Observations on the potantial for sodium substitutes, i.e. other metal chlorides and potassium sorbate for controlling microbial growth.

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Introduction

Sodium chloride has proven an important preservative in processed meat products.However, sodium chloride is also the most important contributer of sodium in cured meat products. Excess sodium intake has been related to high blood pressure in sensitive groups of the human population (Anon,1980).Therefore, there has been a demand for a reduction of sodium content in meat products and other food groups in order to facilitate a decrease in sodium intake. Previous studies have primarily been concerned with technological problems related to sodium replacement (see Terrell, 1983).Furthermore, the microbiological investigations ahve primarily focused on replacement based on equal ionic strength and without control of pH. Broth culture studies with Bacillus stearothermophilus did not show any influence of KCl compared to NaCl(Anagnostopoulos and Sidhu,1981) ,but Clostridium perfringens grew at lower water activity (a_w) in the presence of NaCl than with KCl (Bartsch and Walker,1982). Similarly, growth of Clostridium botulinum was differently affected by NaCl,KCl and $MgCl_2$ (Wagner and Busta,1985). Replacement of NaCl with KCl,$MgCl_2$ and $CaCl_2$, substitution based on equal ionic strength, did not affect numbers of aerobic bacteria in experimental sausages in the absence of nitrite (Terrell et al.,1982). In a meat model system,Lactobacillus plantarum was strongly influenced by a mixture of NaCl and KCl compared to pure NaCl (Raccach and Planck,1985).

Although factors like pH and a_w were not controlled,several experiments have shown that some variation in the inhibitory properties among the different metal chlorides exist.The present study was done to further determine differences in inhibitory activities between NaCl,KCl,$MgCl_2$ and $CaCl_2$ in model systems keeping a_w and pH constant. Further to investigate the effect of sodium replacement with potassium in a Bologna-type sausage and to follow deterioration both microbiologically and organoleptically. Studies have been made on the effect of sodium nitrite reduction in cured meat products together with incorporation of potassium sorbate (see Sofos and Busta, 1983). This has proven a reliable substitute. We wanted to examine, in model systems, if a substitution of part of the sodium in NaCl with potassium sorbate is microbiologically acceptable.

Materials and methods

Meat model studies were made using a 1:1 mixture of minced pork and veal meat with a water content of ca. 62% and a fat content of ca. 19 %. The salts were added on the basis of a a_w equivalent to that of 4 g NaCl/100 g water.The mixed salt system of NaCl/KCl, NACl/$MgCl_2$ and NaCl/$CaCl_2$ were made by adding only 2 g NaCl/100g water. The rest of the a_w reduction was then accomplished by adding appropriate amounts of the other metal chlorides, calculated using tables by Robinson and Stokes (1965).As pH in the meat emulsion containing $MgCl_2$ and $CaCl_2$ was lower than with the other salts, 1N NaOH was used in controlling pH.The meat slurries were divided into sterile glas flasks (50g/flask), and pasteurized in a heating cabinet at 75°C for 30 min, cooled and subsequently inoculated. Meat emulsions in experiments with potassium sorbate were made using 4 g NaCl/100 g water as control, and mixtures of 3 g NaCl/100g water (25 % reduction) plus 0.1 % or 0.26 % potassium sorbate, the latter emulsions resulting in a higher a_w than the control.

The meat emulsion experiment with spices was made by adding a Bologna-type mixture of spices and dried onion in concentrations used in the Bologna sausage. After mixing, the meat emulsion was pasteurized,during which the vegetative bacteria died and spores were heat activated.

For experiments with Bologna-type sausages, two batches of sausage were produced according to a standard recipe. The sausages were made with 2.2 % NaCl or with 1.2 % NaCl plus 1.4 % KCl, the mixture resulting in the same a_w as pure NaCl. Sausages were pasteurized to a center temperature of 70°C, cooled,sliced, inoculated with the relevant bacteria and vacuum-packed.

Bacteria used were Brochothrix thermosphacta,Serratia liquefaciens,Lactobacillus sp.,Bacillus cereus and Yersinia enterocolitica (serotype 03).Appropriate incubation temperatures relevant to refrigeration and the different bacteria were used.

During storage duplicate samples were investigated using plate-count-agar (Difco).Lactobacilli were plated onto all-purpose-medium-with-tween (Difco) .Plates were incubated at 25°C ,except the samples with spices which were incubated at 30°C.

Water content and pH were measured on meat emulsions and Bologna-type sausage.Water activity was determined using Novasina equipment calibrated at 25°C.

Sensory analyses were done using a trained panel by evaluating odor and overall appearance (not shown).An eleven point scale was used (-5 extremely bad, 0 neither good nor bad, +5 ideal).

Statistical analyses were done on mean values of log_{10} nubmers of bacteria and mean odor scores.

Results

Water activity of meat emulsions with chloride salt were 0.972-0.973, and that of emulsion with potassium sorbate 0.978. Water activity of Bologna-type sausage was 0.961-0.962. pH in meat emulsions were 6.1-6.2 and in Bologna sausage 6.2-6.4 .

The combination of reduced levels of NaCl plus potassium sorbate is a better preservative than pure NaCl in the presence of B.thermosphacta (Table 1). Inhibition is influenced by both sorbate concentration and incubation temperature. Serratia liquefaciens grows equally well in emulsion with 4 % NACl and with 3 % NaCl plus 0.1 % sorbate (Table 2), but is completely inhibited when sorbate concentration is increased to 0.26 %.

Development of Lactobacillus is similar in emulsion with pure NaCl and in mixed NaCl and sorbate (Table 3).

The emulsion with spices and onion was incubated at moderately abusive temperature (12°C) (Table 4). Sorbate addition strongly inhibits spore development at the 0.26 % level during 17 days, while numbers are higher after 17 days with only 0.1 % , but significantly lower than with pure NaCl.

Results with chloride salts in meat emulsion show that S.liquefaciens grows equally well in pure NaCl and in mixed NaCl and KCl (Table 5), while $MgCl_2$ addition strongly inhibits growth, and numbers decreases in the presence of $CaCl_2$. The B.thermosphacta develops in the presence of all four chloride salts (Table 6), with no significantly different numbers during storage. While KCl and $MgCl_2$ does not influence growth of Lactobacillus lower numbers are observed with $CaCl_2$,although the bacterium also grows with this salt (Table 7).

The experiments with Bologna-type sausage shows that growth of B.thermosphacta is not influenced by potassium addition and neither is the organoleptic deterioration significantly different in the two series (Table 8). Growth of S.liquefaciens is also equally well controlled by pure NaCl and mixed NaCl and KCl (Table 9).

The odor assessments show that development of spoilage is similar in the two series. Numbers of Lactobacilli are slightly lower during part of the storage period in mixed salt batch,however , after 14 days, numbers in both series are above 1 mill/g (Table 10). Spoilage develops equally fast in the two series. Similar results are obtained for the three bacteria in overall assessments of packages (not shown).
The B.cereus ,which does not grow at 5^oC and only very slowly at 8^oC,develops at moderately abusive temperature of 12^oC, and during most of the storage period numbers in the two series are not significantly different (Table 11). Results with the pathogenic bacterium Y.enterocolitica, show that numbers during logarithmic growth generally are not significantly different in the two series, although maximum numbers in the series with KCl , at 5^oC, are slightly lower than with pure NaCl (Table 12).

Discussion and conclusion

The study showed that the bacteria tested were differently affected by the salt used. Partial replacement of NaCl with potassium sorbate, showed that this has a positive effect when controlling growth of S.liquefaciens and B.thermosphacta, both important spoilage bacteria in vacuum-packed cured,cooked meat products (Nielsen 1982). Results for B.thermosphacta showed that the effect is dependent of concentration and storage temperature, but at the recommended storage temperature of 5^oC, the mixture of 2.3 % NaCl and 0.1 % sorbate is equally effective as 3 % NaCl .The Bacilli spores developing due to the addition of spices and dried onion are effectively controlled by 0.26 % sorbate, and also 0.1 % has an inhibitive effect. The inhibition of Lactobacillus was less than for the other bacteria, although the mixture was equally good as pure NaCl. This is in accordance with other experiments showing that sorbate addition could be used in selective media for lactic acid bacteria (Emard and Vaughn,1952),although it is not always satisfactorily (Reuter, 1985). The addition of sorbate proved effective, even at the relativey high pH in the meat emulsion,and even though the a_w of the sorbate emulsion was higher than with pure NaCl. Considering the problems of getting decontaminated spices, sorbate had a positive effect on Bacilli which are important bacteria in vacuum-packed meat products stored at moderately abusive temperatures. The stronger inhibition of B.thermosphacta and S.liquefaciens compared to Lactobacillus may prove beneficiel, considering that these former bacteria result in spoilage at lower numbers than lactobacilli (Egan et al 1980,Nielsen 1982).
Calcium chloride was the most inhibitive salt in mixed salt emulsion looking at Lactobacillus and S.liquefaciens However, B.thermosphacta is not influenced, and therefore $CaCl_2$ does not seem to be a good substitute. Further, this salt is organoleptically unacceptable (unpublished results). The same is the case for $MgCl_2$ (Hand et al 1982), which was a good substitute for NaCl, not resulting in increased growth. Replacement with KCl showed that development was similar as with pure NaCl, and this is in accordance with results by Terrell et al (1982 and 1983) and Anagnostopoulos and Sidhu (1981), but not with the results by Bartsch and Walker (1982) studying broth cultures of C.perfringens , and Koenig and Marth (1982) studying S.aureus in Chedder cheese. The meat emulsion studies, combined with the knowledge about the organoleptic properties of the different chloride salts led to the experiments with only NaCl and KCl in a Bologna-type sausage . In this product, produced under commercial conditions, it was shown that there was no negative effect of substituting part of NaCl with KCl on the basis of equal a_w. Although there was not a stronger inhibition by KCl addition, the results showed that replacement with KCl is not hazardous, whether considering spoilage or development of the pathogenic bacteria B.cereus, which grows at slightly abusive temperatures, and Y.enterocolitica ,which grows at refrigeration temperatures. Studies with other strains of these bacteria at similar and other temperatures,has also shown that KCl is a reliable substitute in vacuum-packed Bologna sausage (Nielsen and Zeuthen, 1986).

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Table 1. Growth of Brochothrix thermosphacta in meat emulsion with NaCl and potassium sorbate

NaCl %	3.1		2.3		2.3	
sorbate %	0.0		0.10		0.26	
°C	2	5	2	5	2	5
days						
0	2.20	2.20	2.20	2.20	2.20	2.20
4	-	4.97[a]	3.41[b]	3.94[b]	1.93[c]	2.27[c]
5		6.59[a]	-	-	2.18[b]	2.90[c]
7	6.01[a]	7.74[b]	3.41[c]	4.23[d]	2.32[e]	3.25[c]
8	6.47[a]	-	-	-	-	4.12[b]
10	-	-	4.01[a]	5.32[b]	-	4.49[a]
11	-		4.42[a]	5.77[b]	2.85[c]	4.35[a]
14	7.52[a]		4.92[b]	5.81[c]	4.33[b]	4.74[b]
17	8.21[a]		5.55[b]	7.34[c]	5.15[b]	6.54[d]
22	8.31[a]		-	-	6.85[b]	-
27	9.35		8.62	-	8.73	-

[a] means (log_{10} cfu/g) in the same row with different with superscript are significantly different(P<0.05)

Table 2. Growth of Serratia liquefaciens in meat emulsion with NaCl and potassium sorbate

NaCl %	3.1	2.3	2.3
sorbate %	0.0	0.10	0.26
°C		5	
days			
0	3.12	3.12	3.12
2	3.40	-	3.04
6	4.37[a]	3.58[b]	2.86[c]
8	5.55[a]	-	2.83[b]
10	7.00[a]	7.18[a]	2.90[b]
11	-	7.30	-

[a] see Table 1

Table 3. Growth of Lactobacillus in meat emulsion with NaCl and potassium sorbate

NaCl %	3.1	2.3
sorbate %	0.0	0.26
°C	10	
days		
0	3.27	3.27
5	4.01	4.10
9	4.92	5.33
13	6.64	6.20
19	6.50	6.52

Table 4. Growth of sporogenic bacteria from spices and dried onion in meat emulsion with NaCl and potassium sorbate

NaCl %	3.1	2.3	2.3
sorbate %	0.0	0.10	0.26
°C		12	
days			
0	2.32	2.32	2.32
3	2.66[a]	1.58[b]	1.76[b]
7	6.16[a]	1.37[b]	1.31[b]
9	7.77[a]	1.93[b]	1.59[b]
17	9.17[a]	4.80[b]	1.22[c]

[a] see Table 1

Table 5. Growth of Serratia liquefaciens in meat emulsion with different chloride salts

Cl-salt	NaCl	NaCl/KCl	$NaCl/MgCl_2$	$NaCl/CaCl_2$
%	3.1	1.6 /2.1	1.6 /1.8	1.6 /2.1
°C			5	
days				
0	1.90	1.90	1.90	1.90
3	2.40	2.36	2.10	-
5	2.78[a]	2.61[a]	2.02[b]	1.58[b]
10	7.61[a]	7.34[a]	2.23[b]	1.23[c]
12	7.73[a]	7.41[a]	2.04[b]	1.08[c]

[a] means (log_{10} cfu/g) in the same row with different superscript are significantly different (P<0.05)

Table 6. Growth of Brochothrix thermosphacta in meat emulsion with different chloride salts

Cl-salt	NaCl	NaCl/KCl	$NaCl/MgCl_2$	$NaCl/CaCl_2$
%	3.1	1.6 /2.1	1.6 /1.8	1.6 /2.1
°C		5		
days				
0	2.95[a]	2.95	2.95	2.95
1	3.25	3.62	3.42	3.29
3	5.94	6.29	6.24	6.19
6	8.12	8.28	8.52	8.12
8	8.42	8.48	8.58	7.95

[a] means (log_{10} cfu/g)

Table 7. Growth of Lactobacillus in meat emulsion with different chloride salts

Cl-salt	NaCl	NaCl/KCl	$NaCl/MgCl_2$	$NaCl/CaCl_2$
%	3.1	1.6 /2.1	1.6 /1.8	1.6 /2.1
°C		5		
days				
0	2.31	2.31	2.31	2.31
3	3.01	2.89	2.82	2.41
7	4.25[a]	3.97	3.58	3.30[b]
14	6.21[a]	6.01	5.92	5.18[b]
21	8.50[a]	8.62[a]	8.57[a]	7.55[b]

[a] see Table 5

Table 8. Growth of Brochothrix thermosphacta in Bologna-type sausage

Cl-salt	NaCl		NaCl/KCl		NaCl		NaCl/KCl	
%	2.2		1.2 /1.4		2.2		1.2 /1.4	
°C	2	5	2	5	2	5	2	5
days								
0	2.51[a]	2.51[a]	2.51[a]	2.51[a]	0.2[b]	0.2[b]	-0.4[b]	-0.4[b]
6	5.56	6.18	5.44	6.36	-	-	-	-
8	7.23	7.74	6.91	7.89	-	-	-	-
9	7.52	8.62	7.30	8.55	1.0	-1.5	-0.4	-1.0
15	8.15	-	7.98	-	-0.6	-1.6	-0.7	-0.7
20	-	-	-	-	-1.1	-1.1	-0.9	-1.6
26	-	-	-	-	-1.3	-2.7	-1.7	-2.4

[a] means (log_{10} cfu/g), [b] means of odor scores

Table 9. Growth of *Serratia liquefaciens* in Bologna-type sausage

Cl-salt	NaCl	NaCl/KCl	NaCl	NaCl/KCl
%	2.2	1.2 /1.4	2.2	1.2 /1.4
°C		5		
days				
0	2.51[a]	2.51[a]	0.23[b]	-0.30[b]
6	2.39	2.35	-	-
9	5.30	5.33	0.28	-0.10
14	7.42	7.62	0.30	-0.14
20	-	-	-1.00	-1.60
23	-	-	-2.40	-2.40

[a] means (log_{10} cfu/g), [b] means of odor scores

Table 10 Growth of *Lactobacillus* in Bologna-type sausage

Cl-salt	NaCl	NaCl/KCl	NaCl	NaCl/KCl
%	2.2	1.2 /1.4	2.2	1.2 /1.4
°C		5		
days				
0	3.26[a]	3.20[a]	0.0[b]	-0.2[b]
4	3.22	3.27	0.0	-0.2
7	4.36[c]	3.41[d]	-	-
11	6.38	5.44	-1.0	-0.8
14	6.40	6.44	-	-
18	-	-	-1.4	-1.6
21	-	8.79	-	-
25	-	-	-1.7	-1.9

[a] means (log_{10} cfu/g), [b] means of odor scores, [c] see Table 1

Table 11. Growth of *Bacillus cereus* in Bologna-type sausage

Cl-salt	NaCl	NaCl/KCl
%	2.2	1.2 /1.4
°C	12	
days		
0	3.56[a]	3.56
3	3.97	3.41
6	5.18	4.43
7	6.50	-
11	7.14	6.65
13	6.88	6.82
18	7.23	6.97

[a] means (log_{10} cfu / g)

Table 12. Growth of *Yersinia enterocolitica* in Bologna-type sausage

Cl-salt	NaCl		NaCl/KCl	
%	2.2		1.2 /1.4	
°C	5	8	5	8
days				
0	3.80[a]	3.80	3.80	3.80
4	4.45	4.60	4.64	4.62
6	4.80	5.62	4.68	5.19
8	5.12	5.79	5.18	5.25
12	6.23	-	5.95	-
16	7.02	7.66	6.63	7.27
26	-	8.18	-	7.86

[a] means (log_{10} cfu / g)