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A RATIONALE FOR THE SAFETY OF CANNED SHELF-STABLE CURED MEAT: PROTECTION = DESTRUCTION + INHIBITION.

by

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The safety of canned, cured shelf-stable meat with respect to Clostridium botulinum, depends on a mild heat treatment (Fo 0.2 - 0.6) in the presence of curing salts, especially sodium chloride and sodium nitrite. As a 12 D thermal process is not imposed, it appears that safety depends on destruction of some spores and inhibition of those that survive. Thus, protection (Pr) against C. botulinum can be expressed by the equation, $x\ Pr = y\ Ds + z\ In$, where Pr, Ds, and In are equivalent to 1 $\log_{10}$, Ds is destruction and In is inhibition. Sodium chloride, nitrite and a reaction product of nitrite appear to contribute most of the In values; the contribution of each can be quantified, but depends in part on heat damage to the spores that escape destruction.

ANALYSE RAISONNEE SUR LA SECURITE DES VIANDES SALEES EN BOITES SANS BESOIN DE REFRIGERATION: PROTECTION = DESTRUCTION + INHIBITION.

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Les viandes en boîtes qui peuvent être gardées sur tablettes, sont considérées comme étant sans contamination de Clostridium botulinum, àpres un léger traitement à la chaleur (Fo=0.2-0.6) en présence de sels curateurs tel le chlorure de sodium et le nitrite de sodium.

Comme le traitement 12D n'est pas imposé, il appert que la sécurité dépend sur la destruction de certaines spores et l'inhibition de celles qui survivent. Ainsi la protection (Pr) contre le C. botulinum peut être exprimée par cette équation, x Pr=y Ds+y In, où Pr, Ds et In sont équivalent à 1 $\log_{10}$, Ds étant la destruction et In l'inhibition.

Le chlorure de sodium, le nitrite de sodium et un produit de la réaction du nitrite, semblent contribuer le plus à la valeur de l'In; la contribution de chacun peut être quantifiée, mais elle dépend en partie du dommage fait par la chaleur sur les spores qui échappent à la destruction.

EINE ERKLARUNG DER HALTBARKEIT VON POKELFLEISCHKONSERVEN: "PROTECTION = DESTRUCTION + INHIBITION".

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Die Haltbarkeit von Pökelfleisch in Vollkonserven in bezug auf Clostridium botulinum wird durch eine milde Hitzebehandlung (Fo = 0,2 - 0,6) in Gegenwart von Pökelsalzen, vor allem Kochsalz und Natriumnitrit, gewährleistet. Da die Hitzebehandlung weit unter dem 12 D-Wert liegt, ist auzunehmen, dass einige Sporen zerstört, die übrigen dagegen nur an ihrer Vermehrung gehindert werden. Die Sicherheit (Pr = Protection) einer Konserve könnte somit durch die Gleichung Pr = y Ds + z In ausgedrückt werden, wobei Ds (Destruction) und In (Inhibition) Zerstörung bzw. Hemming von Sporen, beide in $\log_{10}$-Einheiten darstellen. Der In-Wert scheint vor allem vom Kochsalz, Nitrit und einem Reaktionsprodukt des Nitrits abzuhängen. Der Beitrag jeder dieser Komponenten wird wiederum von der Hitzeschädigung der überlebenden Sporen bestimmt.

ПОДХОД К ОБЕСПЕЧЕНИЮ СОХРАННОСТИ КОНСЕРВИРОВАННОГО СКЛАДОУСТОЙЧИВОГО ОБРАБОТАННОГО МЯСА: ЗАЩИТА = РАЗРУШЕНИЕ + ЗАДЕРЖИВАНИЕ

Сохранность консервированного обработанного складоустойчивого мяса в связи с "Clostridium botulinum" зависит от тепловой обработки /Fo = 0,2 - 0,6) в присутствии сохраняющих солей, особенно хлористого натрия и азотистого натрия. Так как тепловой процесс 12 D не применяется, то очевидно, сохранность регулируется путем уничтожения отдельных спор и торможением тех, которые выживают. Следовательно, защита /Pr) от "C. botulinum" может быть выражена уравнением $Pr = yDs + zIn$, где Pr, Ds и In эквивалентны I $\log_{10}$, Ds - разрушение и In - задерживание. Хлористый натрий, азотистый натрий и продукты реакции азотистого натрия, очевидно, определяют большую часть значения In насколько они влияют на In, можно определить количественно, но это зависит частично и от степени теплового разрушения спор, избежавших полного уничтожения.

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The thermal process given to non-acid or low-acid canned foods to prevent botulism is Fo=2.8. This thermal process will reduce 10^{12} spores of C. botulinum to 10^{0} spores, and this is usually referred to as a 12D process. In contrast, canned cured shelf-stable meats are usually processed to Fo=0.1-0.6, and the $\log_{10}$ of spores killed can be estimated to be in the order of <1.0 to about 4 (Riemann, 1966; Riemann, 1967; Pivnick et al, 1969; Schack et al, 1958; Spencer, 1966). Unfortunately, there is little published literature on the numbers of spores of C. botulinum that are actually destroyed during the usual thermal process of canned meat although there is abundant information on destruction in phosphate buffer and some information on destruction in food products other than cured meat.

Canned, cured shelf-stable meats, despite the low thermal process, and the small number of spores that are killed compared with a 12D process, are remarkably safe. Undoubtedly, the safety depends on factors other than destruction of spores, and these factors are inhibitory in that they act on the spores that survive the thermal process, either to prevent their germination or to prevent their outgrowth if they should germinate. It is likely that most of the inhibitory factors act by preventing outgrowth. Table 1 presents a list of inhibitory factors.

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Table 1

FACTORS WHICH INHIBIT GROWTH FROM SPORES OF C. BOTULINUM THAT HAVE SURVIVED HEAT PROCESSING.

1. NaCl in the water phase; %brine; Aw.
2. $NaNO_2$.
3. Reaction product of $NaNO_2$ with meat--the Perigo-type factor.
4. Fatty acid peroxides--inhibitory effect is reversed by starch.
5. pH.
6. The extent of thermal processing--the more severe the thermal process, the greater the heat damage to the spores that survive.
7. Temperature of storage of product after thermal process.
8. Duration of storage of product at a given temperature after thermal process.
9. Factors unknown at this time.

As the inhibitory factors are an integral part of a system that provides safety, it is highly desireable that their activity be quantified with respect to preventing outgrowth of C. botulinum spores that survive thermal processing. This is especially important because changing tastes have dictated a decrease in the concentration of sodium chloride used, and the present concern about nitrosamines may lead to a decrease in the concentration of nitrite. Although the effects of salt, nitrite, and heat damage have received considerable attention, less is known about the more subtle effects of pH, fatty acid peroxides, the Perigo-type factor (Perigo et al, 1967; Johnston et al, 1969), and time and temperature of post process storage. It is for this reason that we proposed an equation (Pivnick, 1970a; 1970b) to facilitate measurement of the extent of inhibition. The equation is:

Protection = Destruction + Inhibition

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In the equation Protection (Pr)=Destruction (Ds)+Inhibition (In), we have purposely avoided the term, D, because D is precisely defined as the time in minutes at a given temperature to destroy 90% of a population (Stumbo, 1965). Implicit in the definition of D is that the organism is not capable of recovering when given optimum conditions to grow.

Besides being used in the defined sense, the term D has been widely used in other ways to signify a 90% destruction without reference to temperature of exposure, e.g., by irradiation (Anellis et al, 1965), and to express the effect of inhibition (Duncan and Foster, 1960) and the combined effect of destruction +inhibition (Anellis et al, 1965). The misuse of "D", in our opinion, has diverted attention from the task of quantitating the inhibitory effect: in many cured products safety due to inhibitory effect may be much greater than that contributed by destruction of spores.

We believe that in order to define the factors responsible for safety against C. botulinum and to quantitate them, an equation is needed. The following equation appears to fit this need.

$$x\ Pr = y\ Ds + z\ In$$

Quantitatively, each of the components Pr, Ds and In is expressed on the basis of 1 $\log_{10}$. The equation is simple, easily comprehended, and workable.

The experimentation to exploit use of this equation is simple. Meat devoid of curing salts is inoculated with known numbers of spores at different concentrations, stuffed into commercial sized cans, processed to the desired degree, and incubated for 1 month. Assuming that meat devoid of curing salts is a perfect medium for growing the heat-damaged spores that survive, the most probable number (MPN) of spores that survive can be calculated from the ratio of cans processed/cans not swelling (Stumbo et al, 1950). To test the effect of individual or combinations of inhibitors, it is only necessary to treat meat to which inhibitors are added in an identical manner. There are three additional points to be

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considered: (1) the concentration of spores must be such that only a portion of the cans become toxic in at least one lot devoid of inhibitor and one lot with inhibitor, (2) meat with inhibitors must receive higher concentrations of spores than meat without inhibitors, and (3) the processed meat must be incubated for at least one year (Pivnick et al, 1969).

Table 2 illustrates the calculation of destruction of spores using data from an experiment where meat devoid of curing salts was inoculated to obtain 1 spore per g and 100 spores/g. Eleven cans with meat inoculated at each level were processed to Fo=0.6. On incubation, 1/11 and 8/11 cans, respectively, became toxic. A most probable number (MPN) pooled estimate of the spores surviving was 14.67. This pooled estimate was obtained by weighting the estimated proportion of spores surviving in each set of 11 cans inversely proportionally to their variances (Finney, 1971). The total number of spores in the 22 cans was 377,740; the number of spores killed was 377,725, and the ratio of spores added to the meat, to spores that survived the thermal process was 27,749:1 (Log_{10}=4.41).

Table 2 CALCULATION OF DESTRUCTION OF SPORES IN CANNED MEAT WITHOUT SALT OR NITRITE HEATED TO Fo 0.6.

No. of spores added.			377,740.0
Pooled estimate of No. of spores that survived.			14.67
No. of spores that were killed.			377,725.0
Ratio $\frac{\text{spores added}}{\text{spores survived}}$	$=\frac{377,740}{14.67}$	=	25,749.0
$Log_{10}\frac{\text{spores added}}{\text{spores survived}}$		=	4.41

Experiment: 1 spore/gm; 340 gm/can; 11 cans; Fo=0.6; 1/11 toxic.

100 spores/gm; 340 gm/can; 11 cans; Fo=0.6; 8/11 toxic.

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There is considerable evidence that heating of spores of C. botulinum in the presence of salt and nitrite does not influence the rate of destruction (Pivnick et al, 1970; Pivnick and Thacker, 1970; Ingram and Roberts, 1971). For this reason, we have assumed that spores heated in meat containing curing salts would be destroyed at the same rate as in meat devoid of curing salts.

In Table 3, we present calculations for an experiment in which meat containing 5.5% salt in the water phase was inoculated with 10^6 spores/gm. Ten cans were processed to Fo=0.6 and then incubated at 30 C for 18 months: 8 of the 10 cans became toxic. The total number of spores added was 3,400,000,000; the number that survived the heat treatment was 132,022; the number of survivors that grew was 16.1; and the ratio of total survivors to survivors that grew was 8,200 (Log_{10}=3.91). Thus in the equation, Pr=Ds+In, 8.32 Pr=4.41 Ds+3.91 In.

Table 4 presents similar calculations for 45 cans in which the meat contained both salt and nitrite.

Table 5 summarizes data for 10 lots of cans obtained from 3 separate experiments (Pivnick et al, 1969). The greatest protection was obtained in meat with 150 ppm of $NaNO_2$.

The units of inhibition due to salt appear to increase with salt concentration in the meat. Fig. 1 shows a curve fitted by the method of least squares for 5 lots of cans. This increased inhibition of heat-damaged spores of C. botulinum by increasing concentrations of salt has been shown in test tube experiments (Pivnick and Thacker, 1970).

The units of inhibition due to increased nitrite in the presence of a constant concentration of salt is shown in Table 6; the data are from Table 5. The presence of 150 ppm of nitrite increased the units of inhibition from 4.12 to 5.76.

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Table 3 CALCULATION TO DETERMINE Pr, Ds AND In UNITS IN MEAT WITH SALT.

	Log_{10} Units
1. No. spores added 3,400,000,000	9.53
2. Units of destruction or ratio: $\frac{\text{spores added}}{\text{spores survived}}$ (from Table 2).	4.41
3. No. spores that survived=132,022, i.e., antilog 5.12.	5.12
4. No. survivors that grew=16.1.	1.21
5. Units of inhibition (3-4) or ratio: $\frac{\text{spores survived}}{\text{spores grew}} = \frac{132,022}{16.1} = 8200.$	3.91
6. Units of protection (2+5)	8.32

8.32 Pr=4.41 Ds+3.91 In.

Experiment: 10^6 spores/gm; 340 gm/can; processed to Fo=0.6; 8/10 cans toxic; meat contained 5.5% NaCl in water phase.

Table 4 CALCULATION TO DETERMINE UNITS OF Pr, Ds AND In IN MEAT WITH SALT AND NITRITE.

	Log_{10} Units
1. No. spores added in 45 cans=1.53×10^{10}.	10.18
2. Ratio: $\frac{\text{spores added}}{\text{spores survived}}$ (from Table 2).	4.41
3. No. spores that survived=594,100, i.e., antilog 5.77.	5.77
4. No. survivors that grew=12.61.	1.10
5. Ratio $\frac{\text{spores survived}}{\text{spores grew}} = \frac{594,100}{12.61} = 47,112.$	4.67
6. Units of inhibition in $\log_{10}$ (3-4).	4.67
7. Units of protection in $\log_{10}$ (2+5)	9.08

9.08 Pr=4.41 Ds+4.67 In.

Experiment: 10^6 spores/gm; 340 gm/can; process Fo=0.6; 11/45 cans toxic; meat contained 5.16% NaCl in water phase and 75 ppm $NaNO_2$.

Table 5 SUMMARY OF DATA AND CALCULATIONS FOR DETERMINING Ds, In AND Pr.[a]

Lot	Spores /gm	Cans Toxic / Total	Brine %	$NaNO_2$ (ppm)	Logarithim$_{10}$ of Spores: Added	Added[b] / Survived (Ds)	Survived	Grew	Survived / Grew (In)	Protection (Ds + In)
1	10^6	4/10	5.80	0	9.53	4.41	5.12	0.71	4.41	8.82
2	10^6	8/10	5.50	0	9.53	4.41	5.12	1.21	3.91	8.32
3	10^4	1.45	5.16	0	8.18	4.41	3.77	0.004	3.766	8.176
4	10^6	29/46	5.16	0	10.19	4.41	5.78	1.66	4.12	8.53
5	10^6	43/46	5.00	0	10.19	4.41	5.78	2.10	3.68	8.09
6	10^6	3/44	5.30	150	10.18	4.41	5.77	0.49	5.28	9.67
7	10^6	11/45	5.16	75	10.18	4.41	5.77	1.10	4.67	9.08
8	10^6	1/45	5.16	150	10.18	4.41	5.77	0.004	5.766	10.176
9	10^6	9/10	4.90	75	9.53	4.41	5.12	1.36	3.76	8.17
10	10^6	6/10	5.50	75	9.53	4.41	5.12	0.96	4.16	8.57

[a]Experiment: all cans contained 340 gm meat and were processed to Fo=0.6.

[b]Experiment: based on growth in meat devoid of curing salts following process of Fo=0.6.

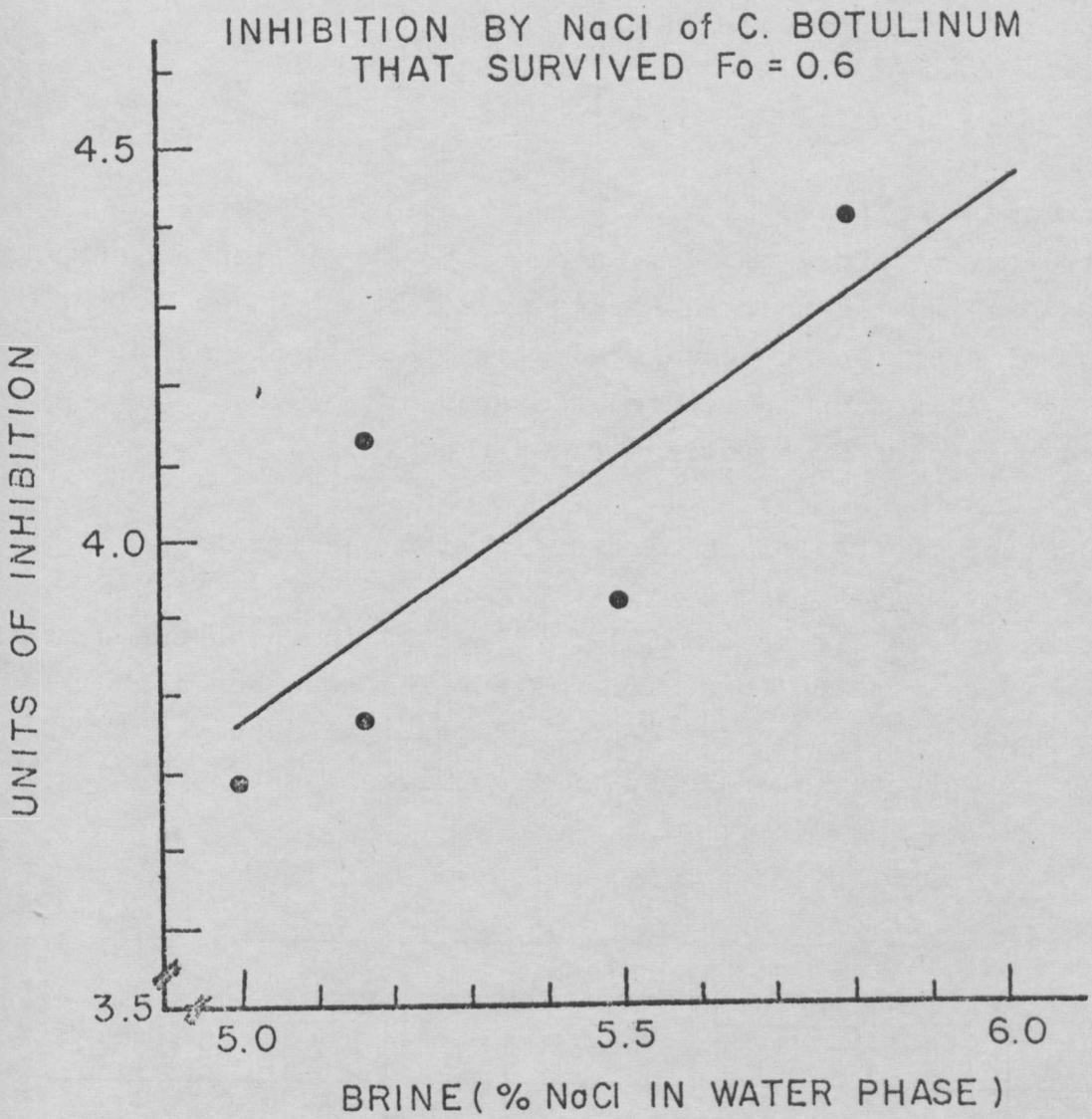

Fig. 1 INHIBITION BY SALT OF SPORES OF C. botulinum IN CANNED MEAT AFTER PROCESS OF Fo = 0.6.

Table 6 INCREASED INHIBITION DUE TO NITRITE.

Brine (%)	Nitrite (ppm)	Cans Toxic/Total	Units of Inhibition
5.16	0	29/46	4.12
5.16	75	11/45	4.67
5.16	150	1/45	5.76

Recent data (Chang and Pivnick, unpublished) indicates that the effect of nitrite may be due, in part, to a reaction product between nitrite and meat--a Perigo type factor. Meat made with 200 ppm of nitrite and inoculated after the nitrite had decreased to less than 2 ppm prevented the growth of 1 $\log_{10}$ heat-damaged spores of C. botulinum (1 unit of In).

The equation, Pr=Ds+In, is also useful for quantitating the inhibitory activity toward spoilage organisms. Table 7 shows data (calculated from Riemann, 1960) for PA 3679 in which cured meat was heat processed to Fo=0.6 or pasteurized and then irradiated to one megarad. The inhibition of spores that survived irradiation is far greater than those that survived Fo=0.6.

Table 7 PROTECTION = DESTRUCTION + INHIBITION FOR PA 3679.

	Logarithm$_{10}$ of Spores					
	Added	Added Survived (Ds)	Survived	Grew	Survived Grew (In)	Protection (Ds+In)
Fo = 0.6	6.49	0.29	6.20	5.20	1.00	1.29
One Mrad	6.49	0.97	5.52	1.67	3.85	4.82

Experiment: Cured ham; 5% brine; 30 ppm $NaNO_2$; heated to Fo=0.6 or heated to 65 C and then irradiated (Riemann, 1960)

We believe that the equation x Pr=y Ds+z In will facilitate the quantitation of inhibitory factors in canned, cured meats and thus lead to a rational assessment of the safety of the product. Certainly, such assessment is necessary whenever new formulations or thermal processes are likely to decrease the safety of the product.

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