

NITRITES AND NITROSAMINES IN PROCESSED MEATS

SOME OBSERVATIONS ABOUT THE HEAT RESISTANCE
OF ENTEROCOCCI IN PORK MEAT SUSPENSIONS

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Strains of heat resistant enterococci isolated from pasteurized meat products have been incubated and heated both in the same pork meat suspensions. Heating tests were done at 68,9°C. At 8°C. the heat resistance of the enterococci incubated in the pork meat suspensions declined rapidly. In suspensions to which brine was added the heat resistance was much higher, but also declined at a fast rate during incubation at 8 and 18°C.

Partially these processes were not coupled to growth. The curing ingredients as a whole appeared to increase the heat resistance of the enterococci markedly, di- and tripolyphosphate exerting however a great influence in this way.

EINIGE BEOBSACHTUNGEN ZUR HITZERESISTENZ VON
ENTEROKOKKEN IN SCHWEINEFLEISCHSUSPENSIONEN

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Einige Stämme von hitzeresistente Enterokokken isoliert aus Fleisch-
halbkonserven wurden bebrütet und erhitzt in dieselbe Schweinefleisch-
suspensionen. Die Erhitzungsversuche wurden durchgeführt bei 68,9°C.
Beim bebrüten bei 8°C ergab sich dass der Hitzeresistenz in den Schweine-
fleischsuspensionen schnell abnahm. Wenn Pökkel verwendet wurde, war der
Hitzeresistenz viel höher, aber nahm auch hier schnell ab beim bebrüten
bei 8 und 18°C. Diese Vorgänge wurden auch beobachtet wenn kein Wachs-
tum gemessen wurde.

Es ergab sich, dass die Pökelingredienze zusammen der Hitzeresistenz
von Enterokokken erhöhen, di- und tripolyphosphat hatten jedoch ein
grosser Einfluss hierauf.

QUELQUES OBSERVATIONS SUR LA THERMORESISTANCE
DES ENTÉROCOQUES DANS DES SUSPENSIONS DE

VIANDE DE PORC

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Souches d'entérocoques de haute thermorésistance isolées des produits
carnés pasteurisés ont été incubé et puis chauffé dans les mêmes
suspensions de viande de porc. Les tests de chauffage sont faits à 68,9°C.
On observait que la thermorésistance dans les suspensions de viande de
porc diminuait rapidement pendant l'incubation à 8°C. Dans les sus-
pensions auxquels de la saumure a été ajouté la thermorésistance était
bien plus élevée, mais diminuait aussi rapidement durant l'incubation
à 8 et 18°C.

Partiellement ces phénomènes n'étaient pas liés à la croissance.
Les ingrédients de la saumure en totalité augmentaient la thermo-
résistance des entérocoques, le di- et le tripolyphosphate de sodium
avaient toutefois une grande influence dans ce sens.

НЕСКОЛЬКО НАБЛЮДЕНИЙ НАД ТЕРМОСТОЙКОСТЬЮ ЭНТЕРОКОККОВ В
С УСПЕНЗИЯХ СВИНИНЫ

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Цепи термостойких энтерококков, изолированных из пастеризо-
ванных мясных продуктов, вывелись и нагрелись в одних и
тех же суспензиях свинины. Делали тепловые опыты при 68,9°C.
Оказалось, что при 8°C термостойкость энтерококков быстро
уменьшалась. В суспензиях, к которым добавился рассол, термо-
стойкость была намного выше, но уменьшалась и здесь во время
выращивания при 8 и 18°C. Эти процессы, частью, не были свя-
заны с ростом. Оказалось, что рассольные ингредиенты вместе
повысили термостойкость, однако, в этом отношении оказали
большое влияние ди- и триполифосфат.

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Introduction

Enterococci often appear to survive the pasteurization process of meat products as is also reflected in some recent publications (4,10,13). During storage they may then develop even at lower temperatures. A lot of work has been done towards the heat resistance and thermal injury of enterococci (1,2,3,5,7,8,11,12,15,16,17,18,19) however not so much has been reported about these phenomena in meat products. So we started a project of this kind and tried to get more information about the heat resistance of enterococci by working with meat suspensions, which seemed easier manageable and moreover in a reproducible way. We worked at a pH of about 6,3. Heating tests were done at 68,9°C (156°F) immediately after the addition of bacteria and after several incubation periods at lower temperatures. We also investigated the influence of brines of varying compositions.

Materials and methods.

Selection of the test strains.

In collaboration with the Netherlands Centre for Meat Technology at Zeist (CIVO/TNO) we collected a great number of enterococcus strains which survived the pasteurization process of cooked hams. After isolation the strains were heavily inoculated in litmus milk and without incubation stored at -40°C. Their heat resistance was determined by heating double washed cells at 68,9°C in a phosphate buffer (Sørensen; pH 6,8). The bacteria were grown 24 hours in TDYM-broth at 37°C. TDYM is a rich medium (14) containing (g/l): 1,5 tryptone, 0,3 meat extract, 0,5 dehydrated yeast extract, 1,5 peptonized milk, 0,1 dextrose in distilled water; pH: 7,0±0,1. For the experiments described in this paper we used three strains, all variants of *Streptococcus faecium*, in code E₁, E₁₅ and E₂₀. E₂₀ possesses a high, E₁ and E₁₅ a moderate heat resistance.

Preparation and inoculation of the meat suspension

The meat was derived from hams of normal pigs, slaughtered at about 95 kg liveweight. The ultimate pH of the hams varied between 5,7 and 6,0. After removing bones, visible fatty tissue and tendons, the meat was passed through a mincing machine and finally comminuted and homogenized with a high speed chopper. Portions of 100 g were vacuum packaged and stored at -40°C. Before each experiment a package was first thawed in water at c. 40°C for about 30 min. The meat was then transferred to a waring Blendor jar and minced with 200 ml of distilled water during 1 min. at the highest speed. After deaeration and pH adjustment (experiments without brine) the whole was lightly sterilized (20 min. 112°C). After cooling down and mincing we got a suspension which would not coagulate again during heating.

In the experiments with brine 18% upon weight (meat suspension) was added aseptically prior to the addition of enterococci. The brines were filter sterilized.

Composition of the brine (type injection brine for cooked hams)

Sodium chloride	132,20 g
Sodium nitrite	0,800 g
Phosphate	33,000 g
Dextrose	33,000 g
Sodium-L(+)-glutamate	6,000 g
L(+)-ascorbic acid	3,000 g
made up to 1000,0 g with distilled water.	

All reagents were of an analytical grade except glutamate and sodium diphosphate (Na₂H₂P₂O₇) being chemically pure, sodium tripolyphosphate and "curafos" being commercial phosphates.

After addition of the brine the meat suspension contained about 90% water, 2% sodium chloride and 120 ppm sodium nitrite.

The enterococci were first cultivated in the litmus milk during 24 hours at 37°C and then another 24 hours at 37°C in TDYM-broth. From these cultures double washed cell suspensions were prepared in 0,1% peptone saline. After inoculation the meat suspensions were heated, but also incubated at different temperatures.

After several incubation periods the heat resistance of the enterococci in the meat suspension was determined again.

Execution of the heating tests.

The meat suspensions were dosed in equal amounts (about 5 ml) in sterile 6 oz Whirl-Pak bags, these were heat sealed. The bags were heated in a suspended position in a precision water bath at 68,9±0,1°C. Heating up times were about 30 sec. After heating the bags were cooled in ice-water. Counts were done in duplicate using the agar plate method (TDYM agar; incubation 2-3 days at 37°C).

D-values were calculated from the survivor curves for heat treatments up to 75 min. Over this period the curves were linear, which appeared to be significant.

Results and discussion

The most relevant results are summarized in table 1. Some of them have also been visualized in figure 1.

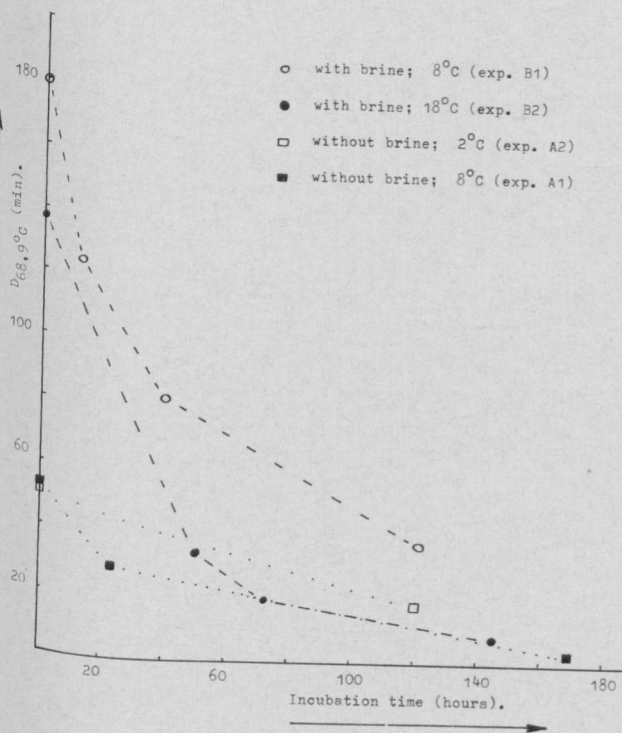
Table 1 Summary of results

Exp	Strain	Meat Sample	Incubation Time (hours) - Temperature	pH	10 ⁶ Log(Count/ml)	D _{68,9°C} (min)	Remarks
A1	E ₂₀	VI	0	6,5	3,74	49,7	Without brine
			24h - 8°C	6,5	3,84	34,4	
			168h - 8°C	6,5	5,65	4,4	
A2	E ₂₀	VI	0	6,5	4,71	48,6	Without brine
			120h - 2°C	6,5	4,79	25,0	
B1	E ₂₀	VI	0	6,2	4,11	180	Brine added,
			16h - 8°C	6,2	4,18	125	this brine
			40h - 8°C	6,2	4,15	78,8	contained
			120h - 8°C	6,2	4,18	38,0	curafos
B2	E ₂₀	VI	0	6,3	4,23	135	Brine added,
			48h - 18°C	6,3	4,74	36,7	this brine
			72h - 18°C	6,3	5,57	25,8	contained
			144h - 18°C	6,2	7,70	9,0	curafos
C1	E ₂₀	VI	0	6,3	4,96	139	"Ditri"-brine
			192h - 8°C	6,3	5,11	31	added. This brine contained a mixture of di- and tripolyphosphate (Na ₂ H ₂ P ₂ O ₇ : Na ₅ P ₃ O ₁₀ = 1 : 10). This composition gave the sauce brine pH as with curafos
C2	E ₁	VI	0	6,3	4,76	26,6	"Ditri"-brine
			168h - 8°C	6,3	4,72	19,7	added
C3	E ₁₅	VI	0	6,3	4,82	33,8	"Ditri"-brine
			168h - 8°C	6,3	5,63	16,3	added
C4	E ₂₀	VII	0	6,3	3,20	143	"Ditri"-brine
			144h - 8°C	6,3	3,23	46,6	added
C5	E ₂₀	VI	0	6,3	5,23	122	Raw meat
			24h - 8°C	6,3	5,08	40,6	suspension (1 : 3), "ditri"-brine added
D1	E ₂₀	VI	0	6,3	4,85	131	"Di"-brine
			24h - 8°C	6,3	4,88	118	added. This brine contained
			168h - 8°C	6,3	5,23	36,5	Na ₄ P ₂ O ₇ . The pH of the brine was adjusted with 8N HCl

Table 1 continued

D2	E ₂₀	VI	0	6,3	4,93	100	"Tri"-brine
			24h - 8°C	6,3	4,80	99	added.
			168h - 8°C	6,3	4,84	32,5	This brine contained Na ₅ P ₃ O ₁₀ . The pH of the brine was adjusted with 8N HCl
D3	E ₂₀	VI	0	6,6	4,67	39,5	"Ortho"-brine
			24h - 8°C	6,6	4,73	45,0	added. This brine contained
			144h - 8°C	6,6	6,04	20,5	a mixture of NaH ₂ PO ₄ and Na ₃ PO ₄ . A composition was chosen which gave about the same brine pH as with curafos
D4	E ₂₀	VI	0	6,1	4,73	88,2	A brine was
			24h - 8°C	6,1	4,76	59,9	added from
			192h - 8°C	6,1	6,67	19,3	which the phosphates were omitted

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Fig. 1. Decline of the $D_{68,9^{\circ}\text{C}}$ - value during incubation.

The accuracies of the plate count (9) and the D-value estimation have been calculated only for experiment B1 (Table 2). In the other experiments the reported values have been estimated in the same way and are thus of a comparable level of accuracy.

Table 2.

Accuracy of the plate count and the D-value estimation

10^6 Log (Count/ml)	Limits (95% probability)		$D_{68,9^{\circ}\text{C}}$ (min.)	Limits (95% probability)	
	Lower	Upper		Lower	Upper
4,11	4,03	4,18	180	167	189
4,18	4,10	4,25	125	115	131
4,15	4,07	4,22	78,8	78	79
4,18	4,10	4,25	38,0	37,6	38,6

Experiments A1 and A2 show a decrease in the heat resistance of E_{20} during incubation in the pork meat suspension. At 8°C there is some growth during one week and the heat resistance falls to a low level. At 2°C there is hardly any measurable growth during 5 days, the heat resistance however declines rather rapid.

In experiments B1 and B2 a "curafos" brine was added to the meat suspension resulting in a much higher heat resistance of the enterococci. At 8°C growth was not measurable during 5 days, the heat resistance however declined rapidly. At 18°C growth was rather good during 6 days, the heat resistance declining more again.

A similar decline in the heat resistance was demonstrated with a brine containing a known mixture of polyphosphates instead of curafos (exp.C1). The same was shown for two other test strains E_1 and E_{15} and for another meat sample (experiments C2 to C4). In a raw meat situation (dilution 1:3) a similar process could be demonstrated (exp. C5). The experiments D1 to D4 were drawn up to study the influence of the brine composition (with regard to the phosphates) upon this phenomenon. With the "ortho"-brine (exp.D3) the heat resistance was immediately much lower, even lower than with the "no phosphate" brine (exp.D4). A first hypothesis that polyphosphates are largely converted to "ortho" phosphates and thus could lower the heat resistance during incubation could not be proved by thin layer chromatography.

The experiments with brines (except "ortho"-brine) showed an increase of the heat resistance caused by the curing ingredients together, di- and triphosphates demonstrated however a great influence in this way.

The theoretical background of the reported decrease in heat resistance remains to be elucidated and also must be cleared if such a process may take place during the preparation of a meat product, for instance cooked ham.

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