TOTAL SCREENING, IN VITRO, OF INHIBITORY AND STIMULATORY EFFECTS OF NATRIUM CHLORIDE, NATRIUM NITRITE AND TRIPOLIPHOSPHATE ON PATHOGENIC AND SAPROPHYTIC MEAT MICROFLORA

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Summary

About 3600 growing curves got by turbidity measurements technique in a "Cobas-Bact" instrument were tested by the potency of both "single" and "in combination" chemicals to inhibit and/or stimulate bacterial growth in Nutrient Broth No 2 (Oxoid). Six bacterial strains: Escherichia coli, Salmonella agona, Proteus vulgaris, Streptococcus faecalis, Bacillus subtilis, Staphylococcus aureus were examined. Very strong inhibitory action of tripoliphosphate (0.5%) was detected on Staphylococcus aureus, weaker one on Bacillus subtilis, Proteus vulgaris and Streptococcus faecalis. No action was noticed on Escherichia coli and Salmonella agona. Stronger inhibitory action was noticed in application of NaCl (1.5% and 3.0%), stronger than in a case of $NaNO_2$ (0.0050% and 0.0125%), except of Staphylococcus aureus, where stimulatory effects resulted. "In combination" NaCl and $NaNO_2$ were exerting a stronger inhibitory action than in "single" application, and were stimulatory to Staphylococcus aureus. Stronger inhibition or less stimulation was noticed with the pH 5.5, than 6.5. Other additives: sodium ascorbate and sodium citrate brought additional inhibitory effects to the agents mentioned above.

1. NaCl could act stronger as inhibitor than $NaNO_2$, especially at lower pH. 2. Stimulatory effects of NaCl and $NaNO_2$ on Staphylococcus aureus could be canceled by tripoliphosphate, sodium ascorbate or sodium citrate. 3. Tripoliphosphate could be considered the potential chemical agent for partial replacement of NaCl and $NaNO_2$ in meat products due to its strong bacteriostatic activity on Gram-positive microflora and on condition that it could act as efficiently in meat environment as in nutrient broth.

Introduction

Shelf stability of meat cured products is based on the degree of thermal destruction of microflora, if such a process takes place, and subsequent inhibition of residual microflora by the number of chemical factors, mainly salt and nitrite. For greater destruction of vegetative microflora than in case of spores during pasteurization of meat products, great deal of experiments were directed toward controlling the spores count, mainly Clostridium botulinum spores PIVNICK, PRTRASOVITS (1973); HAUSCHILD (1982); HAUSCHILD et al (1982); ROBERTS (1986).

Relatively little is known about the influence of chemical factors in meat products on other bacteria than sporulating Clostridia. The following paper attempts to spotlight the influence of some chemical factors (the constituents of curing mixtures) on the dynamics of growth of saprophitic and pathogenic bacteria, that are common meat products contaminants. The experiments are done in microbial medium, so they have to be considered as total screening. The tendencies running from these experiments could not reappear in meat products, but they could be taken into account for farther less model experimental planning. Instead of probabilistic approach to the protection features of chemicals, the inhibitory/stimulatory coefficient was proposed, which in contrary to the probability could be easily applied to dynamic experiments. After all the temperature 37°C is much higher than storage temperatures for meat products, consequently weak inhibitory tendencies noticed in such model experiments seem to be very likely to occur in meat products, for the fact commonly known that weak inhibitory action of chemicals becomes stronger during diminishing of storage temperature.

Materials and methods

The following bacterial strains were used:

1.Escherichia coli NCTC 8196;2.Proteus vulgaris NCTC 4635;3.Staphylococcus aureus NCTC 4163;
4.Salmonella agona, from National Institute of Hygiene in Warsaw;
5.Streptococcus faecalis , from Institute of Biotechnology in Warsaw;
6.Bacillus subtilis , from Institute of Biotechnology in Warsaw

The following factors in two pH variants 5.5 and 6.5 were studied:

	concentration (% w/v on medium):
- NaCl	1.5 , 3.0
- $NaNO_2$	0.0050 , 0.0125
- tripoliphosphate "Almina" got from the factory at Alwernia near Kraków	0.5
- four curing mixtures:I, II, III, IV	(content in table 1)

Microbial media and fluids

Experimental medium was prepared by solution of tested chemical agents to the desired concentration in redistilled water, then dry bullion component (Difco broth No 2) was added. After pH correction to the desired value ± 0.1, medium was filter sterilized through membrane filter.

Sodium ascorbate, prepared and separately filter sterilized , was added to the medium directly before starting cultivation for the possibility of its reaction with natrium nitrite.

Dilution fluid was applied according to the Polish Standard PN-83/A-82054 Meat and Meat Products. Bacteriological Examinations.

For the passaging of bacterial strains before the main experiment, liquid nutrient broth No 2, sterilized by autoclaving at 121°C for 20 min., showing the final pH 6.5 was applied.

Experimental plan

After preparing the liquid media with two pH values and with or without chemical factors studied, plastic rotors for the automatic analyzer "Cobas - Bact" were filled with cultivation media and cultures taken from the 3 rd passage from over - night cultures at 37°C. (Over-night cultures were ran by successive passaging by loop inoculation in the same medium). Rotors were automatically analyzed by increase in optical densities (λ: 430, 546 nm); incubation temperature was fixed to 37°C.

Growing curves were approximated with the polynomials of the order from 2 to 6. Polynomials orders were estimated by taken into account the best fit (maximal correlation factors). Several parallel (under the same conditions) growing curves were averaged and fields under the curves were estimated by integration. Growing curves were passed through three growing stages. It is not possible to present about 3600 growing curves in this paper, therefore only one example is presented (fig. 1). Intensity of growth of every bacterial strain was estimated by comparison of the growing curve, obtained with the addition of tested chemical factor, to the growing curve got without chemicals (used as a control curve). Inhibition/stimulation coefficients were calculated by dividing the fields obtained under the growing curves obtained after addition of the tested chemical or a combination of chemicals by the corresponding fields under the curves obtained without the addition of any chemical factor. Every tested combination (version) was repeated a few times in experiments. Some of the inhibition/stimulation factors were rejected after negative verification with Q - Dixon test for the probability 90%. Not rejected factors in the form of diagrams are presented in figs from 2 to 7.

Cultures before being added to the rotors and cultures at the end of experiments were enumerated several times by Koch method, in order to estimate the approximate limits of their counts. Nutrient broth solidified by supplementing with the agar (1.5%), and dilution fluid

for preparing serial dilutions were then applied.

Results and discussion

Bars in figs from 2 to 7 placed below the level of 1 represent inhibitory action of tested chemicals, while above this level they indicate the stimulatory action on the intensity of bacterial growth.

Comparison of the results presented in figs 2 and 3 indicates that NaCl exerts a stronger inhibitory action than $NaNO_2$ on Escherichia coli, Streptococcus faecalis and Bacillus subtilis, especially at lower pH. Inhibitory action in these cases is stronger in higher doses of NaCl and $NaNO_2$. Staphylococcus is eminently stimulated for growth by NaCl and to a little bit smaller extent by $NaNO_2$, although $NaNO_2$ at higher concentration (0.0125%) and at pH 5.5 shows distinct inhibitory action. Proteus vulgaris undergoes small stimulation by NaCl, except NaCl in higher concentration (3.0%) and in higher pH (6.5), where small inhibition results. $NaNO_2$ seems to have distinct inhibitory effect on Proteus vulgaris in higher concentration and in lower pH. $NaNO_2$ exerts a stimulatory action in lower concentration and in higher pH on Escherichia coli. Small inhibitory effects of NaCl and $NaNO_2$ on Salmonella agona are equivalent.

Effect of tripoliphosphate on Gram - positive bacteria (fig. 4) is eminently inhibitory - being very strong on Staphylococcus aureus. On Gram - negative bacteria tripoliphosphate exerts a slightly stimulatory action at higher pH values.

Influence of joined action of NaCl and $NaNO_2$ (fig. 5) shows a stronger inhibitory action on bacteria in comparison to the factors acting separately. Stimulatory action of NaCl on Staphylococcus aureus is slightly diminished by the influence of less stimulatory action of $NaNO_2$. Stimulatory action of NaCl on Proteus vulgaris is not reduced by $NaNO_2$ however, except the case of higher doses of factors in lower pH.

As it matters curing mixtures higher inhibitory action of factors is noticed in lower pH values. Despite the junction of the potentially inhibitory constituents in the mixtures, small stimulatory effect is exhibited on Salmonella agona of the mixture No II, containing lower levels of NaCl and $NaNO_2$. Smaller inhibition of the mixture No II, in comparison to other mixtures, on other bacteria is also noticeable. Comparison of the mixtures differing in the content of sodium ascorbate and citrate shows that these components are bringing additional inhibitory effects to the mixtures.

Conclusions

Lowering NaCl and $NaNO_2$ levels could give the effect of fluent transition of the inhibitory action of the above mentioned factors to stimulatory action on bacterial growth intensity.

Tripoliphosphate, sodium ascorbate and citrate bring farther inhibitory action to NaCl and $NaNO_2$.

NaCl and $NaNO_2$ act more efficiently as inhibitors "in combination" than separately, but it is not truly synergistic action.

References

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TAB. 1

Curing mixtures content (in % w/v on medium)

Ingredient	Number of curing mixture			
	I	II	III	IV
NaCl	3.0	1.5	3.0	3.0
$NaNO_2$	0.0125	0.0050	0.0125	0.0125
Sodium ascorbate	0.03	0.03	0.00	0.00
Tripoliphosphate "Almina"	0.5	0.0	0.5	0.5
Sodium citrate	0.3	0.3	0.3	0.0

FIG. 1

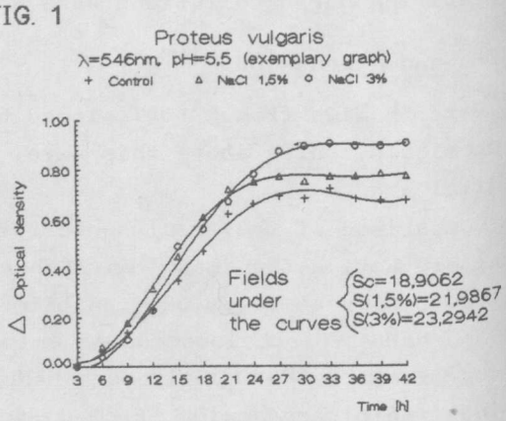

FIG. 2

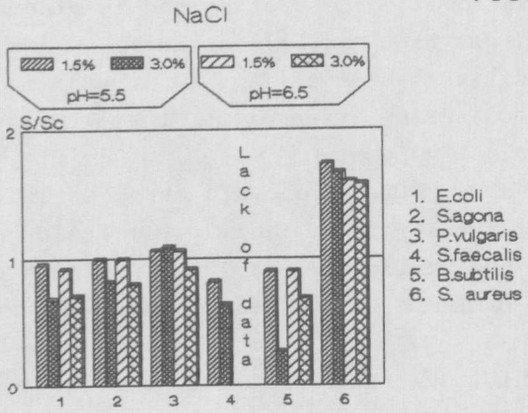

FIG. 3

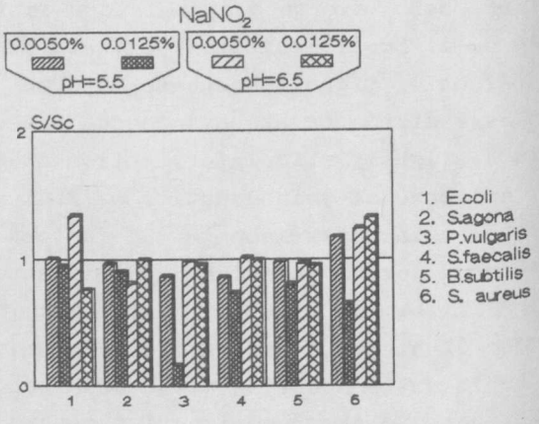

FIG. 4

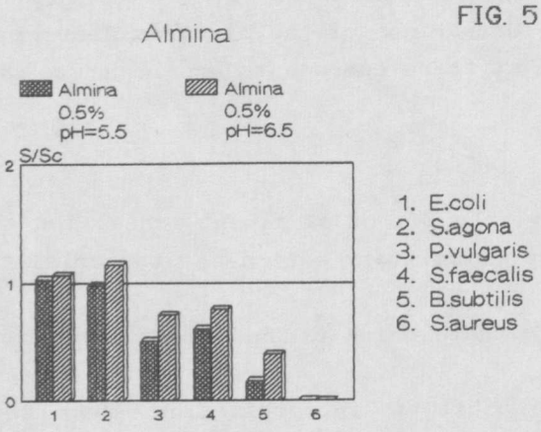

FIG. 5

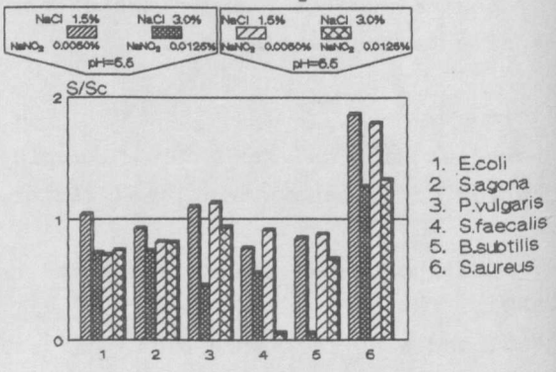

FIG. 6

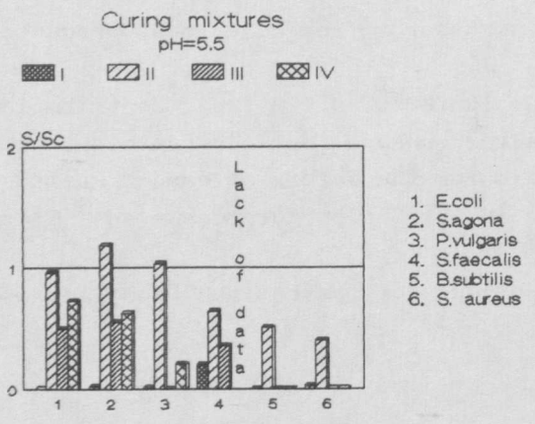

FIG. 7