth in a manner similar to S.aureus (Fig.2).
Salmonella enteritidis (not shown) developed equally well with NaCl and KCl at 20^{o}C (pH 6.0 and 5.5) . At 10^{o}C, there was a small inhibition due to KCl, as was found with other bacteria. No growth was observed at any temperature with $MgCl_2$. Bacillus cereus (not shown) did not develop with $MgCl_2$. At 20^{o}C, but not at 8^{o}C, the bacterium grew at a pH of 6.0 with NaCl and, after a longer lag period with KCl as well.
The OD measurements clearly show that although chloride salts and pH values may not have any influence on bacterial growth at optimum temperature, reducing the storage temperature make the combination of inhibiting factors more effective,i.e. there is a stronger inhibition when NaCl is replaced with other metal chlorides or if pH is reduced at suboptimal temperatures. It has been shown in other studies that sodium replacement may have an effect at refrigerated temperatures (Nielsen and Zeuthen 1987).
The study on minimum a_w for growth did not show any difference between NaCl and KCl, the minimum a_w allowing growth was similar for the two salts, for all the 30 different bacterial strains tested. Results from other studies vary, growth of B.stearothermophilus took place equally well with NaCl and KCl (Anagnostopoulos and Sidhu 1981). but Clostridium perfringens grew at lower a_w values with NaCl than with KCl (Bartsch and Walker 1982).Minimum a_w for growth with the divalent cations was far higher than for the monovalent cations (Table 1). This is consistent with the inhibition observed at low levels in model systems (Nielsen and Zeuthen 1987) at refrigerated and moderately abusive temperatures.However, it does not seem possible to rely on bacterial inhibition through the presence of divalent metal chlorides, both for technological and organoleptical reasons.Some bacteria are inhibited at very low levels,which could be used in

a metal chloride salt mixture (Nielsen and Zeuthen 1987). It is obvious from the table, that bacteria which grow at high salt concentrations of monovalent metalions will also grow at relatively high concentrations of the divalent metalions (S.aureus, B.thermosphacta), reflecting a capability of developing at lower a_w irrespective of the a_w reducing compound. Although B.thermosphacta grew at a salt/water ratio of 7.6 % (a_w 0.950), this is surprisingly high this bacterium is generally considered fairly salt resistant. However, 9 strains were tested with more or less the same result. The minimum a_w for growth of S.aureus corresponds with other studies (Noterman and Heuvelman 1983), and this is also the case with S.typhimurium which grew at a a_w of 0.951 , which is a value similar to B.thermosphacta. Yersinia enterocolitica grew only at a_w 0.962 under aerobic conditions. This is similar to results obtained with vacuum-packed Bologna, where no growth was observed at a concentration of 1.1m NaCl at 2-12°C (Nielsen and Zeuthen 1985) or at 7 % NaCl in broth cultures (Stern et al. 1980). The Gram negative spoilage bacterium S.liquefaciens grew at low a_w values controlled by NaCl or KCl. This organism is observed fairly often in cooked, cured meat products.

In the meat product models, the sodium chloride concentration comprised 2.2 %, 3.0 % and 3.8 % in the three batches, corresponding to salt/water ratios of 3.8 % ; 5.1 % and 6.4 % respectively. These salt levels correspond to water activities of 0.98 ;0.97 and 0.963. Instead of giving the 45 growth curves which comprise the results of this experiment, a few curves of logarithm of number of days to a specific microbial number (10^6/g) at 2 , 5 and 10°C are shown. Initially total numbers, consisting mostly of micrococci, B.thermosphacta and yeast , were quite low, c. 10^2/g. Fig. 3 shows that the time to 1 mill/g of total aerobic bacteria varied little with salt level. This happened rather quickly at 10°C, but very slowly at 2°C. Lactic acid bacteria were highly affected by salt and temperature. 1 mill/g was reached after increasing storage time with increasing salt concentration. The effect was highly influenced by storage temperature. Brochothrix thermosphacta developed to high numbers (above 10^6/g) in all series. The curves for salt/water ratios 3.8 and 6.4 are parallel (Fig.5), i.e. increasing salt concentration has the same effect at all temperatures. At 5°C, the curve has a different slope, the numbers are probably influenced by other bacteria. Gram negative bacteria did not develop at a salt/water ratio of 6.4 at any temperature; at lower salt concentrations growth to high numbers happened at 5 and 10°C, but not at 2°C. Only at a salt/water ratio of 3.8, did the Gram negative bacteria grow to high levels at all temperatures (Fig.6). The yeast grew in all series to levels of 10^{4-5}/g; they were only slightly affected by salt concentration.

Numbers of days during storage to a definite decrease in odour scores were highly influenced by salt at high temperature, but completely unaffected by salt at 2°C (Fig.7).

The total aerobic count was not related to salt concentration. However, total counts covers a range of bacterial groups, some of which dominated at low, others at high a_w values. At high salt content, the dominating group was micrococci, especially at salt/water ratio of 6.4 at all temperatures, but also at a salt/water ratio of 5.1, especially at 2 and 5°C during the first 6 weeks. At a salt/water ratio of 3.8, B.thermosphacta grew rapidly and dominated the flora within a week at 2 and 5°C. However, they were later overgrown by the Gram negative bacteria (after c. 3 weeks storage). At 10°C, growth of Gram negative bacteria was abundent. These bacteria dominated the flora after 1 1/2 weeks storage. The packages were stored for 1 1/2 to 3 month depending on salt concentration and temperature. The lactics also grew to quite high numbers, but in most cases they did not dominate the flora. The figures indicates why. At high salt concentration, and to a lesser extent also at intermediate concentration, micrococci developed fast and dominated the flora. At lower and intermediate salt content B.thermosphacta grew rapidly and dominated for some time. Afterwards the batches were influenced by rapid growth of the Gram negative bacteria, especially at salt/water ratio of 3.8 and at 10°C also at salt/water ratio of 5.1 .

Although the composition of the total flora varied with salt concentrations at 2°C , this had only small influence on the organoleptic quality, probably because the microbial activity was slow at this temperature, irrespective of the flora.

CONCLUSION

The model experiments showed that replacing NaCl with other metal chlorides may be beneficial at otherwise suboptimal conditions, but replacing NaCl especially with KCl is without effect at otherwise optimal conditions. This is also the case with minimum a_w values for growth; replacing with KCl at optimum temperature gives similar results, while the divalent cations inhibit growth at high a_w. This and other studies have shown that substituting NaCl with KCl generally has little effect on growth. Reducing NaCl levels in vacuum-packed cooked, cured meat products may highly affect microbial growth and quality of the product. Especially at high temperature (10°C) organoleptic quality will rapidly decrease when Gram negative bacteria are present. Keeping a low temperature throughout the storage period (2°C), will not inhibit growth of these bacteria or B.thermosphacta, but this does not seem to have any great effect on the organoleptic quality. Thus, reducing the salt concentration is acceptable only when maintaining a low storage temperature.

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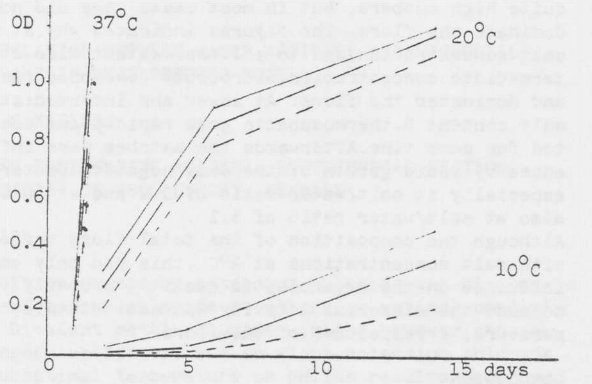


Fig.1. Growth of S.aureus (a_w 0.974) with NaCl (——); KCl (— —) and $MgCl_2$ (- -). pH 6.0: ——, 5.5: —•—.

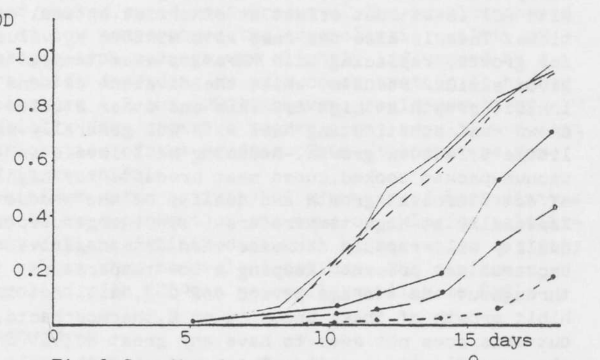
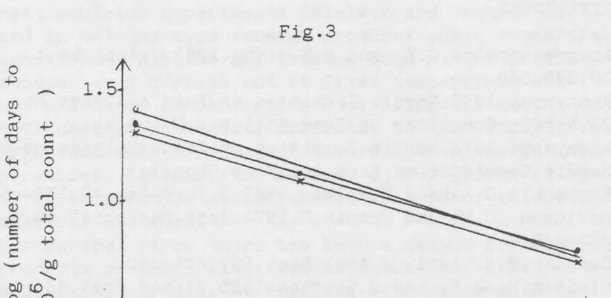


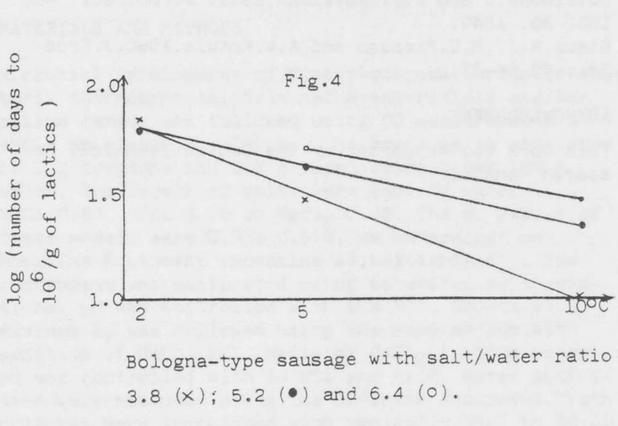
Fig.2. Growth of B.thermosphacta at 2°C (a_w 0.974) NaCl (——); KCl (— —) and $MgCl_2$ (- - -). pH 6.0: ——. 5.5: —•—.

Fig.3



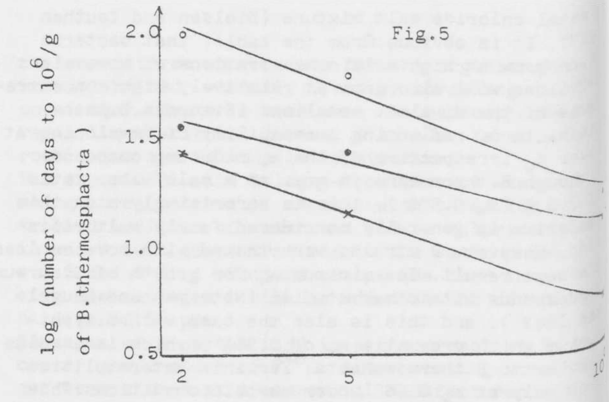
Bologna-type sausage with salt/water ratio: 3.8 (×), 5.1 (•) and 6.4 (○)

Fig.4



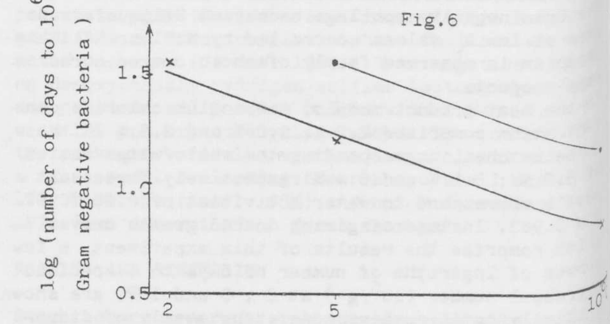
Bologna-type sausage with salt/water ratio: 3.8 (×); 5.2 (•) and 6.4 (○).

Fig.5



Bologna-type sausage with salt/water ratio: 3.8 (×); 5.1 (•) and 6.4 (○).

Fig.6



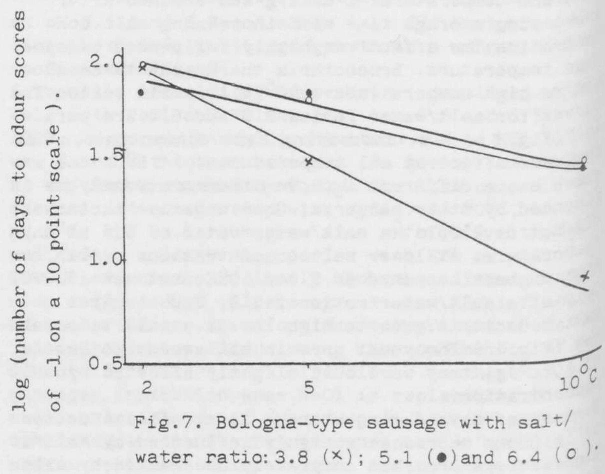


Fig.7. Bologna-type sausage with salt/water ratio: 3.8 (×); 5.1 (•) and 6.4 (○).

Table 1. water activities controlling development of bacteria as influenced by chloride salt.

chloride salt	NaCl	KCl	$MgCl_2$	$CaCl_2$
growth	+ /	+ / -	+ / -	+ / -
S.liquefaciens[1]	0.949/0.946	0.949/0.946	0.991/0.982	0.989/0.982
B.thermosphacta[2]	0.951/0.948	0.952/0.947	0.977/0.966	0.974/0.967
B.thermosphacta[3]	0.948/0.944	0.949/0.943	0.966/0.960	ND
Y.enterocolitica[4]	0.962/0.957*	0.961/0.957*	0.984/0.978	0.989/0.980
B.cereus[5]	0.959/0.953	0.959/0.952	0.990/0.982	0.992/0.981
S.aureus[6]	0.867/0.858*	0.868/0.857*	0.949/ND	0.967/ND
S.aureus[7]	0.875/0.867*	0.876/0.869*	0.960/ND	0.966/0.960
S.typhimurium[8]	0.950/0.947	0.953/0.946	0.984/0.979	0.989/0.984

[1] 1 strain; [2] 5 strains ; [3] 4 strains including ATCC 11509; [4] 8 strains including ATCC 27709 ; [5] 5 strains ; [6] 1 strain; [7] 4 strains; [8] 2 strains. ND not done. * aerobic incubation.