EVALUATION OF THE PERFORMANCE OF ALTERNATIVE PRESERVATIVES TO SULPHUR DIOXIDE IN FRESH PORK SAUSAGES.

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BACKGROUND: The microbiological status of fresh pork sausage has been well documented (Surkiewicz et al., 1972, El-Nawawi and Nouman, 1981) and is influenced by meat quality, handling, and processing. Sulphur dioxide (SO_2), in the form of sodium metabisulphite, is permitted in sausage products at levels not exceeding 450 ppm to control microbial spoilage. The development of an acceptable replacement to SO_2 would be advantageous since a number of undesirable side effects have been attributed to its addition to foods. These include the aggravation of asthmatic and other respiratory conditions; urticaria; angiodema; headache and gastrointestinal dysfunction, in both sulphite sensitive and normal individuals (Simon 1990). Many organic acids and their salts, which are generally recognised as safe, are active against spoilage microorganisms and a number of foodborne pathogens and could be regarded as alternatives to SO_2. Bacteriocins, including nisin, a broad spectrum antimicrobial agent produced by *Lactococcus lactis* subspecies *lactis* (Harris et al., 1992), are already widely used in the dairy industry and may provide another option in the replacement of sulphite.

Objectives: The aim of this study was to evaluate the effect of replacing SO_2 with alternative preservatives, such as sodium lactate (SL), sodium citrate (SC), nisin (NIS.) and combinations of these on the survival of *Salmonella kentucky* AT 1 and *Staphylococcus aureus* MMPR 3, and also on the the total aerobic plate counts of fresh pork sausage.

METHODS:

Bacterial cultures: *Staphylococcus aureus* MMPR 3 was obtained from the culture collection at the Department of Microbiology University College Cork. *Salmonella kentucky* was obtained from the Veterinary Department of Cork Corporation. Stock cultures were maintained by monthly subculture on brain heart infusion (B.H.I.), (Oxoid, Basingstoke, Hampshire) agar slants and stored at 4°C.

Sausage Model Systems: 2 kg batches of 70% visual lean pork trimmings were ground using a 12 mm plate, vacuum packaged and frozen at -20°C. Meat to be used in sausage production was removed from the freezer and allowed thaw overnight at 4°C. Treatment additives included, 450 ppm SO_2, 500 I.U. g^{-1} NIS., 2% SL, 2% SL + 500 I.U.g^{-1} NIS., 1.5% SL. + 1.5% SC, 1.5% SL + 1.5% SC + 500 I.U.NIS.. Three replications of the six treatments were formulated independently, pre-minced fat/lean mixture was added to a Kenwood mixer (Hampshire, England), and mixed at high speed (setting 5) for three minutes with prepared sulphur dioxide-free pork sausage seasoning (National Rusks Ltd. Limerick, Ireland) and the appropriate treatment combinations during the first thirty seconds. Subsequent to mixing, each batch was divided into three equal portions, one of which was inoculated with *S. kentucky* and one inoculated with *St. aureus*, both at levels of ~ 10^6 C.F.U.g^{-1}. The final portion remained uninoculated to determine the total aerobic bacteria during storage. The sausage meat was then stuffed into regenerated collagen (Nojax NST) casing (Viskase,Croydon, England), overwrapped with PVC film and stored at 4°C for ten days.

Microbiological analysis: Microbiological evaluation was conducted at 1, 3, 5, 7 and 10 days. Samples were evaluated for total aerobic counts, staphylococci and salmonellae. Meat (10g) was aseptically weighed into a stomacher 400 bag (Seward Medical, London, England) with 90 ml 0.1M pH 7.2 peptone water (Oxoid) and homogenised for two minutes in a Seward 400 Stomacher. Serial dilutions were prepared in peptone water and 0.1 ml was inoculated, in triplicate, onto the listed media using the spread plate technique. Aerobic (48 hr @ 32°C) organisms were cultivated on standard plate count agar (Oxoid) and results were expressed as C.F.U. g^{-1} . Salmonellae were enumerated on both Rappaport / Vassiliades Soy (Oxoid) agar and Brilliant Green Agar (Oxoid) while staphylococci were determined from Baird Parker agar plates (Oxoid). Results in this case were expressed as the difference between the initial bacterial load (day 1) and final bacterial load (day 10) i.e. $\Delta \log_{10}$ C.F.U. g^{-1}.

RESULTS and DISCUSSION: When the preservatives were introduced to the sausage system it was noted that all combinations performed better than sulphur dioxide in their ability to control of aerobic total viable counts. The 2% SL + 500 I.U.NIS. showed the smallest increase in bacterial load and demonstrated a better preservative effect than either of the components used separately (FIG. 1.). In challenge tests, where *St. aureus* was inoculated at a level of ~ 10^6 C.F.U. g^{-1}, increases in the staphylococcal populations occurred only in those sausages preserved with sulphur dioxide and, to a lesser extent, in sausages containing other treatments (FIG. 2.). Again the treatment containing 2% SL + 500 I.U. NIS. showed the greatest net reduction of *St. aureus*. This result was also achieved in the parallel trial with *S. kentucky* .

CONCLUSION: The results of this study indicate that the preservation regime containing 2% sodium lactate together with 500 I.U.g^{-1} nisin is superior, in the control of total aerobic counts, to the sulphur dioxide treatment currently in use. It also appears that this combination of preservatives provides increased protection against common pathogenic contaminants of fresh pork sausage i.e. *Salmonella* species and *St. aureus* . This is particularly encouraging in the case of Salmonellae since the inclusion of lactate appears to facilitate nisin in the control of this Gram negative pathogen.

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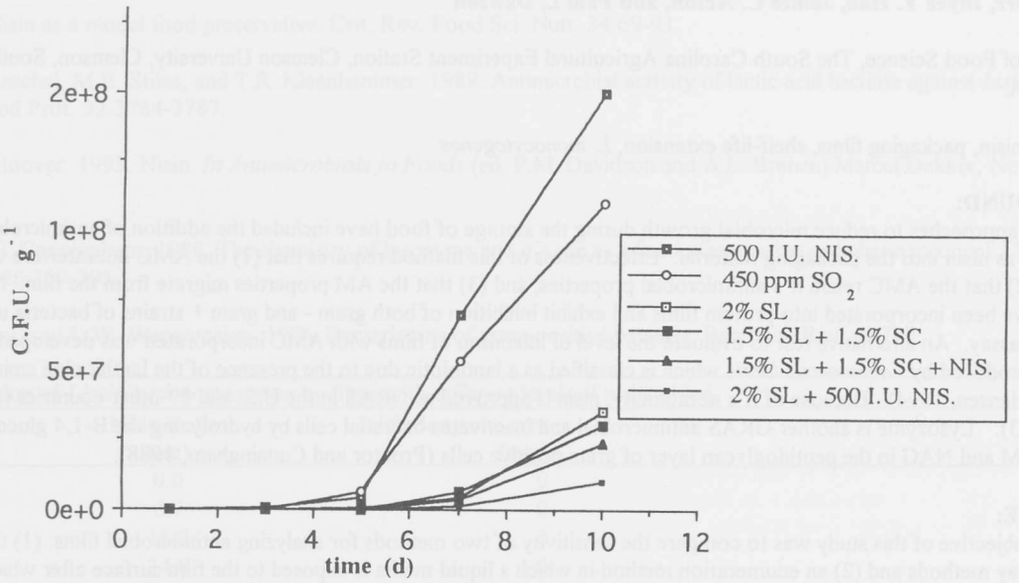

Fig . 1 . Increase in total aerobic plate counts of fresh pork sausage stored for 10 days at 4° C.

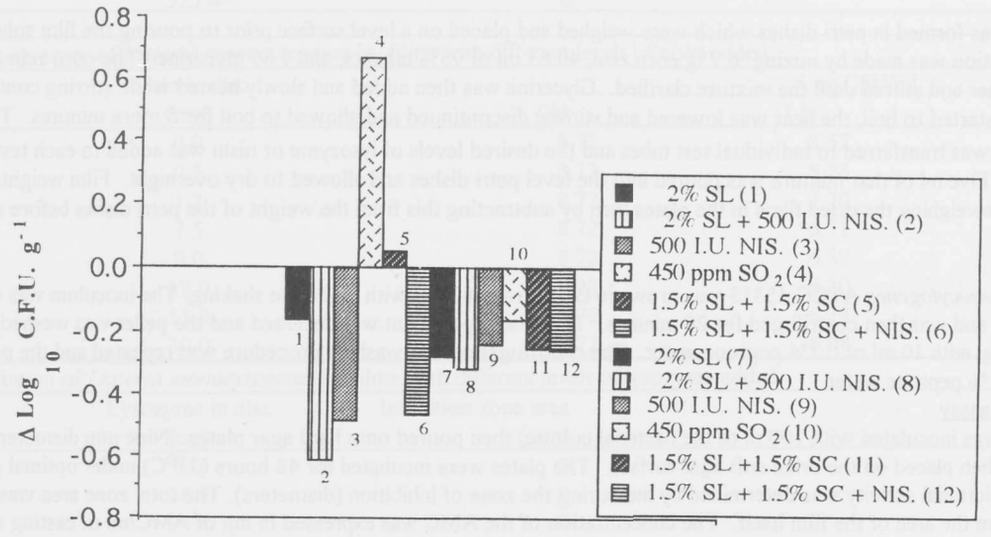

Fig. 2 . Histogram showing the net effect of preservation regimes on the survival of *Staphylococcus aureus* MMPR 3 , (columns 1-6) , and *Salmonella kentucky* AT 1 , (columns 7-12) , in fresh pork sausage stored for 10 days at 4° C .