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## INFLUENCE OF SODIUM CHLORIDE UPON HEAT RESISTANCE OF *BACILLUS STEAROTHERMOPHILUS* F.S. NCA 1518 SPORES IN FISH MUSCLE HOMOGENATES

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Key words: *Bacillus stearothermophilus*, sodium, chloride, fish muscle homogenates, thermo-resistant spores.

Highly thermoresistant spores of the known test organism F.S. NCA 1518 were used to study influence of sodium chloride upon bacterial spores during heating in fish muscle tissues. Homogenates of herring and mackerel were used as suspending media. Heat resistance determinations were made with use of Thermal Death Time Tube method (10 000 spores per tube) in four temperatures of the range 115-124°C. On the basis of Thermal Reduction Time values, there were computed parameters of thermal destruction curves representing 99.9999% destruction of initial spore load. Results were compared with thermoresistance in the buffer. Thermal resistance of spores had highest value in the M/15 phosphate buffer of pH 7.0 ( $F = 25.8$ ,  $z = 8.0$ ). Protective action of salt was discovered only in mackerel homogenates where 3% addition raised  $F$  value from 12.3 to 16.4 min. while in herring homogenates its action was less pronounced, and generally resistance of spores was lower. Values of 'z' have shown variations depending on suspending medium used.

### INTRODUCTION

Sodium chloride should be regarded as one of the most commonly used food additives. From the microbiological point of view the preserving activity of salt is linked at the same time to a reduced hydroactivity of food [2]. It is also reported that salt 's' effect on spores also results from reactions of chemical type [17].

Application of the new thermal processes in canned food industries which show a high capacity for spore-killing makes it unnecessary to use high concentrations of NaCl for preservation effect. Thus salt becomes largely responsible for organoleptic properties of processed food.

It is also reported, however, that low NaCl concentrations can alter the dynamics of thermal inactivation of spores. The destruction of the

latter conditions the safety of consumption and durability of low-acid canned foods. A number of studies concerning thermal inactivation of spores in foods of both plant and animal origins [8, 23] as well as in the generally used reference environment—the neutral phosphate buffer [14]—proved that 0.5 to 3.5% NaCl present in may induce protective action on heated spores of *Cl. botulinum*. There are also studies indicating absence of any protective effects of 3% and 6% NaCl on heated BA 3679 spores [15], and also reporting a progressively growing destructive effect of heat on *Bacillus thermoacidurans* heated in a suspension medium containing 1%, 2%, 4% and 8% NaCl [1].

Considering NaCl an integral component of fish canned products it can be surmised that effects of the salt on spores will depend on species of fish being processed [25].

Taking into account the three alternatives of NaCl effect on heated bacterial spores in fish muscle, it seemed interesting to determine how concentration of this salt and species of fish may alter heat destruction of highly thermoresistant spores of a strain inducing "flat sour" spoilage.

## MATERIALS AND METHODS

### MATERIALS

Lyophilized culture of *Bacillus stearothermophilus* strain NCA No. 1518 was supplied by American Type Culture Collection (Rockville, Maryland, U.S.A.).

For culturing and recovery of spores of test organism the following media were used: a) nutrient agar 2.5% — N A medium [12] for maintaining of active culture; b) nutrient agar supplemented with 0.1% of soluble starch and 0.01%  $MnSO_4$  — N A S medium [4, 20] was used as sporulation medium; c) glucose tryptone agar 2.5% — A G T medium [14] was used for making plate counts of spore suspensions; d) glucose tryptone agar supplemented with 0.5% of soluble starch — A G T S medium [13, 17] for making plate counts of heated spores; e) glucose tryptone broth — B G T medium [14] was used as diluent for quantitative transferring of heated samples with spores onto recovery medium. Instead of Bacto Tryptone (Difco), pepton of domestic brand "Peptobak" (Bacutil, Warsaw) was used in all media.

Spores were suspended and heated in: a) 1/15 M phosphate buffer pH 7.0; b) mackerel and herring muscle tissue homogenized with distilled water (1:1 proportion w/w) without additives, and with addition of 1.0, 2.0 and 3.0% of NaCl w/w, pH of herring homogenate was 5.9-6.1, and of mackerel was 6.2-6.4.

Herring and mackerel were of North West Atlantic origin, and came

from deep-sea fleet landings at autumn 1972 as frozen fillets. They were used for preparation of mince which were stored in 50 g portions at  $-20^{\circ}\text{C}$ , protected against access of air.

#### PREPARATION OF SPORE SUSPENSION

Spores were obtained using method of Thompson and Thames 20 with use of N A S,  $\text{MnSO}_4$ , supplemented medium [4]. Temperature of sporulation was  $55^{\circ}\text{C}$ . Spore crop was suspended in sterile distilled water in 250 ml flask to which several of glass beads were added and stored at  $4^{\circ}\text{C}$  until used.

Viable counts of stock spore suspension were made with use of A G T medium. Before platings spore suspension was heat activated 30 min at  $95^{\circ}\text{C}$ . Replicate platings of decimal dilutions were incubated 48 hrs at  $55^{\circ}\text{C}$ . Concentration of suspension was computed from colony counts by use of formula of Farmiloe et. al. [9]. Concentration of spores estimated after above mentioned heat shock was  $8.1 \times 10^6$  per ml.

#### DETERMINATION OF SURVIVAL CURVES IN PHOSPHATE BUFFER

Buffer suspension (pH 7.0) of 10 000 spores per ml was distributed into  $10 \times 100$  mm Termisil tubes in 2 ml quantities. Tubes were cotton plugged and hermetically stoppered. For each time-temperature combinations 5 tubes were heated. During heating the test tubes were closed in brass statives [25]. Which enabled their total immersion in glycerol bath with temperature maintained with accuracy of  $\pm 0.1^{\circ}\text{C}$ . Survival at four temperature levels was determined e.g. 115, 118, 121 and  $124^{\circ}\text{C}$ . After heating, tubes were cooled down in iced water, and 1 ml of heated suspension was divided between ten Petri dishes with A G T S and spreaded on the surface. Subcultures were incubated 48 hrs at  $55^{\circ}\text{C}$ .

#### DETERMINATION OF SURVIVAL CURVES IN FISH MEAT HOMOGENATES

Spore loads of 10 000 per ml of fish meat homogenates were prepared. The same concentration was used in all versions of homogenates. Homogenates were placed in test tubes with aid of syringe and were heated under the same conditions as buffer suspension. For each time-temperature combination 9 test tubes were heated. After cooling, to each test tube about 4 ml of B G T were added, and mixed with fish meat by glass rod. Whole mixture was next transferred into the 100 ml Erlenmeyer flask containing 40 ml of melted A G T S, and test tube was additionally washed with the same portion of BGT. Washing portion of broth was added to the same flask. Content of flask gently mixed and divided between 2 Petri dishes. Plates were incubated 32-38 hrs at  $55^{\circ}\text{C}$  and colonies surrounded by yellow zone of acidification were counted.

At each temperature level heating programme was designed to determine "end points of survival" as was suggested by Stumbo [19]. End points of survival could be here defined as numbers of spores recovered after final intervals of duration of heating which means gradual decrease of plate counts from 300 to less than 10 colonies per plate.

#### DETERMINATION OF PERCENTAGE DESTRUCTION CURVES

Counts of spores recovered after heating were expressed as percentage survival of initial population. Percentage survival points were plotted against logarithmic scale of survival and appropriate time of heating on the linear scale. Semilogarithmic survival curves were drawn by the experimental points which made possible easy and reliable reading of destruction times required at given temperatures to reduce spore population up to 0.0001%. Because some of these curves had bi-phasic form, typical for *Bac. stearothermophilus* spores [5] e.g. initial curved portion ("shoulder") was followed by linear part, this procedure was fully justified [16].

Times of heating were corrected for each temperature level for heating lag. Corrections have been made on the basis of temperature measurements using Ellab Electric Thermometer type TEC (Ellab, Denmark) with needle probe type TCK-9.

Destruction of spores during heating of each version of fish meat homogenate was described by values of time (min.) required at four temperatures to obtain 99.9999% destruction of initial spore load. Those data were plotted in a semilogarithmic arrangement: log of time against temperature of heating. That enable drawing of straight line destruction curves. Drawing of these graphs followed calculation of  $F$  (min) and  $z$  ( $^{\circ}\text{C}$ ) parameters of destruction curves. Those parameters were calculated by the least square method with use of linear regression equation in a form proposed by Worthing and Geffner [14].

#### RESULTS

Thermal Reduction Times required to achieve 99.9999% reduction of initial spore load in the tested suspending media are listed in Table 1. Comparison of their values for media not containing NaCl shows that buffer values are exceeding values obtained in the same set of conditions in both fish media, the differences being slightly more distinctive comparing with results for herring homogenate. Considering influence of increasing concentrations of NaCl in the tested homogenates varying patterns are observed. Namely, in mackerel homogenates increase of NaCl content results in constant raise of TRT-s values in all temperatures tested.

Exception is noted at higher temperatures of test for 3<sup>0</sup>/<sub>0</sub> of NaCl which suggests influence upon dynamics of heat inactivation.

In the herring homogenates, where higher dynamics of inactivation was detected, dependences were inverse comparing mackerel substrates. Generally, the highest TRT-s values were found in herring homogenate without NaCl addition but it could be noted that general order of data is slightly irregular comparing to mackerel homogenates.

Table. Thermal resistance of *Bacillus stearothermophilus* FS NCA 1518 spores suspend in neutral phosphate buffer, mackerel and herring meat homogenates

Suspending medium	Thermal Reduction Time (min.) required for 99.9999% destruction of spores during heating at:				Parameters of thermal destruction curves representing 99.9999% destruction	
	115°C	118°C	121°C	124°C	F (min.)	z (°C)
M/15 phosphate buffer pH 7.0	149.8	76.1	25.4	10.4	25.8	8.0
Mackerel meat homogenate:						
— without NaCl	70.6	29.8	13.3	5.2	12.3	8.0
— with 1% NaCl	74.7	34.4	13.7	—	13.1	8.2
— with 2% NaCl	94.5	35.5	15.5	6.6	15.1	7.8
— with 3% NaCl	95.2	45.0	14.1	6.3	16.4	7.4
Herring meat homogenate:			8.2			
— without NaCl	67.8	26.6	12.0	4.8	10.4	7.6
— with 1% NaCl	52.0	24.5	11.1	4.6	10.0	8.6
— with 2% NaCl	61.8	23.8	10.2	3.7	9.3	7.3
— with 3% NaCl	49.1	20.7	11.6	4.6	10.1	8.8

F values in herring homogenates are the lowest and reflect negligible response to increasing NaCl content.

Probably, observed variations of “z” values reflect changes of dynamics of heat inactivation of FS 1518 spores in applied substrates.

The average “z” values of thermal destruction curves determined in both kinds of fish muscles amounted to 8.1°C showing the variation not exceeding 7.5<sup>0</sup>/<sub>0</sub> for mackerel homogenates and 10<sup>0</sup>/<sub>0</sub> for herring homogenates. In phosphate buffer observed “z” value of 8.0°C was very close to the average “z” value in fish muscle homogenates.

## DISCUSSION

The simplest arrangements for this type of determinations were applied in reported experiments. In fish canning practice the environments suspending bacterial spores during heat treatment are getting more complex by application of several other additives beyond sodium

chloride and therefore, the purpose of model investigations was fully justified and attained. Presented work proved that, each environment even slightly different in respect of chemical composition, provides different conditions of heat destruction of microorganisms.

Heat lethal effects were influenced not only by the species of fish used but were also dependent on percentage of sodium chloride in fish muscles. Observed interrelationship, however, could not be evaluated univocally.

Mackerel and herring homogenates have had approximate pH values so this factor could be considered as having rather minor effects on differences of thermal resistance. Presumably, by analogy to other thermo-bacteriological works [14] they are resulting from distinction and specificity of chemical composition. There are some data [25] that specific origin of fish muscle may influence dynamics of thermal destruction of *Bacillus subtilis* spores suspended in water extracts prepared out of 8 species of fresh-water and marine fishes under identical pH values, and destitution of fat. Mechanisms of this type of action remain obscure.

Marked differences were noted in this work between thermal resistance shown by FS NCA 1518 spores in fish homogenates and neutral phosphate buffer. In this case the use of buffer resistance as a basis for thermal process evaluation would have caused unnecessary severe heating.

On the other hand differences of resistance in fish homogenates e.g. much higher resistance of spores in mackerel homogenates bear evidence on thermobacteriological distinctness of both media. This is additionally emphasized by different mode of action of sodium chloride on bacterial spores during heating in mackerel and herring homogenates. Namely, increase of sodium chloride content in mackerel homogenates caused progressive rise of thermal resistance of spores. This kind of action of sodium chloride was not observed in herring homogenates. Data from this work confirm the controversial opinions about sodium chloride action on thermal resistance of bacterial spores presented in available literature.

Accordingly to Walker and Matches [24] heating of bacterial spores in water media results in progressive loss of dipicolinic acid (DPA), peptides and other spore constituents. This opinion is supported by Duncan [6] who showed that presence of sodium chloride (0-3%) in suspending medium partially prevents spore from loss of DPA, and furthermore that this effect was more marked at pH 7.0 than at pH 6.0.

It might be also presumed that observed protective action of sodium chloride was due to water activity modifications of mackerel homogenates caused by this solute [2].

It is difficult however to find any explanation for lack of distinctive action of sodium chloride on spores heated in herring homogenates.

In this work variations of "z" values determined in both kinds of homogenates did not exceed 10% of average value. It was shown [14] that

"z" value of thermal death curve of *Clostridium botulinum* type A spores in phosphate buffer was influenced by presence of sodium chloride in this medium e.g. after incorporation of 3.5% of salt "z" value was about 50% higher than without salt. On the other hand it was found [1] that "z" values of thermal destruction curves of *Bac. thermoacidurans* spores in tomato juice had very small variations independently on salt concentration.

Opinion was presented [11] that variability of "z" value for thermal destruction curves of bacterial spores in different low-acid foods does not really exist, and that  $z = 10^{\circ}\text{C}$  has universal meaning in calculations of lethal values ( $F_0$ ) of thermal processed regardless of the variety of food. Up to date many calculations are made with such belief.

Value of "z" reported for FS NCA 1518 in the literature show variations in the range from  $6.6^{\circ}\text{C}$  to  $12.8^{\circ}\text{C}$  depending on suspending medium [3, 14, 18]. Similar variations were found for putrefactive anaerobe spores PA 3679 heated in different foods [3, 7, 19].

Presently, it was shown [22] that slope of thermal reduction curves of *Clostridium sporogenes* spores in two types of fish conserves had value of  $12^{\circ}\text{C}$ , and that for their sterilization different lethal thresholds were required. Seems purposeful to make reference to Hicks [10], who stated that use of  $z = 10^{\circ}\text{C}$  is justified as easy to use approximation when actual value of "z" is unknown but it does not make undoubtful that in all causes "z" equals  $10^{\circ}\text{C}$ .

Determination of actual "z" value for spores of important organisms in variety of canned foods has essential meaning because underestimating of this parameter in relation to actual value may lead to overheating of product, while use of "z" higher than actual may create danger of not adequate destruction of pathogenic organisms spores in food. None of this is the best for the consumer, and therefore the thesis that sterilization parameters should be evaluated individually for each assortment is fully justified.

## CONCLUSIONS

1. Intensity of sodium chloride protection during heating of bacterial spores depends upon kind of substrate to which it is added. This phenomenon should not be neglected when effectiveness of heat treatment of canned fish in brine is estimated.

2. Much milder heat treatment is required to obtain desired level of spore destruction in the herring muscle tissue than in the mackerel tissue.

3. The slope values of determined curves of thermal destruction allow a conclusion that  $z = 8^{\circ}\text{C}$  is sufficiently accurate approximation which may be used for evaluation of thermal destruction of FS NCA 1518 spores in herring and mackerel muscle tissue.

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*Manuscript received: December, 1977.*

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WPLYW CHLORKU SODOWEGO NA CIEPŁOOPORNOŚĆ SPOR *BACILLUS STEAROTHERMOPHILUS* F.S. NCA 1518 W HOMOGENIZATACH TKANKI MIĘŚNIOWEJ RYB

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Streszczenie

Przetrwalniki znanego szczepu testowego *Bacillus stearothermophilus* F.S. NCA 1518 ogrzewano w homogenizatach mięsa ryb atlantyckich — makreli i śledzia, z dodatkiem i bez dodatku NaCl. Oporność termiczną określano techniką próbkową w czterech temperaturach 115, 118, 121 i 124°C stosując stały ładunek spor — 10 000/próbkę. Wyznaczono krzywe redukcji termicznej i na tej podstawie wyliczono parametry krzywych śmierci termicznej spor na poziomie 99,9999%. Wyniki porównywano z parametrami krzywej zniszczenia w M/15 obojętnym buforze fosforanowym. Oporność przetrwalników posiadała najwyższą wartość w buforze ( $F = 25,8$ ,  $Z = 8,0$ ), w homogenizacie makreli była niższa ( $F = 12,3$ ,  $Z = 8,0$ ). Najniższą oporność termiczną spor stwierdzono w homogenizacie śledzia ( $F = 10,4$ ,  $z = 7,6$ ). Wyraźnie zaznaczone ochronne oddziaływanie soli stwierdzono tylko w homogenizacie makreli, gdzie 3% dodatek powodował wzrost wartości  $F$  z 12,3 do 16,4 min. Oddziaływanie NaCl w homogenizacie śledzia było zmienne i znacznie słabiej zaznaczone. Wartość "z" krzywych śmierci termicznej wykazała niewielką zmienność uzależnioną od zawartości soli w środowisku zawieszającym.