KURZE MITTEILUNG ÜBER DIE HITZERESISTENZ EINES VON DER KERNERWEICHUNG IN DOSENSCHINKEN ISOLIERTEN STREPTOCOCCUS FAECALIS

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ZUSAMMENFASSUNG

Der Mikroorganismus, welcher Kernerweichungen bei Dosenschinken verursacht, wurde isoliert und als Streptococcus faecalis var. liquefaciens identifiziert. In APT-Bouillon mit 3%igem NaCl betrug der F_{150}-Wert 11,7 Minuten und der z-Wert 7,6°F. Die Hitzeresistenz wurde auch in gepökelten Schinkenmuskeln bestimmt, und es zeigte sich eine Beziehung zwischen dem Kochsalzgehalt und dem F-Wert. Bei Schinken mit einer Kochsalzkonzentration von 3% betrug der F_{150}-Wert 24 Minuten und der z-Wert 6°F. Der F_{150}-Wert erhöhte sich zehnmal für einen Anstieg von 4,6% NaCl im Fleisch bei Schinken mit einem NaCl-Gehalt im Bereich von 1-8%, was als ein z(Kochsalz)-Wert bezeichnet werden darf. Mit steigendem F-Wert erhöhte sich auch der z(Temperatur)-Wert, wobei mit einem Anstieg des F-Wertes von 31 Minuten der z-Wert um 1°F zunahm. Also kann man zum Ausdruck der Hitzeresistenz dieser Art Streptococcus faecalis eine Erweiterung der üblichen Formel verwenden:

$$\frac{z_2}{r_2} \quad \frac{z_1}{r_1} \qquad F = x \text{ Min}$$

z_1 = z-Wert (Temperatur)
r_1 = Bezugstemperatur
z_2 = z-Wert (NaCl)
r_2 = NaCl Bezugskonzentration
x = Zeit (Min), in der eine 7 log Reduktion der Population erzielt wird

z.B. Für Strep. faecalis im Schinken

$$\frac{4.6}{3.0} \quad \frac{6.0^{+}}{150} \qquad F = 24 \text{ Min}$$

+ Für je einen Anstieg des F-Wertes von 31 Minuten ist 1.0 hinzuzufügen.

NOTE SUR LA THERMORESISTANCE D'UNE SOUCHE DE STREPTOCOCCUS FAECALIS ISOLEE DU RAMOLLISSEMENT DU NOYAU CENTRAL DES CONSERVES DE JAMBON

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RESUME

L'organisme qui cause le ramollissement du noyau central dans des conserves de jambon cuit a été isolé et identifié comme Streptococcus faecalis var. liquefaciens. Dans un bouillon APT avec 3% NaCl la valeur F_{150} était 11.7 min et la valeur z 7.6°F. La thermorésistance a été également déterminée dans des muscles de jambon salé, et une relation a été trouvée entre la teneur en sel et la valeur F. Dans du jambon contenant 3% NaCl la valeur F_{150} était 24 min et la valeur z 6°F. Dans des jambons ayant une concentration de NaCl dans la gamme 1-8% la valeur F_{150} s'augmentait dix fois pour une augmentation de sel de 4.6% dans la viande. Cela peut être exprimé comme la valeur z (sel). D'ailleurs, à mesure que la valeur F s'augmentait, la valeur z (température) montait aussi; pour chaque hausse de F de 31 minutes, la valeur z s'augmentait de 1°F. La thermorésistance de cette souche de Strep. faecalis peut être exprimée ainsi au moyen d'une extension de la formule normale:

$$\frac{z_2}{r_2} \quad \frac{z_1}{r_1} \qquad F = x \text{ min}$$

z_1 = valeur z (température)
r_1 = température témoin
z_2 = valeur z (NaCl)
r_2 = niveau témoin de NaCl
x = temps (min) pour réaliser une réduction 7 log de la population

Par exemple: Pour Strep. faecalis dans le jambon

$$\frac{4.6}{3.0} \quad \frac{6.0^{+}}{150} \qquad F = 24 \text{ min}$$

+ Ajouter 1.0 pour chaque augmentation de F de 31 min.

A NOTE ON THE HEAT RESISTANCE OF A STREPTOCOCCUS FAECALIS ISOLATED FROM A "SOFT CORE" IN CANNED HAM

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SUMMARY

The causative organism of soft core in canned cooked ham was isolated and identified as Streptococcus faecalis var. liquefaciens. In APT broth with 3% NaCl the F_{150} value was 11.7 min and the z value 7.6°F. Heat resistance was also determined in cured ham muscles and a relationship was found between salt content and F value. In ham containing 3% NaCl the F_{150} value was 24 min and the z value was 6°F. In hams with NaCl levels within the range 1-8%, the F_{150} value increased 10 fold for an increase of 4.6% salt in the meat. This may be expressed as a z (salt) value. Also as the F value increased, so did the z (temperature) value; for a rise in F of 31 min the z value increased by 1°F. Thus the heat resistance of this strain of Strep. faecalis may be expressed in an extended version of the normal formula:

$$\frac{z_2}{r_2} \quad \frac{z_1}{r_1} \qquad F = x \text{ min}$$

z_1 = z value (temperature)
r_1 = reference temperature
z_2 = z value (NaCl)
r_2 = reference NaCl level
x = time (min) to effect a 7 log reduction of the population

e.g. For Strep. faecalis in ham

$$\frac{4.6}{3.0} \quad \frac{6.0^{+}}{150} \qquad F = 24 \text{ min}$$

+ Add 1.0 for every rise in F of 31 min.

A NOTE ON THE HEAT RESISTANCE OF A STREPTOCOCCUS FAECALIS ISOLATED FROM A "SOFT CORE" IN CANNED HAM

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Of the enterococci 2 species, Strep. faecalis and Strep. faecium may be regarded as ubiquitous in meat plants and commonly occur in most meat products, particularly those which have received pasteurising heat treatments. These species are among the most heat resistant of non-sporing bacteria, and a considerable amount of work has been carried out on their heat resistance, mostly in non-meat media such as buffer, broths and other natural fluids (e.g. skim milk).

For the meat processor, the main concern is their ability to cause spoilage of the product, which can occur as acid (sour) flavour defects or as colour problems in the ham when sliced. In addition there can occur a "soft core" condition in canned hams. Such softening of the meat occurs at the centre of canned hams, luncheon meats, canned ribs and pork loins, due to gross enzymatic breakdown of the intramuscular and intermuscular connective tissue. In most cases this was found to be due to the gelatinase activity of Strep. faecalis var. liquefaciens (Coretti & Enders, 1964).

The "soft core" condition was recently recognised in our experience from a batch of canned hams (10 lb) which were approximately 4 months old. When the hams were cut, there was no off odour or discolouration, even when left for 24 hr at room temperature. However, the consistency of the core (ca. 40 mm diameter) was that of a meat paste, although the muscle structure macroscopically appeared normal.

In this paper the isolation, identification and in particular the heat resistance of the causative organism in both broth and ham media are described.

MATERIALS AND METHODS

Examination of "soft core" in canned ham. 5 gram samples of both the "soft core" and normal tissues from the ham were aseptically removed and homogenised with 45 ml diluent (% w/v, NaCl, 4.0; peptone, 0.1), serial dilutions prepared and plated out by the drop and spread technique on a total count medium (TCM) (Gardner, 1968). Plates were incubated for 4 days at 37°C. The analysis of curing salts in the meats is described elsewhere (Gardner, 1971). The methods and tests used to identify the organism were those of Sharpe, Fryer & Smith (1966).

From this, F values, the time in minutes to completely kill a given population at a specific temperature, were calculated by the following method:

$$F = 7 \times D + \text{lag period}.$$

In each experiment the z value, the number of degrees Fahrenheit increase or decrease for a tenfold increase or decrease in F value, was determined by plotting Log F against temperature.

RESULTS AND DISCUSSION

The results of analyses of the soft core and normal tissues are summarized in Table 2.

TABLE 2. Levels of curing salts (on a wet weight basis) and total colony counts of ham.

	pH	NaCl %	$NaNO_3$ ppm	$NaNO_2$ ppm	Total colony count/g
Normal firm meat	6.3	5.90	<5	24	275,000
Soft core meat	5.4	5.90	<5	12	700,000,000

The soft core tissues differed from the surrounding firm meat in two features. Firstly, the pH was approximately 1 unit lower in the soft core, and this was associated with very large numbers of bacteria ($>10^8$/g). Colonies on the plates from both samples appeared similar and a number from each were found to be streptococci.

One strain from the soft core sample was identified further and used for the heat resistance studies. This strain was a Gram positive, catalase negative, homofermentative coccus, which has the following characteristics: grew at 10° and 45° but not 50°; grew in the presence of 0.1% methylene blue, 0.04% potassium tellurite and 6.5% NaCl; reduced tetrazolium; produced acid from arabinose and melibiose, but not sorbitol or melezitose, and was able to liquefy gelatin. These criteria would indicate that the species is Strep. faecalis var. liquefaciens.

Thus the soft core condition was caused by the growth and activity of Strep. faecalis resulting in a type of spoilage in the centre of canned ham similar to that described by Coretti & Enders (1964). The organism had survived the heat treatment given to the cans, the aim being to achieve a centre temperature of 156°F, and had multiplied over a period of months at temperatures probably nearer ambient than normal refrigeration to a level at which gross degeneration of the connective tissue in the meat became evident.

Heat Resistance Determination

Media. 1. APT broth (Difco) with a final level of 3.0% w/v NaCl. 2. Cured ham muscles. Samples of uncooked cured ham muscles were trimmed free of fat and connective tissue and finely minced. A sample was taken for analysis of curing salts and the remainder inoculated with the Strep. faecalis. The inoculated material was then minced a further 3 times, dispensed, using a sterile syringe and a Pasteur pipette attachment, in 1.0 g ± 0.05 g amounts into 1 ml glass ampoules (Adelphi (Tubes) Ltd., London), and sealed.

Inoculum. The Strep. faecalis was streaked out on APT agar (Difco) and incubated at 37°C for 24 h. A loopful of cells was shaken with 45 ml diluent (as above) and 10 ml of inoculum was added to ca. 350 g of prepared ham.

Heat treatments. The heat treatments were carried out in a water bath and the sampling times are given in Table 1.

TABLE 1. Temperatures and times of heat treatments.

	Temperature (°F) of heat treatment			
	140	143	147	150
Sampling times (min)	30	10	2.5	2.5
	60	20	5.0	5.0
	120	40	10.0	7.5
	180	60	15.0	10.0
	240	90	20.0	12.5
	300	120	30.0	15.0

Enumeration of survivors. For each meat at each sampling time, 2 ampoules were removed from the bath and cooled in industrial alcohol. The ampoules were then flamed (including unheated zero samples) and transferred aseptically to a bottle containing 45 ml diluent (as above). The bottles were shaken vigorously to break the ampoules and were then homogenised for 10 sec, using an Ultra-Turrax homogeniser, the use of which has been described elsewhere (Gardner & Kitchell, 1973).

On each quarter of a plate of TCM agar, 0.02 ml of serial dilutions of the homogenate were dispensed and spread using a Davis dropping pipette (Astell Cat. No. 851) and a wireloop. Colonies were enumerated after the plates had been incubated at 37°C for 3 days.

Heat Resistance Calculations. At each temperature survivor curves were plotted and from these the lag period, if present, and decimal reduction time, D, were calculated. D = the time in minutes at a specific temperature for a 90% reduction in the population.

The Heat Resistance of Strep. faecalis in APT broth

In each of 3 experiments the heat resistance of the organism was determined in APT broth, which had been supplemented with NaCl to a final level of 3.0% w/v. The pH of the medium was 6.82. The results, found to be very reproducible between experiments, are given in Table 3.

TABLE 3. F_{150} and z values for Strep. faecalis in APT broth containing 3% NaCl

Experiment No.	z value	F_{150} value*
1	7.8	12.0
2	7.8	11.2
3	7.3	12.0
Mean	7.6	11.7

* $F_{150} = D_{150} \times 7$

No lag period was noted in any of the survivor curves in experiments with the broth media.

The Heat Resistance of Strep. faecalis in Ham

As has been shown by most workers, the medium in which streptococcal cells are processed has a pronounced effect on their heat resistance. Thus it was decided to measure the heat resistance of the Strep. faecalis in raw freshly cured ham which was destined for cooked ham manufacture, so that the data would be directly applicable to the practical situation. Sixteen experiments were carried out on a range of different cured meats (Table 4) in an attempt to demonstrate whether there were variations in heat resistance which could be correlated to the levels of curing salts in the ham. Of all the criteria measured, there appeared to be a close relationship between the salt content of the ham and the F value (Fig. 1). An increase in salt content resulted in an increase in the F_{150} value.

The heat resistance of the Strep. faecalis was much higher in ham media than in broth. At the same salt content of 3%, the F_{150} value in broth was 11.7, while in ham the F_{150} value was 24.5. Such an effect has been noted by Hansen & Riemann (1963). Generally speaking the heat resistance of enterococci is much greater in foods than in media such as broths, buffers and salt solutions which are widely used in this type of work.

The influence of NaCl content, or a_w, on the heat resistance of many non-sporing bacteria has been recognised for many years. The effect of increased salt or lowered a_w tends to increase the heat resistance of the cells (e.g. Horner & Anagnostopoulos, 1975). This has been demonstrated for

Table 4. Levels* of curing salts and heat resistance characteristics (F_{150} and z) of Strep. faecalis in hams.

Ham No.	Curing salts NaCl (%)	$NaNO_3$ (ppm)	$NaNO_2$ (ppm)	Moisture (%)	pH	Heat resistance of Strep. faecalis z	F_{150}
1	1.02	<5	44	76.11	6.00	6.0	12
2	1.96	36	40	75.73	6.05	6.7	22
3	2.10	29	56	74.81	5.80	5.5	20
4	2.32	<5	26	76.31	6.00	6.8	28
5	2.34	21	40	76.94	6.30	5.9	26
6	2.92	49	60	76.38	6.00	5.4	39
7	3.20	200	152	76.37	6.00	8.4	73
8	3.40	34	72	76.86	6.10	6.6	33
9	3.58	84	112	75.92	6.80	8.4	82
10	3.90	<5	28	77.61	6.00	8.5	76
11	4.50	200	196	76.27	6.10	8.0	67
12	4.66	<5	52	76.93	6.40	8.4	126
13	4.86	48	76	76.02	6.60	9.8	162
14	5.82	50	72	74.31	6.20	14.0	269
15	6.20	20	12	72.38	6.50	8.5	120
16	6.60	30	14	74.16	6.45	9.4	123

* On a wet weight basis.

lactobacilli (Vrchlabsky & Leistner, 1971) and also enterococci (Vrchlabsky & Leistner, 1970). In the latter study, the authors adjusted broth media to various a_w values and found that the enterococci were most heat resistant at a value of 0.95, which would be equivalent to 6.6% NaCl in the aqueous phase. However, Incze (1968) using broth media found no difference in D value of a Strep. faecalis, when 2% NaCl was included.

There also appeared to be a relationship between F_{150} value and z value for the Strep. faecalis (Fig.2), when determined in ham. Within the range examined, the z value increased 1°F for each rise in the F value of 31 min. Thus as the F_{150} value increases, changes in temperature in the critical range associated with canned ham processing have less effect on the rate of thermal destruction.

We can then express the heat resistance characteristics of the Strep. faecalis more comprehensively by an extension of the normal formula:

$$F^{\frac{z_2}{r_2}}_{\frac{z_1}{r_1}} = x \text{ (min)}$$

z_1 = z value (temperature)
r_1 = reference temperature
z_2 = z value (NaCl)
r_2 = reference NaCl level
x = time (min) to effect a 7 log reduction of the population.

e.g. for the Strep. faecalis in ham

$$F^{\frac{4.6}{3.0}}_{\frac{6.0*}{150}} = 24 \text{ min}$$

* add 1.0 for each rise in F of 31.

Many factors are known to affect the heat resistance of faecal streptococci, such as age of culture (White, 1953; Beuchat & Lechowich, 1968) and pH of the medium (White, 1963). Those directly applicable to the heat resistance in the meat environment include fat or oil content, which increases heat resistance (Jensen, 1954; Zakula, 1969). Also the length of time the cells are in the meat before the heat treatment is carried out has a marked effect, probably associated with the stage of growth of the organism. Hansen & Riemann (1963) found a marked reduction in the heat resistance of streptococci in ham after a 3 hr incubation at 42°C before heat treatment. This has been confirmed by Houben (1974) at lower incubation temperatures 8°C and 18°C for longer periods.

The heat resistance of a coccus, most probably an enterococcus, was found to increase from 40 min to 400 min at 150°F, when the cells were grown in meat rather than broth (Brown, Vinton & Gross, 1960).

Curing salts other than NaCl can influence the heat resistance of streptococci in meats. Greenberg & Silliker (1961) found that 100 ppm nitrite in broth increased the death rate of Strep. faecalis and Strep. faecium at 155°F and 158.5°F, but not at 148°F, an effect which could be reversed with ascorbic acid. They concluded that nitrite plus a crucial amount of thermal energy can render enterococci incapable of outgrowth in pasteurised hams. However, Incze (1968) found that nitrite in the level of 2 mg% in broth had no effect on the D value of Strep. faecalis. Polyphosphates in ham have been reported to markedly increase the heat resistance of Strep. faecium (Houben, 1974). The converse has also been reported (Kniewallner & Prandl, 1972).

Thus in conclusion, much more data is required to elucidate individual and combined factors which influence the heat resistance of enterococci in cured meats. Marked changes in product formulation (e.g. NaCl) will undoubtedly affect the minimum safe process, to ensure ham stability, and this must always be considered with the organoleptic qualities of the product.

Fig. 1. Relationship between log F_{150} value for Strep. faecalis and salt content of the ham.

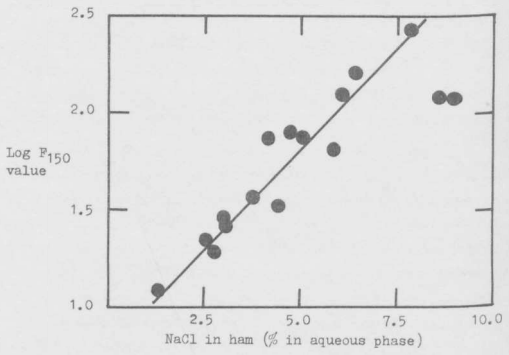
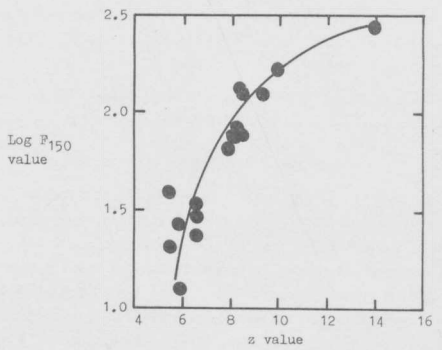


Fig. 2. Relationship between F_{150} and z value of Strep. faecalis determined in ham.



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