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THE INHIBITORY EFFECT OF GARLIC ON BACTERIA*

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Sarımsağın bakteriler üzerine inhibitör etkisi

Özet : Bazı patojen ve toksik bakterilerin *(Staphylococcus, Micrococcus, Bacillus, Salmonella, Citrobacter, Proteus, E.Coli* ve *Klebseiella)* gelişmeleri üzerine sarımsağın etkisi araştırılmıştır.

Gram negatif bakterilere nazaran, gram pozitif bakterilerin sarımsağa karşı daha duyarlı oldukları tesbit edilmiştir. Bakterilerin gelişmesi üzerine bu inhibitör etki, bir familya içindeki çeşitli tür bakterilerde aynı olduğu saptanmıştır.

Sarımsağın bakteriyostatik etkisi üzerine sodyum klörürün potansiyel bir etkisi olduğu görülmüştür. Özellikle *Staphylococcus aureus* (alfa toxin) sodyum klorürün bu potansiyal etkisine karşı çok hassastır.

% 5 sodyum klorürle % 1 oranında sarımsağın birlikte kullanılmasının, gıda preservasyonu konusunda bakteriyostatik etki bakımından iyi sonuçlar alınacağı neticesine varılmıştır.

Summary : Inhibitory effect of garlic on a number of well known pathogenic and toxinogenic bacteria *(Staphylococcus, Micrococcus, Bacillus, Salmonella, Citrobacter, Proteus, E.coli* and *Klebsiella)* has been examined.

The gram positive bacteria were found to be more sensitive to garlic than the gram negative. The inhibitory effect was the same on different species within the same family.

Sodium chloride was found to have a potential effect on bacteriostatic ability of garlic. Especially *Staphylococcus aureus* (alfa toxin) is very sensitive to NaCl's potential effect.

The practical aspects is discussed and the conclusion is that garlic valve in the food preservation must be taken into consideration when the garlic concentration is 1 % and the NaCl concentration is 5 % or more.

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Introduction

The purpose of this investigation was to study the inhibitory effect of garlic on various bacterial species, known as potential pathogens or spoilers of sausages and similar products.

Garlic extract was prepared in an atomizer in different water solutions (from 50 % to 0.1 %) of fresh French garlic. As test organisms were used representative members of the genera Micrococcaceae, Enterobacteriaceae and some Bacillus species.

Furthermore the potential effect of different NaCl concentrations was examined in order to determine the inhibitory effect of garlic at the different water activities commonly used in sausages. The medium used was 5 % blood agar, incubation temperature was 37°C.

Material and Method

Bacterial cultures used were: Staph. aur. alpha-tox., Staph. aur. beat-tox., anhaemolytic micrococcus, B.subtilis, B.cereus, Salmonella typhimurium, Citrobacter ballerup bethesda, E.coli I, Klebsiella, Proteus and as representatives for the enterotoxin producing strains of Staph.: Staph. A 100, Staph. B 56, Staph. C 361, Staph. D.315 and Staph. epidermis 12.288.

All strains were obtained from the Institute of Veterinary Microbiology and Hygiene, the Royal Veterinary and Agricultural University, Copenhagen. Four hour old trypsine-digested ox heart broth cultures were used in the experiment. And the effect of the garlic extract was examined on blood agar according to the agar cup method, with a 12 mm hole drilled in the centre.

To compare the potential inhibitory effect of the different NaCl concentrations, the blood agar was mixed with 5% and 10% NaCl to obtain water activities of 0.97 and 0.94.

The agar was surface-inoculated with the 4 hour broth culture and garlic extract was poured into the hole in the centre. Then the plates were stored at 4°C for one hour to obtain good diffusion of the garlic extract before the bacterial multiplication started.

The plates were incubated at 37°C for 24 hours and the diameter of the zone of inhibition was measured. The proportion between the garlic concentrations and the diameter of the inhibition zone was measured.

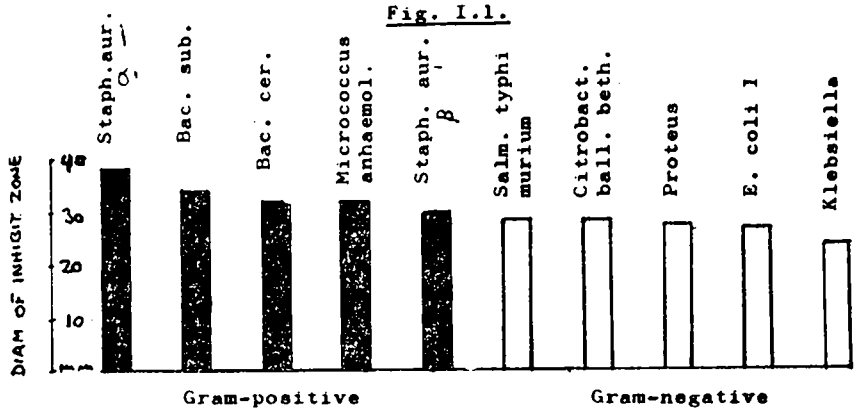
Results

The results obtained in the experiments are shown below.

TABLE I . 1 .

Micro-Organisims	Staph. aureus alpha	Bac. subt.	Bac. cer.	Micro-cocus anha-em.	Staph. aur. beta	Salm. typhi mur.	Citro. ball. beth.	Pro-teus	E.coli	Kleb-siella
Diam. of inhibit. zone	38 mm	34 mm	32 mm	32 mm	30 mm	28 mm	28 mm	27 mm	26 mm	24 mm

Fig. I.1.



A clear difference in the sensitivity between Gram-positive and Gram-negative bacteria is noted (10 % garlic consentration).

The difference in the sensitivity of the species within the same genera was examined in different garlic concentration. The results were as fallow.:

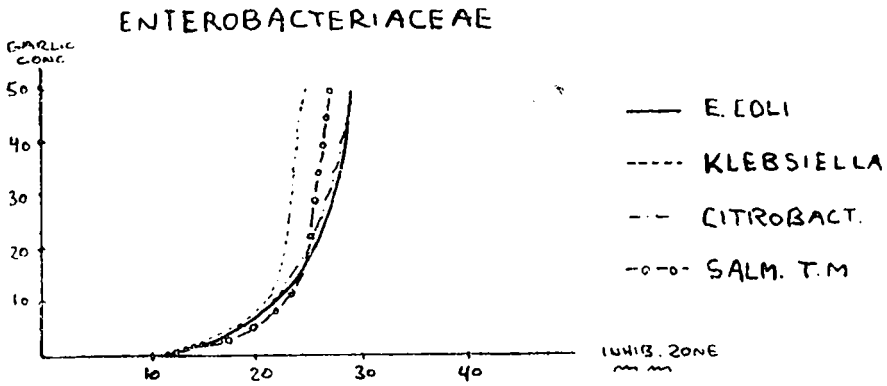
TABLE II . 1

ENTEROBACTERIACEAE

Diameter of inhibition zone, mm.

Garlic Concentration	E.Coli I	Kleb siella	Citrobacter B.B	Salmonella T.M.
50 %	29	25	29	27
25 %	27	23	26	25
10 %	22	21	22	23
1 %	13	13	14	14

Fig. II.1.



The table and the graph show that Klebsiella has greater resistance in high garlic concentrations (capsule formation).

TABLO II.2.

Bacillus sp.
Diam. of inhibition zone, mm.

Garlic Concentration	Bac. subtil.	Bac. cereus.
50 %	36	37
25 %	34	32
10 %	29	23
1 %	13	13

Fig. II.2.

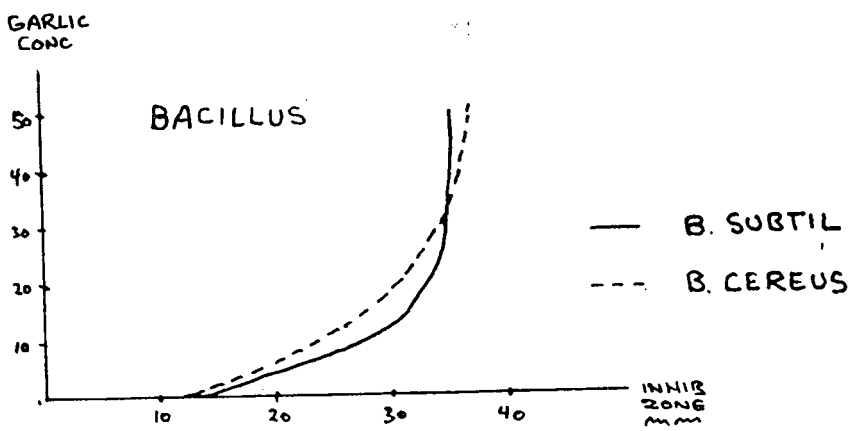
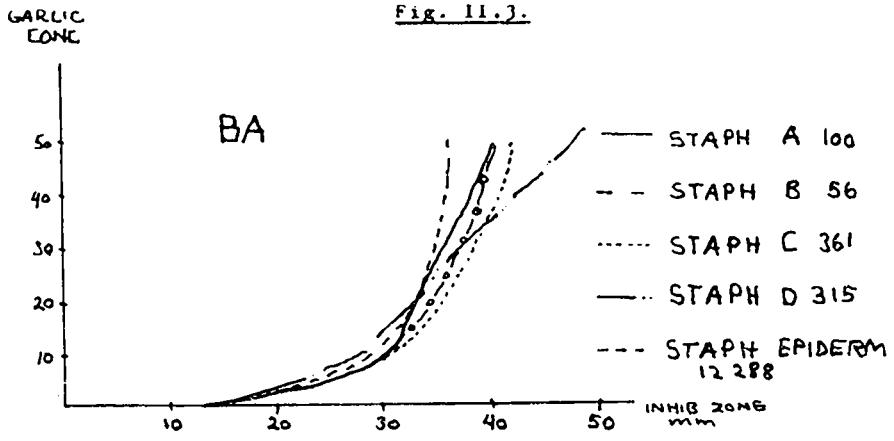


TABLE II. 3

MICROCOCCACEAE

Carlic conc.	Staph. A 100	Staph. B 56	Staph. C 361	Staph. D 315	Staph. D 494	Staph. epiderm 12 288
50 %	40	40	42	48	36	40
25 %	34	36	36	35	33	35
10 %	31	31	32	28	29	32
1 %	13	13	13	13	13	13

Fig. II.3.



The graphs Fig. II. 1,2,3 show that sipecies within the same genus have equal sensitivitz; but the maximum effect of garlic differs (cf. fig. I. 1),

In practice a synergistic preservative effect is obtained by using garlic in combination with NaCl. The inhibitory effect of garlic on Staph. aureus alpha toxin, Bac. cereus, and Salm. typhimurium in relevant salt concentrations was examined.

TABLE III. 1.

STAPHYLOCOCCUS AUREUS (alpha toxin)

Garlic Conc.	Blood agar (BA)	BA + 5 % NaCl	BA + 10 % NaCl
50 %	54	80	>85 Diam. of
25 %	48	62	74 inhibition
10 %	39	46	56 zone, mm

TABLE III. 2.

BACILLUS CEREUS

Garlic Conc.	BA	BA + 5 % NaCl	BA + 10 % NaCl
50 %	39	54	>85
25 %	36	49	>85
10 %	29	42	>85

TABLE III. 3.

SALMONELLA TYPHİMURİUM

Garlic conc.	BA	BA - 5 % NaCl	BA + 10 % NaCl
50 %	31	55	No growth
25 %	28	50	"
10 %	26	37	"

The figures of Table III, 1,2,3 represented in a system of coordinates.

Fig. III. 1. shows a highly increased potential inhibitory effect of garlic in combination with NaCl. (The curve is flattened). It appears from Fig. III. 2 and 3 that 5 % NaCl gives the same maximum effect of the garlic.

The increased bactericidal effect of garlic with 5% NaCl gave rise to an examination of the effect of garlic concentrations below 5%. As test organisms were used enterotoxic Staphylococcus aureus strains.

Fig. III.1.

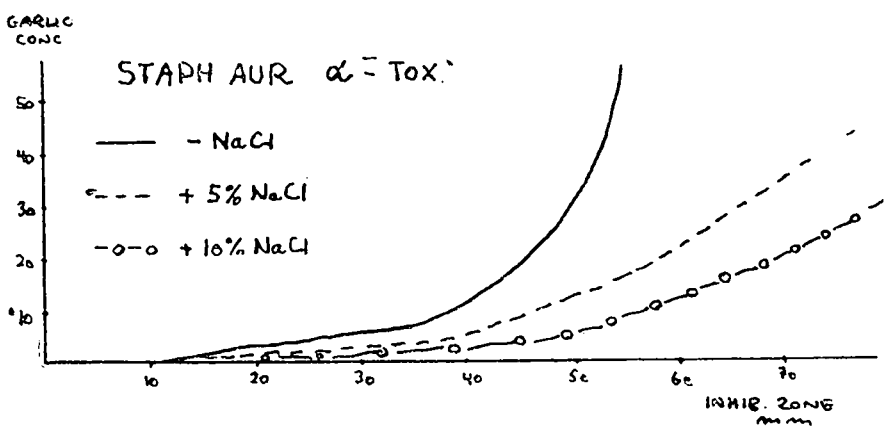


Fig. III.2.

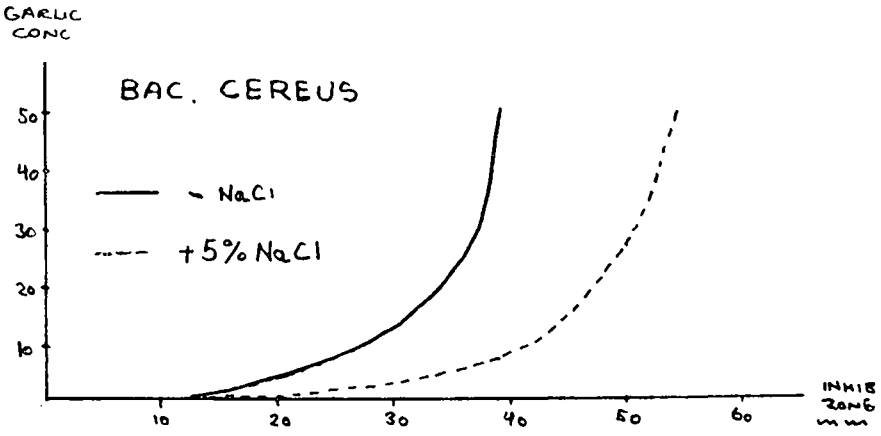


Fig. III. 3.

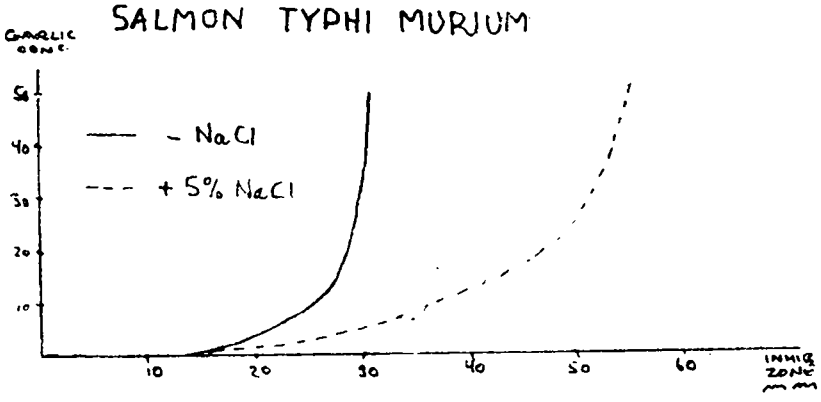


TABLE III. 4.

BLOOD AGAR (BA) + 5 % NaCl
Diam. of inhibition zone, mm.

Garlic conc.	Staph. A 100	Staph B 56	Staph. C 631	Staph. D 315	Staph. D 494	Staph. epiderm
5 %	29	30	29	26	29	31
1 %	13	13	19	15	13	13
0.1 %+	<12	<12	<12	<12	<12	<12
0.01 %	<12	<12	<12	<12	<12	<12

+) Diameter of the hole drilled in the centre: 12 mm.

The table shows bactericidal action of 1% garlic (and below) in 5% NaCl environment.

The antibacterial effect of diallyl-disulphide was also tested. A minor inhibitory effect was recorded with 1% diallyl-sulphide solution for Salmonella, Staphylococcus aureus, Bacillus cereus and Pseudomonas.

Discussion

It has been known for centuries that the storage life of food can be prolonged by the addition of spices. And a number of reports on the inhibitory effect of spices on bacterial growth have been published.

In 1964, Pride (7) reports on the effect of clove oil and Özer and Özalp (8) described the bactericidal effect of garlic on Staph. aureus. The latter investigation was carried out in a liquid medium with 48 hrs old cultures, and an incubation period of 5 days (up to 15 days). Besides the effect of the garlic, a natural reduction of the number of bacteria may be expected.

In order to prolong the shelf-life of fresh camel meat, Al-Delaimy and Barakat (1) treated fresh lean camel meat with 5, 10 and 15 % (by wt.) of fresh ground garlic segments and stored at room temperature (20°-22°C), 12°C and in a refrigerator (2°–3°C) respectively. They found that the period of shelf-life was increased 2,3 and more than 4-fold respectively, compared with the corresponding control samples. They stated that after 4 days storage at room temperature, 12 days at 12°C and 28 days refrigration, the meat treated 15 and 25 % garlic shown no sign of any organoleptic spoilage. They claimed that after frying for 15 minutes the meat samples were found to be acceptable in taste and flavour by local people in Saudi Arabia.

Fletcher and co-workers (5) studies the inhibition of coagulase activity and growth of Staph. aureus in garlic extracts. They found that garlic extract (1 mg dry weight/ml) inhibited the coagulase reaction and increased the time of coagulation by a factor of 1.5, whereas 4 mg dry weight/ml increased the coagulation time factor by 2.75. It was also shown by these workers that 1.4 mg garlic (dry weight) /ml reduced growth in nutrient broth while 5.6 mg dry weight / ml was completely inhibitory. They did not observed these effects until 8 h after exposure of the organisms to the garlic extracts. It was found that 5.9 % more survivors among garlic treated mice compared to non-treated animals.

At the Fourth International Symposium on Food Microbiology, Gothenburg, 1964, Ingram et al. (6) expressed a desire for a suitable procedure for estimating the preservative effect of natural inhibitors.

The concents of diallyl disulphide and mercaptanes in garlic are well-known. A synthetic garlic oil consisting of

diallyl-disulphide	85	%
allyl mercaptane	5	%
dimethyl sulphide	2	%
buthyl thiocyanate	5	%
Conc. acetic acid	3	%

has been used in the meat industry for a long time (2).

The general inhibitory effect of enzymes by means of allyl groups and SH groups has been studied in detail and the phenomenon "biochemical lesion" defined by Peters (4) may be dealt with in a more detailed discussion on the effect of the inhibitory mechanism.

The varying sensitivity of microorganisms to natural inhibitors such as garlic is of considerable interest to the understanding of keeping quality problems. Cavallito (3) isolated a bactericidal agent from garlic. He described the agent as a colourless substance containing 40 % sulphur, which was decomposed by dry distillation. The substance in called allicin. Natural diallyl dissulphide showed practically no antibacterial effect.

Considering the minimum effect level of garlic demonstrated in the present experiment, it is obvious that the concentrations of garlic used in Danish meat industries (0.02 %) have no noticeable inhibitory effect, with or without the addition of salt.

The Turkish product "Pastırma" has a coating (çemen) with a very high content of garlic providing the surface of the meat with approximately 10 15 % garlic. In the presence of 1 % garlic and 5 % NaCl all the tested Staphylococcus strains were inhibited.

The antimicrobial effect of the "natural" flora (e.g.Lactobacillaceae) in fermented meat products resulting in a decrease of pH and/or the antogonistic effect of spices other than garlic (e.g.pepper), and the effect of the meat itself is a problem too complex to be discussed here.

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