INFLUENCE OF TECHNOLOGICAL INGREDIENTS ON THE INHIBITION OF *Listeria monocytogenes* BY A BACTERIOCINOGENIC STARTER CULTURE IN DRY FERMENTED SAUSAGES

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BACKGROUND

The presence of *Listeria monocytogenes* during the production of fermented sausages has been reported by several authors (Farber et al, 1988; Johnson et al, 1988). Bacteriocinogenic (BC) cultures of Lactic Acid Bacteria (LAB) have been probed effective in inhibiting *L.monocytogenes* in meat and meat products (Berry et al, 1991; Foegeding et al, 1992). In Spanish dry fermented sausages, *Lb.sakei* CTC494 producing sakacin K was able not only to suppress the growth of listeria but to diminish their number significatively compared to a bacteriocin negative standard starter strain (Hugas et al, 1995). In a further study, on the investigation of the formula and fermentation technology on the antilisterial effect of some BC producing cultures where two different processes and formulae were used (German-style sausages with nitrate curing and 3 g/Kg of glucose and Spanish style process with nitrate and nitrite curing, 7 g/Kg of glucose and other ingredients) the treatment with CTC494 as starter culture reduced *Listeria* by 4 log cycles in three days in the nitrate-nitrite process, while no reduction was observed in the nitrate-curing process (Hugas et al, 1996).

OBJECTIVES

The purpose of this study was to investigate the effect of the additives used in the standard formulation of Spanish fermented sausages in implementing the effect of sakacin K produced by *Lb.sakei* CTC494 towards *L.monocytogenes*.

METHODS

"In vitro" assays.TSBYE (Triptone Soya Broth Yeast Extract, Difco) was supplemented with NaCl (2.5%) and dextrose (0.7%) for all experiments, the rest of additives ($NaNO_2$, 0.01%; KNO_3, 0.03%; Sodium Ascorbate, 0.05%; Lactose, 1%; Skimmed Milk, 1%; Sodium caseinate, 1%; black pepper, 0.3%) were added filter sterilized one per tube. Sakacin K was added to each tube (400 AU/ml) and inoculated with an exponential growing culture of *L.monocytogenes* CTC1011. After incubation at 30ºC, counts of *L.monocytogenes* CTC1011 were determined at 0, 1.5, 3, 5, and 6 hours to evaluate its inhibition degree. A similar experiment was carried out with MRS supplemented with the same additives and inoculated with sakacin K producer, *Lb.sakei* CTC494, to determine the influence of technological ingredients in the production of bacteriocin.

Model sausage experiments were carried out as previously described (Hugas et al, 1995). Different treatments were manufactured consisting in lean pork and backfat pork (3:1) with different additives (Table 1) and starter cultures. *Lb.curvatus* CTC371 (Bac-) was used as a standard starter strain in a control treatment. Three sausages chubs from each treatment were sampled at selected times to determine *L.monocytogenes*, either by the spread plate technique in Palcam Agar (Merck) or by the MPN in *Listeria* enrichment broth base (UVM, Oxoid), lactobacilli populations in MRS (Difco), the pH values and lactic acid concentration.

RESULTS AND DISCUSSIONS

"In vitro" experiments. In absence of sakacin K, the ingredients supplemented to TSBYE had no significative effect on the growth of *L.monocytogenes* compared to the control (Table 2). When sakacin K was added, a growth inhibition was observed at least during the first 6 hours of incubation at 30ºC. All the treatments showed the same bacteriostatic effect except when the supplement was black pepper where a further reduction of 2 log cycles was observed compared to the same treatment without pepper (NaCl, dextrose and sak K) showing a synergism between pepper and the bacteriocin. Surprisingly, *L.monocytogenes* inhibition was higher in the control treatment with plain TSBYE than with TSBYE supplemented with NaCl and dextrose, suggesting sodium chloride affected bacteriocin activity. Gänzle et al, (1997) reported that sakacin P activity was increased at higher concentrations of NaCl (3%) although their experiments were performed in MRS. No synergism was found on the inhibition of *L.monocytogenes* when nitrate and nitrite were added to the media either with or without sakacin K. pH values in all the treatments were alike in the range 5-6.

The above mentioned additives supplemented in MRS had no significative effect on the production and secretion of sakacin K to culture supernatants compared to standard MRS.

"In situ" experiments. Meat fermentation is a vey complex system which is influenced by several ecological factors like temperature, a_w, pH, ingredients used for curing and of course microbial growth. Due to the complexity of the system, it is very difficult that data obtained in "in vitro" studies could be directly extrapolated to the "in situ" systems but it can be usable to orientate "in situ" studies which are more deffinitive.

In model sausages manufactured with NaCl, dextrose, the BC culture *Lb.sakei* CTC494 (Fig 1) and single ingredients like nitrate (Lot B), nitrite (Lot C) and pepper (Lot D) it was shown that nitrite was more effective in reducing listeria counts than nitrate and pepper (Fig 1) compared to the control where the ingredients were NaCl, dextrose and the BC *Lb.sakei* CTC494 (Lot A). The combination of nitrate, nitrite and pepper (Lot F) in the same treatment had a synergistic effect with the Bac+ culture reducing *L.monocytogenes* 2.5 log cycles compared to Lot A. The use of nitrate and nitrite do not produce a higher inhibiton rate towards the indicator strain than nitrite alone, confirming (Gänzle et al, 1997) that during sausage fermentation nitrate exerts only an indirect effect as a source of nitrite.

In model sausages manufactured according to Table 1, a summatory inhibiton effect was observed on the inhibiton of *L.monocytogenes* CTC1011 when more additives where used in the formulation (Fig 2) indicating the Sakacin K effect is reinforced by the effect of additives specially nitrite and black pepper. Pepper stimulates the microbial growth favouring the pH drop. Lots with pepper (4, 5, 6, 7, 9) reduced the pH quicker than lots without pepper (1, 2, 3, 8). The ultimate inhibition observed in lots 5, 6, 7, and

9 can be caused by the binding water capacity of the ingredients in these lots favouring a slight drop in a_w which could produce more inhibition in *L.monocytogenes* by the hurdle theory of Leistner. No experiments evaluating the additive effect with a non BC culture had been performed but from the results obtained from treatment 10 (all the ingredients with a Bac- *Lb.curvatus* CTC371) it can be assumed that the use of a Bac + culture in inhibiting *L.monocytogenes* enhanced the effect achieved by the formulae itself.

CONCLUSIONS

The most effective ingredients in achieving a synergism with sakacin K in reducing *L.monocytogenes* counts were nitrite and pepper. The combination of more ingredients had a summatory effect on the inhibition of the pathogen.

PERTINENT LITERATURE

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Table 1

ADDIT.	LOT 1	LOT 2	LOT 3	LOT 4	LOT 5	LOT 6	LOT 7	LOT 8	LOT 9	LOT 10
NaCl	X	X	X	X	X	X	X	X	X	X
Dextrose	X	X	X	X	X	X	X	X	X	X
$NaNO_2$		X	X	X	X	X	X		X	X
KNO_3			X	X	X	X	X		X	X
Pepper				X	X	X	X		X	X
Ascorbate					X	X	X	X	X	X
Skim. Milk						X	X	X	X	X
Caseinate							X	X	X	X
Lactose								X	X	X
CTC494	X	X	X	X	X	X	X	X	X	
CTC371										X
Listeria	X	X	X	X	X	X	X	X	X	X

Fig 1-*L.monocytogenes* counts in model sausage fermentation

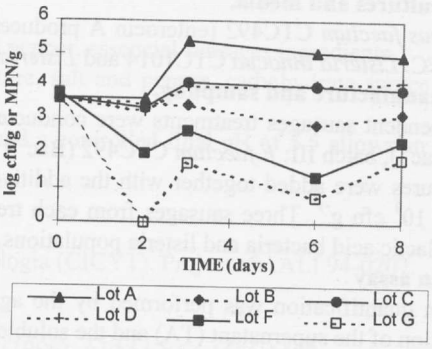


Table 2. Growth of *L.moncytogenes* in TSBYE supplemented with different additives used in the formulation of dry fermented sausages with and without 400 AU/ml of Sakacin K.

ADDITIVES	0 h		1.5 h		2 h		5 h		6 h	
	No Sak	With Sak	No Sak	With Sak	No *Sak*	With Sak	No Sak	With Sak	No Sak	With Sak
Control	6.83	6.83	7.57	6.09	8.05	4.62	8.77	4.33	9.02	4.78
NaCl, dextrose	6.83	6.83	6.99	6.44	7.81	5.2	7.97	6.15	8.85	5.7
NaCl, dextrose, $NaNO_2$	6.83	6.83	6.92	6.36	7.84	6.32	8.05	5.8	8.1	6.05
NaCl, dextrose, KNO_3	6.83	6.83	6.97	6.36	6.86	6.29	8.06	5.73	8.84	5.85
NaCl, dextrose, ascorbate	6.83	6.83	6.94	6.25	7.95	6.05	7.98	6.23	8.88	6.11
NaCl, dextrose, lactose	6.83	6.83	6.98	6.36	7.9	6.39	7.98	5.86	8.48	6.17
NaCl, dextrose, milk	6.83	6.83	6.93	6.38	7.93	6.37	8.08	5.86	8.52	6.63
NaCl, dextrose, caseine	6.83	6.83	6.82	6.32	7.8	6.4	7.96	6.28	8.51	6.33
NaCl, dextrose, pepper	6.83	6.83	6.7	6.16	7.91	5.48	8.17	3.78	8.51	3.81
NaCl, dextrose, $NaNO_2$, KNO_3	6.83	6.83	7.13	6.39	7.87	6.28	8.00	5.45	8.62	6.17

Fig 2-*L.monocytogenes* counts in model sausage fermentation.

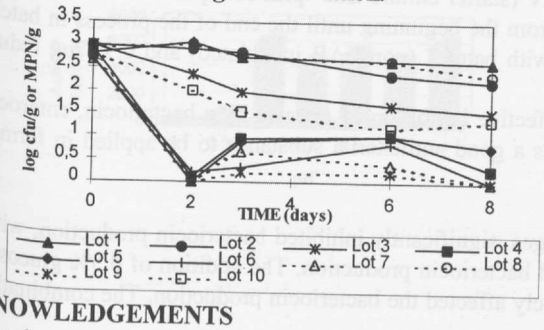
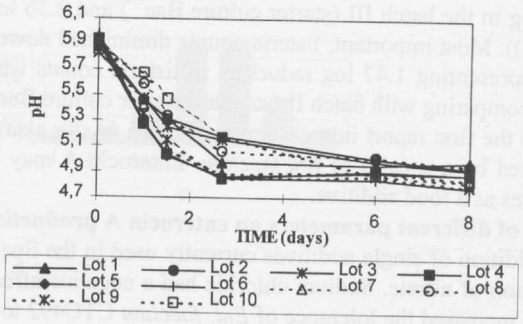


Fig 3-pH in model sausage fermentation



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