EFFECT OF NITRITE- VS. NITRATE-CURING ON STAPHYLOCOCCUS AUREUS

M. DELÉNYI, K. INCZE, H.É. DOLOZSELEK, K.M. VADA, A. NAGY

Hungarian Meat Research Institute
1097 Budapest, Gubacsi ut 6/b.
and

GY. LOMBAI, J. TAKÁCS

University of Veterinary Sciences
1077 Budapest, Rottenbiller ut 50.

Former investigations in our Institute have proved the prevalence of nitrite-curing vs. nitrate-curing regarding raw ham. By the help of the so called equilibrium curing technique /the $NaCl+NaNO_2$+water: meat ratio is known/ a relatively exact and uniform salt- and nitrite-content of the cured meat can be attained. On the other hand the use of KNO_3 as a curing agent causes uncertainty in curing time, meat-colour and nitrite-content potentiating this way also the higher risk of nitrosamine-formation. It was nevertheless to be elucidated, whether the lack of nitrate- as nitrite-reservoir- and /eventually/ lower nitrite-levels in the ham caused a decrease of shelf life or an increase of bacteriological hazard. As first test organism Staph. aureus has been chosen, because of its high tolerance against salts and low water activity.

Materials and methods

Pieces of Musculus semimembranosus /about 1000 g/were removed from swine carcasses 24-48^h after slaughter. With two of the series of experiments DFD-muscles were used /pH 6,3-6,5/, while normal muscles /pH 5,6-5,8/served for the third experiment. External fat and connective tissue were removed from the muscles and they were formed uniformly to 1 kg. In a series 40 pieces of muscles were used altogether.

Staph. aureus strains:

Code No.	Type	Enterotoxin production	Isolated at
4	Bovine	"C"	Univ. of Vet. Sci.
14	Intermediate	"D"	"
73	Intermediate	"A"	"
187	Human	"D"	State Vet. Control Service

24^h slant agar /"Takács basic agar"/ cultures were washed in saline and photometrically determined the viable cell count. 5 x 10^8/ml of cells in 5 ml saline has been injected in the deeper regions of the meat pieces. Immediately after injection, 40 g nitrite-containing or 40,8 g nitrate-containing curing salt had been rubbed uniformly on the surface of each sample. Surface inoculum of Staph. aureus has been administered together with curing salts giving a final concentration of 1 x 10^6 cells per g meat.
Composition of the curing salts /referring to one kg sample/.
1. 34 g NO_2-curing salt /with 0,5 % of $NaNO_2$/ + 6 g NaCl;
2. 40 g NaCl and 0,8 g KNO_3
The cured pieces of meat were kept in polyethylene bags at 6-8°C for the duration of 3 days. As next processing step we performed the pickling in nitrite- vs. nitrate-containing brine, with the following composition per 1000 g meat /calculated for reaching desired salt content/:
1. 48,4 g NO_2-curing salt with 0,5 % $NaNO_2$, 8,5 g NaCl and 453 g water;
2. 55 g NaCl, 1,14 g KNO_3 and 453 g water.
Curing has been continued until maturation i.e. about 7-10 days, at 7°C, 90 % rel. humidity.
After curing one half of the samples has been undergone a 6 week freezing at -18°C. Thereafter all the processing steps were identical with that of the unfrozen hams, namely: Heat treatment for 6 hours at 40°C followed by a 12^h drying at room temperature and storing for 1 and 3 weeks at 12-15°C.
10 g samples have been taken after each processing step i.e. nine times. After the first processing step samples have been taken both from the outer layer /about 1 cm/ and the internal parts. Nitrite-, nitrate- and salt-content of the samples as well as DNase test were performed according to Hungarian standards. Staph. aureus count was determined on Baird-Parker agar. Petri plates were incubated at 37°C for 24 hours.
After further processing steps whole 1 kg samples were ground and homogenized and the tests listed above have been carried out.

Results

As mentioned, after 3-days-salt curing Staph. aur.-count was determined both from the outer and deeper regions of samples. Because of the very similar results /Table 1/ we consider as verified the rather homogeneous distribution of the microbes cited.
With unfrozen samples there is little alteration in Staph. aureus-count after various processing steps compared to initial count. Similarly, no significant difference has been found in Staph. aur.-counts, no matter nitrate- or nitrite curing has been applied /Table 1/.
Nitrite-content of nitrate-cured hams grows continuously and after a storage of 3 weeks it reaches a maximum value of 250-400 ppm. Although final nitrite levels with nitrite cured meats /20-70 ppm/ have been much lower, no significant difference could be found in terms of staphylococcal growth between the samples differently cured.
Nitrite-maximums with normal muscles /3. series/ of nitrate-cured samples show significantly lower levels, without however any influence on growth of Staph. aureus.
After thawing of frozen samples Staph. count corresponds to initial levels, except 1. series, where some reduction can be observed /fig. 1-2/. Due to processing steps after freezing /and thawing/ increase of Staph. activity is detectable and this tendency continues until the end of the experiment, except 2 strains of series 3 with nitrate-cured hams /fig. 1-2 /.
The DNase test performed after each processing step gave always negative results.

Table 1 Staph. aureus count after salting

strain		in NO_2 - cured samples			in NO_3 - cured samples		
		ser. I	ser. II	ser. III	ser. I	ser.II	ser. III
4	O[x]	$1,2x10^5$	$5,1x10^6$	$3,4x10^6$	$1,1x10^5$	$2,9x10^6$	$2,9x10^6$
	I	$2,6x10^5$	$1,3x10^6$	$1,7x10^7$	$3,3x10^5$	$3,0x10^6$	$1,3x10^7$
14	O	$2,8x10^5$	$5,0x10^6$	$1,3x10^6$	$1,7x10^5$	$2,9x10^6$	$2,6x10^6$
	I	$1,0x10^5$	$1,3x10^6$	$2,9x10^6$	$1,7x10^5$	$3,0x10^5$	$6,9x10^6$
73	O	$1,8x10^5$	$2,1x10^6$	$3,2x10^6$	$2,9x10^5$	$2,9x10^6$	$2,3x10^6$
	I	$4,0x10^5$	$2,8x10^6$	$1,4x10^7$	$4,6x10^5$	$3,9x10^6$	$5,0x10^5$
187	O	$5,4x10^5$	$1,9x10^6$	$2,8x10^6$	$2,8x10^5$	$2,1x10^6$	$1,7x10^6$
	I	$8,7x10^5$	$1,5x10^6$	$8,5x10^6$	$6,0x10^5$	$6,9x10^6$	$1,0x10^6$

[x] O = outer layer
I = inner regions

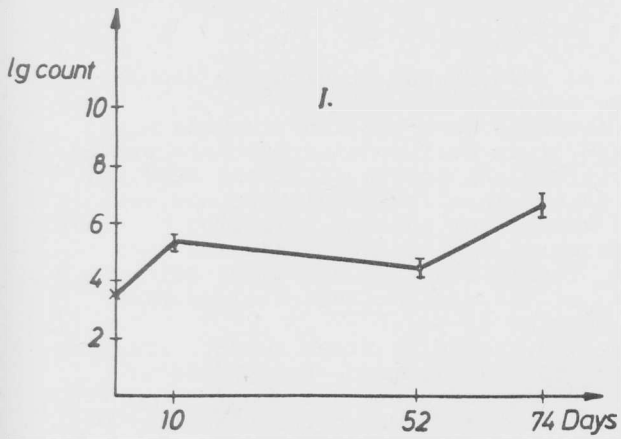
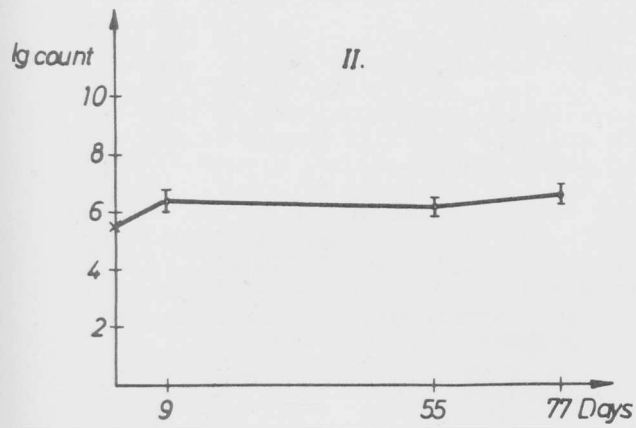
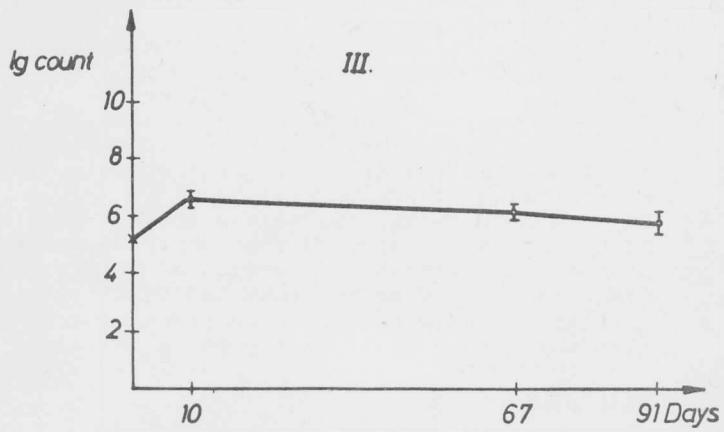


Fig.1. CHANGES OF STAPH. AUREUS COUNT IN NITRITE-CURED SAMPLES





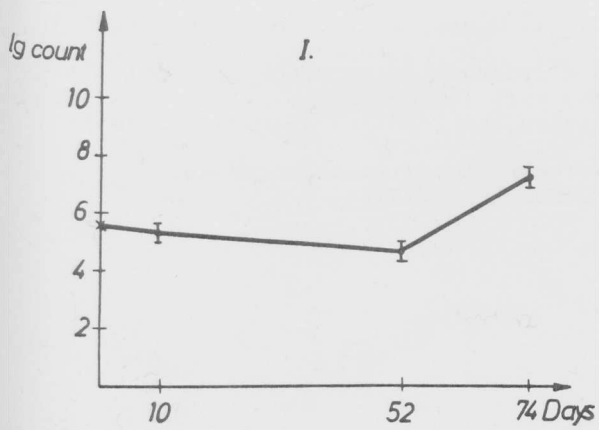
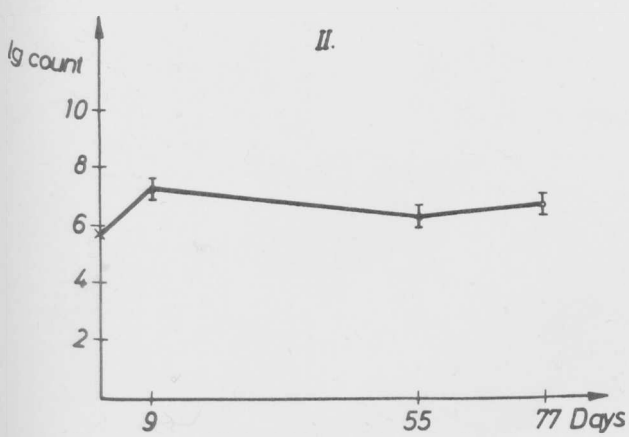
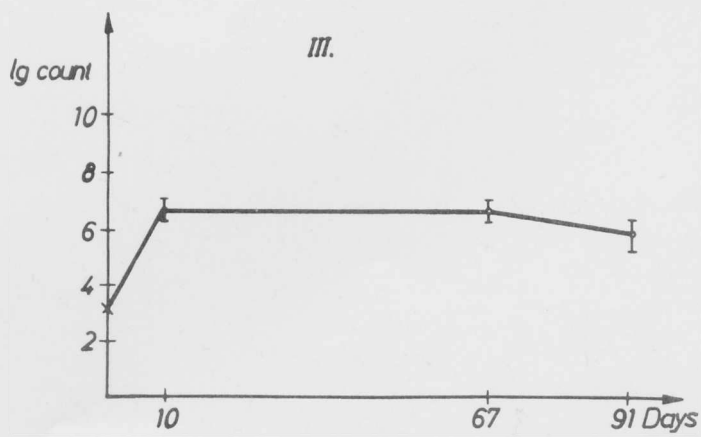


Fig.2. CHANGES OF STAPH. AUREUS COUNT IN NITRATE-CURED SAMPLES





Discussion

It has been thoroughly investigated thus well documented that Staph. strains are unable to compete successfully the "native" microflora in raw meat.
Casman et al. /1/ found that little or no growth was obtained when the inoculum was mixed with raw ground beef. Genigeorgis /2/, McCoy and Faber /3/ as well as other authors proved that the other bacteria present in meat generally inhibited the growth of Staph. aur. So, chances of growth for Staph. aur. inoculated onto or rather into raw muscle are not very good but salting and pickling can improve its growing conditions because competing microflora is eliminated, yet growth or survival of Staph. aureus is not hindered.
Scott found optimum growth rates for Staph. aur. at a_w levels of 0,995 and 0,99. Below these levels growth rates decrease as a function of a_w of the medium. Nevertheless slow growth of 9 of the 13 strains was observed as low as at 0,86 a_w /4/.
Temperature and period of chilling are unable to cause a decrease in Staph. count. Freezing, according to investigations of Zawadzki and Pogorzelska /5/ had limited staphylococcal survival to 15,8 % of the initial population /-22°C, 7-8 days of storing/. Minor and Marth /5/, however, found that freezing and thawing had little or no effect on the viability of Staph. aureus. Our experiments seem to support this latter finding despite of the prolonged freezing period. As to the eventual inhibitory effect of nitrite and nitrate, we refer to the already classic report of Lechowich et al. /5/ who found vigorous Staph. growth in ground pork that contained any permissable and palatable combination of NaCl, $NaNO_3$ and $NaNO_2$.
Genigeorgis /2/ stated that concentrations of NaCl, nitrite, nitrate, initial pH and a_w found in commercial sausage mixes will not inhibit Staph. growth even at numbers below 10^3/g.
It is known that high pH and a_w values as well as law salt concentration promote the capability for enterotoxin formation of Staph. aureus and that critical levels of the mentioned factors are different regarding the inhibition of staphylococcal growth or the enterotoxin production resp /4/.
In view of our findings we may suggest that changes of meat environment due to the various processing steps enabled the Staph. aureus strains for survival or low growth, inhibiting at the same time the production of enterotoxin even with an initial count of 10^6/g. It has been clearly shown, that use of nitrate in curing has no advantages compared to nitrite-curing with regard to Staph. aureus growth and enterotoxin production, which in turn means that equal safety can be attained with both methods in this respect.

Literature cited

/1/ Casman, E.P., McCoy, D.W. Brandly, P.J. /1963/: Staphylococcal Growth and Enterotoxin Production in Meat. Appl. Mb. Vol. 11, p.498.
/2/ Genigeorgis, C.A. /1976/: Quality Control for Fermented Meats. J. Am. Vet. Med. Ass. Vol. 169. No. 11.
/3/ McCoy, D.W., Faber, J.E. /1966/: Influence of Food Microorganism on Staphylococcal Growth and Enterotoxin Production in Meat. Appl. Mb. Vol. 14. No. 3.
/4/ Troller, J.A., Christian, J.H.B. /1978/: Water Activity and Food. p. 133-138. Academic Press, New York-San Francisco-London, 1978.
/5/ Minor, T.E., Marth, E.H. /1976/: Staphylococci and Their Significance in Foods. p. 224-225 Elsevier Sci. Publ. Co. Amsterdam-Oxford-New York, 1976.