Evaluation of Bacteriological Stability of Minced Canned Meat Stored Under Simulated Tropical and Subtropical Conditions

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ABSTRACT

Thermal inactivation of spoilage microorganisms is one of the widely used commercial food preservation techniques. However, its application may be too costly in terms of energy expenditure or inappropriate in terms of product quality. In this study, an attempt was made to produce tropical storage stable canned meat using remarkably milder heating effects than those commonly used by food processors. "Canned beef in its own juice" was used as a model product and *Bacillus stearothermophilus* (Merck Art No. 11499), *Clostridium sporogenes* (ATCC 10000) and *Clostridium thermosaccharolyticum* (ATCC 7956) as reference strains. The raw minced meat used for the formulation of the product was contaminated with spores of the aforesaid strains at 10^4-10^6 spores/g prior to the heat treatment. The heating effects (F_c – values) were measured throughout the experiment at the geometrical middle point (GMP) of the can. The canned meat was thereafter stored up to six months under simulated tropical and subtropical temperatures of 55 ^{0}C and 25 ^{0}C respectively, followed by bacteriological investigations. Results showed that *C. sporogenes* is of no significance in the production of tropical storage stable canned meat products. This strain was eliminated by F_c = 3.0 even at a very high contamination level of 10^6 spores/g. At the same level of contamination, heating values of F_c = 13.0 and 16.0 were needed to eliminate spores of *C. thermosaccharolyticum* and *B. stearothermophilus*, respectively. At a reduced contamination level of 10^4 spores/g F_c values of 11.0 and 15.0 were necessary to completely eliminate the same bacteria, respectively. Addition of 0.4 % benzoic acid resulted into reduction of the F_c – value from 16.0 to 13.0 for the elimination of *B. stearothermophilus* at 10^6 spores/g contamination level. Adding the same amount of citric or acetic acid was enough to lower the heating value further to 11.0. Potassium sorbate was ineffective in increasing the heat sensitivity of *B. stearothermophilus*

even at a concentration of 4 % in the minced meat. It was concluded that the heating effects (F_c – values) recommended for the production of tropical stable and safe meat products are applicable only for highly contaminated raw meat. F_c – values of 16 and above given by some authors seem to be on the higher side. Observing hygienic practices in handling of the raw meat could significantly ($P<0.05$) contribute to the lowering of heating values. Addition of small amounts of benzoic, citric and acetic acids (GRAS-substances) to the product can indeed reduce the heating values remarkably, however their application is limited due unacceptable flavor imparted to the product at levels exceeding 0.5 %.

KEY WORDS: thermal spore inactivation, safe and tropical stable product, F-values

1.0 INTRODUCTION

The preservation of foodstuffs by heating was introduced sometimes in the 15th Century and was further advocated by Papin (Kolb, 1983). According to the same author it was however, Appert who brought the process to application in 1790.

The production of preserved canned foods is in effect, a paradox, on one hand efficient destruction of micro-organisms requires high heat and/or lengthy contact. On the other hand, nonimpairment of sensory qualities and nutritional value requires relatively low heat and as short a contact time with heat as possible. The requirement, lengthy and high heating, is justified by the fact that, all microorganisms including spoilage ones such as *C. sporogenes* and food poisoning ones including *C. botulinum* should be eliminated in the process (Al Delami and Banu, 1982). Moreover, any toxins, which might be present in the food material, should be inactivated in the process. With regards to the production of the so-called safe and " tropical" stable products, heating effects (F_c-values) of between 12 and 15 (Thummel *et al.*, 1987; Oebel, 1988 and Leistner, 1983) have been recommended. Grau *et al.* (1981) is of the opinion that canned foods meant for tropical and subtropical regions should experience enough heating to ensure destruction of *C. thermossacharolyticum* or *B. stearothermophilus*-spores from 10^{12} to 10° per can. Considering the Decimal reduction values (D-values) of these microorganisms, it would mean applying heating effects (F_c-values) of between 36-60. Heat treatments of this order have detrimental effects on sensory and nutritional-physiological quality of the food materials.

Therefore, in the production of tropical products only F-values that ensure a stable and high quality product should be applied (Nnko, 1991). Evaluation of international documentation has revealed that, no systematic investigation has so far been conducted to justify heating values recommended in the literature. In fact, these F-values were only empirically fixed. Grossklaus (1975) demanded that any applied F-value should be both bacteriological and technologically justified. The aim of this study was therefore, to investigate, if tropical stable and safe products could be obtained by applying remarkably mild heating regime as currently recommended in the literatures using a model product, "canned beef in its own juice".

2.0 MATERIALS AND METHOD

Reference Strains

The strains used were: *Clostridium thermossacharolyticum* (ATCC 7956), *Bacillus stearothermophilus* (Merck Art No. 11499) and *Clostridium sporogenes* (ATCC 10,000). Pure cultures of *B. stearothermophilus* and *C. sporogenes* were obtained from the FAO/WHO Collaborating Center for Research and Training in Food Hygiene and Zoonoses, Berlin. *C. thermossacharolyticum* was procured from Germany culture collection center (DSM – Goettingen).

Inoculum preparation

The preliminary experiments (data not shown) indicated that the following protocol was optimal for spore production: For the production of *C. sporogenes*-spores, stock culture was first cultivated aseptically in liver broth (Merck Nr. 5464) for 48 h at 37°C as described by Gawlik (1985). From this subculture a sample of 0.1 ml was spread evenly over the surface of blood agar plates with 0.1 % dextrose (Standard-I-agar, Merck Nr. 7881 + 5% sheep blood), followed by anaerobic incubation in a Gas-pack-system at 37°C for 72 h. The degree of sporulation was assessed microscopically by means of spore staining–technique according to Rackette (Gawlik, 1985). Only plates which showed ≤ 80% sporulation were flooded with 10 ml sterile distilled water and by rubbing gently with a sterile glass loop, spores were harvested, washed three times and concentrated by centrifuging and decanting. The spore suspension was pasteurized in a water bath for 10 min. at 100°C to eliminate any traces of vegetative cells. For the production of *B. stearothermophilus* spores,

stock culture of the same was pre-cultivated in Standard – I – Nutrient broth (Merck Art No. 7882) for 72 h at 55°C. This was followed by inoculation of the strain on a plate count agar (Merck Art No. 5450) supplemented with 0.03% $MnSO_4$ to facilitate sporulation. Incubation was carried out for 3 days at 55°C. Assessment of sporulation, purification and concentration of the spore suspension was done as described by Gawlik (1985). However, elimination of vegetative cells was accomplished by adding 0.1 mg/ml lysozyme (Serva) to the suspension followed by incubation for 2 h at 37°C. Clostridium thermohydrosulfiricum medium (CTHSM) was used for pre-cultivation of the stock culture of *C. thermosaccharolyticum* (DSM, 1993). The medium was, after inoculation covered with liquid paraffin (Merck No. 7139) followed by incubation at 55°C for 72 h.

Preparation of the Model Product

The composition of the standard product was as % mass: minced beef (87%), meat extract preparation (3%), collagen (10%) and salt (2%). Other experimental treatments were treated with potassium sorbate (KS), Benzoic (BA), Citric (CA) and Acetic (AC) acids of varying amounts ranging from 0.4 – 4.0 %. The following procedure was used to prepare the product: First, all parts of the mincer coming into contact with the meat were autoclaved for 2 h at 120°C. The coarsely minced meat was then inoculated with spores of the reference strains so as to bring the contamination level to 10^4 or 10^6 spores/g food material. Finally 2% NaCl, 3% meat extract and 10% preheated collagen were added to the inoculated material. Other treatments were supplemented with KS, BA, AC and CA in their corresponding amounts. Samples were thoroughly mixed to ensure homogeneity. Stuffing was performed in 300g cans. After sealing (Sealing machine Fa. Webra) the cans were immediately autoclaved (120°C, 2.0 bar). Center temperatures were measured in one can per batch by using Cu - Constantan thermocouple. Center Temperature/time curves as well as resulting F-values was recorded.

Analyses

pH, proximate and salt composition

The moisture content was determined by drying in the air oven at 130 °C for 1 hour. Crude fat was extracted using petroleum ether soxhlet method (Kirk and

Sawyer, 1991) while protein content was mathematically derived as recommended by Rauscher (1986). The Vollhard method (Rauscher, 1986) was used for establishing salt content and the brine concentration was calculated using the formula: Brine concentration (%) = g salt/(g salt + g water). The pH-value of the model product was measured in triplicate by using a digital – pH –meter (Russel, No. PH – C – 1018).

Cans storage and examination

Cans inoculated with spores of *C. sporogenes* were stored for a maximum period of 6 months at 25°C and those inoculated with *C. thermosaccharolyticum* or *B. stearothermophilus* were stored at 55°C for the same period. The cans were checked daily for any sign of bulging due to internal gas formation during the first week of storage then after every three days. Hard swells were withdrawn from the incubator, swelling time recorded followed by microbiological examination. Cans with no signs of swelling were also subjected to microbiological investigation after every 8 weeks of storage. The pH-values of the can contents were recorded immediately after picking a sample for microbiological investigation. At the end of 6-month storage, all remaining cans were opened and sensory, microbiological and pH values determined.

Microbiological investigation

Demonstration of the survival of bacterial spores of the reference strains was performed on a sample obtained aseptically from geometrical center (cold point) of the can. Microbiological evaluation was on the basis of presence-absence-test. The media used included SPS-Agar for *C. sporogenes* which was incubated for 24 h at 37°C. For *C. thermossacharolyticum*, CTHSM - medium was used followed by incubation at 55°C for 72 h and *B. stearothermophilus* was detected using plate count agar (Dextrose Tryptone Agar Fa. Oxoid) and incubated at 55°C for 48 h. Categorization of the colonies was done following recommendations of Beelman *et al.* (1988).

Experimental design

Analyses were replicated nine times. Standard deviations (SD) and variance were calculated. The data were analyzed using MMSTAT statistical package as suggested by Steel and Torrie, (1980).

3.0 RESULTS AND DISCUSSION

In Table 1, values of arithmetic mean, maximum, minimum and standard deviation for the physico – chemical data of the model product have been given. There was only a slight variation in the data obtained from the different treatments implying that the model product was homogeneous throughout the experiment. The mean values for water, fat and protein contents were 68.90%, 10.96% and 16.24%, respectively. Brine concentration, which depends on salt concentration and water content, was 2.60 %.

Table 1. Results of the Physico – chemical analysis (n = 9) of the model product

Values	Fat g/100g	Protein g/100g	H_2O g/100g	NaCl g/100g	Brine g/100g	pH	Temp. ^0C
Mean	10.96	16.24	68.90	1.84	2.60	6.00	19.0
SD	0.69	1.11	0.80	0.16	0.17	0.10	1.4
Min.	10.40	14.87	67.40	1.60	2.30	5.90	18.0
Max.	12.10	18.00	69.90	2.10	2.90	6.24	20.0

Mean = mean values; SD= standard deviation; Min. = minimum value; Max. = maximum values. Analysis of 9 independent determinations

In hermetically sealed cans of low acid foods, insufficient heat treatment may result in the growth of thermophilic spore formers and induce spoilage. Results for the investigation on thermal inactivation of *C. Sporogenes* in the model product are shown in Table 2.

Table 2. Survival of *C. sporogenes* (ATCC 10000) in the model product stored at 25^{0}C

Experimental code	Fc–value[1]	Contamination level Spores/g- Fill material	Detection of Microorganisms 0T	8W	6M	pH at time: 0T	8W	6M
HCS1	7.9	10^4	–	–	–	6.0	6.0	6.0
HCS2	5.1	10^4	–	–	–	6.0	6.0	6.0
HCS3	3.3	10^4	–	–	–	6.0	6.0	6.0
HCS4	3.3	10^6	–	–	–	6.0	6.0	6.0

OT = Time zero; 8W = 8 weeks; 6M = 6 months; – = no survival; [1] = measured F – values; HCS1-4 = Experimental treatments with *C. sporogenes* as test organism. The pH analysis of 3 independent determinations gave a SD = ± 0.01.

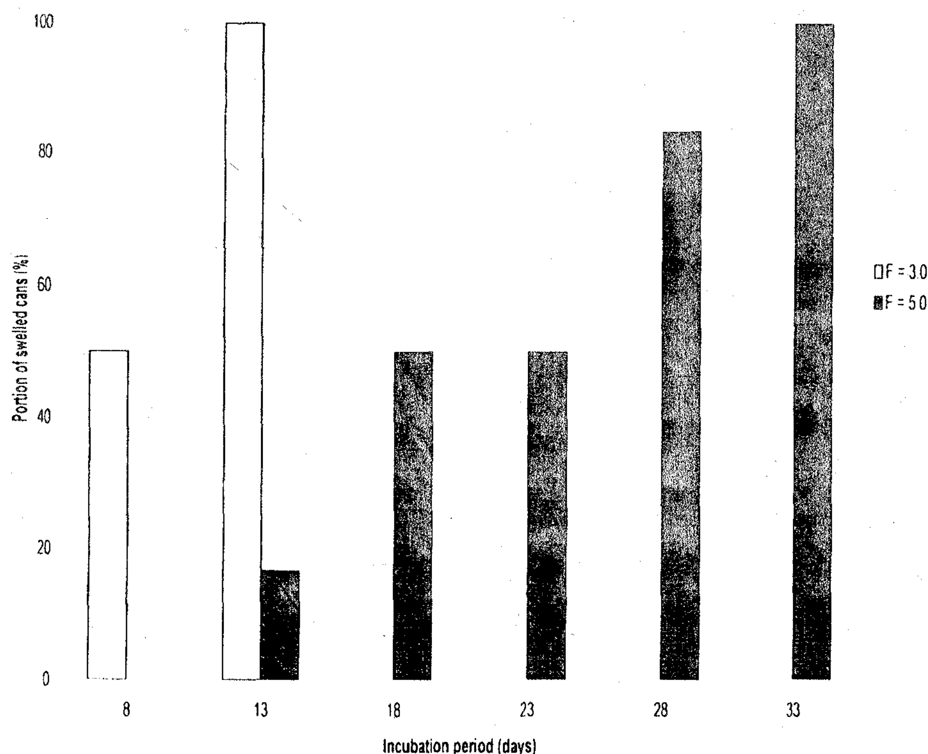

Figure 1. Proportion of the swelled cans during storage at 55°C for treatments contaminated with *C. thermosaccharolyticum*

The results show that this strain poses no problems in the production of tropical stable and safe product even at a high contamination level of 10^6 spores/g of meat. F_c – values of about 3.0 were sufficient to eliminate the microorganism from the product. There was no change of flavor and pH during the entire period of storage at 25^0C implying that there were no heat-stressed spores that could have eventually multiplied during storage at this temperature. The initial pH of the product, which stood at 6.0, did not change during the entire period of storage. This compares well with previous studies which show that even at a contamination level of 10^7spores/g no spores of *C. sporogenes* could survive heat treatment of F_c = 1.4 in "Luncheon meat" product (Havacs and Takacs, 1982). Isolation of mesophilic anaerobic sporeformers particularly *Clostridium* spp., from spoiled low-acid canned food has been traditionally attributed to under-processing spoilage or leaking of the cans, particularly when putrefactive types are recovered (Lake *et al.*, 1985).

In this study it was observed that when the experimental material was contaminated with spores of *C. thermosacharolyticum* and *B. stearothermophilus* at a

level of 10^6 spores/g (Tables 3 and 4), heat levels of at least F_c – values of 13.0 and 16.0 were needed to inactivate them. These F_c – values correspond closely to those recommended by Thummel (1988), although there was no indication of contamination levels of the raw materials used. From this study it is evident that F-values recommended assumed a raw material which was contaminated with at least 10^6 spores/g of thermophilic strains. Considering that the $D_{121.1}{}^0C$ values (time required to reduce by 10-fold the number of microorganisms at 121.1 ^0C) for *B. stearothermophilus* lies between 4-5, arithmetically, F_c – values of 24 –30 would be required to ensure a storage stable product. These F_c - values and those of 30 and 36-60 as suggested by some authors (G[illegible] *et al.,* 1981) appears to be on the higher side. For a product contaminated with 10^6 *C. thermosaccharolyticum* spores/g, application of F_c – values of 11.0 (Table 3) led to a product in which viable spores could still be isolated on the production day but not during the subsequent storage of the product. Application of sub-lethal heat treatment has been reported to induce stress to *Bacillus* spores thereby affecting their ability to germinate, outgrow and divide. This has been linked with the loss of spore cortex lytic activity (Banks *et al.* 1988). The loss of spore germination and multiplication has been associated with the number surviving thermal treatment. Furthermore, a small decrease in pH to the order of 0.1 (Tables 3), has also been observed to interfere with the metabolic activity of the microorganism leading to a kind of "autosterilisation" of the product (Gillepsy and Thorpe, 1968).

Table 3. Survival of *C. thermosaccharolyticum* (ATCC 7956) in the model product stored at 55^0C.

Exp. code	Fc - value[1]	Contamination Spores/g-Fill material	Exterior Can appearance			Survival of Microog.			pH		
			0T	8W	6M	0T	8W	6M	0T	8W	6M
HCT1	3.0	10^4	Flat	Hard swell	Hard swell	+	+	+	6.2	6.0	5.8
HCT2	5.0	10^4	Flat	Hard swell	Hard swell	+	+	+	6.1	5.8	5.8
HCT3	7.8	10^4	Flat	Flat	Flat	+	+	+	6.0	5.9	5.8
HCT4	9.5	10^4	Flat	Flat	Flat	+	+	–	6.0	5.8	5.8
HCT5	11.2	10^4	Flat	Flat	Flat	–	–	–	6.1	6.0	5.9
	11.2	10^6	Flat	Flat	Flat	+	–	–	6.0	5.9	5.9
HCT6	13.2	10^6	Flat	Flat	Flat	–	–	–	6.0	5.9	5.9
HCT8	14.8	10^6	Flat	Flat	Flat	–	–	–	6.0	5.9	5.9

OT = Time zero; 8W = 8 weeks; 6M = 6 months; – = no survival; + = survival; [1] = measured F – values; HCT1-6 = experimental treatments with *C. thermosaccharolyticum* as test organism. The PH - analysis replicated 3 times (SD = ± 0.01)

Table 4. Survival of *B. stearothermophilus* (Merck No. 11499) in the model product stored at 55°C

Experimental code	Fc – value[1]	Contamination level Spores/g Fill material	Detection of the Micro-organisms 0T	8W	6M	pH at time 0T	8W	6M
HBS2	8.0	10^4	+	–	–	6.0	5.7	5.7
HBS4	10.8	10^4	+	–	–	6.1	5.7	5.6
HBS5	12.9	10^4	+	–	–	6.0	5.7	5.7
HBS6	15.0	10^4	–	–	–	6.0	5.7	5.7
HBS7	15.0	10^6	+	+	–	6.0	5.9	5.9
HBS8	16.1	10^6	–	–	–	6.1	5.9	5.9
HBS9	17.0	10^6	–	–	–	6.0	5.9	5.9

OT = Time zero; 8W = 8 weeks; 6M = 6 months; - = no survival; + = survival; [1] = measured F – values; HBS2-9 = Experimental treatments with *B. sterarothermophilus* as test organism. The PH - analysis replicated 3 times (SD = ± 0.01)

When contamination levels was decreased from 10^6 to 10^4 spores/g, the required heating effects were reduced from 13.0 to 11.0 and 16.0 to 15.0 for *C. thermosaccharolyticum* and *B. stearothermophilus* respectively. It can be observed from Figure 1 that all cans which were inoculated with *C. thermosaccharolyticum* and thermally stressed with F_c values of 3.0 and 5.0 developed hard swells within the first 8 (50%) and 13 (16.7%) days of storage at 55°C respectively. After a storage period for 13 and 33 days at 55°C all cans (100 %) showed signs of bulging. Thermophilic spores may be introduced in foods through ingredients such as flour, spices and sugars and can therefore be source of contaminants of formulated meat products. However, primary contamination of the raw material with thermophilc spores at levels exceeding 10^4/g meat, is normally very rare (Nnko, 1991). Kokubo *et al.* (1986) found that the major sources of *Clostridia* in meat products, such as meat itself and added ingredients contain only a small number of spores, generally not exceeding 10-spores/g of raw material. Levels higher than this are only possible if microorganisms had a chance to multiply in the raw material prior to processing. Therefore, the hygienic status of raw meat determines the level of heat treatment necessary for product stability and safety. This in turn has direct implications on the nutritional, physiological and sensory qualities of the product. In this study the contamination levels of *C. thermosaccharolyticum* and *B. stearothermophilus* were reduced from 10^6 to 10^4 leading to reduction of heating values to 11.0 and 15.0 respectively, these

values are still too high and detrimental to the nutritional and physiological qualities of the product. For this reason experiments were conducted to explore the possibility of further reducing the heating values by incorporating small amounts of sorbic, citric or acetic acid in the model product. These chemical substances are widely accepted as food preservatives and are Generally Regarded as Safe (GRAS) substances (Liewen and Marth, 1985).

Results showed that a combination of potassium sorbate and mild heat treatment neither enhanced cellular injury during heating nor increased heat sensitivity of *B. stearothermophilus* in the model product (Table 5). *B. stearothermophilus* was able to survive a combination of heating effects at F_c – value of 13.0 and concentration of 1.0% potassium sorbate in the product inoculated with 10^6 spores/g meat. However, during subsequent storage of the product no viable spores were detected in the product. This was expected because sorbate is known to retard or prevent repair of thermal injury in bacteria and yeast (Beuchat, 1981).

Table 5. The effect of potassium sorbate, benzoic acid, citric acid and acetic acid on thermal inactivation of *B. stearothermophilus* (Merck Art No. 11499) in the model product at a contamination level of 10^6 spores/g.

Preservative	Concentration (%)	F_c-value	Product PH	Detection of Micro-organisms at time:		
				0T	8W	6M
Potassium sorbate	1.0	13.0	6.2	+	–	–
	2.0	11.0	6.2	+	–	–
	2.0	13.2	6.2	+	–	–
	4.0	11.0	6.4	+	–	–
Benzoic acid	0.4	8.0	5.4	+	–	–
	0.4	11.1	5.3	+	–	–
	0.4	13.1	5.4	–	–	–
	1.0	8.0	5.4	+	–	–
	1.0	10.7	5.4	–	–	–
	1.0	12.9	5.4	–	–	–
Citric acid	0.5	8.3	5.2	+	–	–
	0.5	11.0	5.2	–	–	–
	1.0	8.0	4.8	+	–	–
Acetic acid	1.0	11.0	4.8	–	–	–
	0.5	8.0	4.9	+	–	–
	0.5	11.0	4.9	–	–	–
	1.0	8.0	4.8	–	–	–

OT = Time zero; 8W = 8 weeks; 6M = 6 months; + = survival; – = no survival. The PH -analysis replicated 3 times (SD = ± 0.01)

On the other hand, addition of small amounts of benzoic, citric or acetic acids in the model product proved to be effective in lowering the heating values. On the

production day and during subsequent storage there were no viable spores of *B. stearothermophilus* detected in the product containing of 0.4% benzoic acid and thermally stressed with a F_c Value of 13.0. The heating value was further reduced to about 10.0 when the concentration of the acid in the product was raised to 1.0%. Benzoic acid is known to be more potent against heated spores than vegetative bacilli, preventing growth of the former at pH 4.8 and 5.4 with levels of 500 and 1000 µg/ml respectively (Banks *et al.*, 1988). Regardless of the amount of citric acid used application of a F_c value of 11.0 was sufficient to achieve a storage stable product, though acetic acid proved to be the most effective additive in the reduction of the heating values needed to achieve storage stability. Addition of 0.5% acetic acid and application of a F_c -value of 11.0, were enough to achieve a shelf stable product. Increasing the amount to 1.0% led to further reduction of the heating by 50% to achieve the same. Similar observations have also been reported elsewhere (Banks *et al.* 1988). While comparing the effectiveness of sorbates, citric and acetic acid on the growth of thermally stressed cocktail of *Bacillus spp.*, Banks *et al.* (1988) found that 0.5% level, acetic acid was more effective and citric acid least effective against heated spores. The efficacy of benzoic, citric and acetic acids in the reduction of the heating values is most probably associated with the lowering of pH in the model product with *Bacillus* spores being reported to have the highest resistance to heat at a near neutral pH value. The effects of pH on thermal inactivation of spores in various systems have received considerable investigation (Lowik and Anema, 1972; Lynch and Potter, 1988; Swerdlow *et al.*, 1981). Experiments utilizing minced meat at various pH values showed that D-values of *C. sporogenes* spores decreased as the pH decreased from 6.0 to 4.8 (Lowik and Anema, 1972). Lynch and Potter (1988) observed a greater inactivation rate of *B. stearothermophilus*-spores in Frankfurter emulsion slurry treated with citric or acetic acids at a lower pH of 4.6 and none at 5.2. The reduction of the heat resistance at low pH has been associated with the lowering of the internal pH of the spore or the loss of spore cations that are essential for the resistance (Swerdlow *et al.*, 1981). Generally the addition of small amounts of benzoic, citric and acetic acids (GRAS-substances) to the product can indeed reduce the heating values remarkably, however their application is limited due unacceptable flavor imparted (sensory evaluation data not shown) to the product at levels exceeding 0.5%.

4.0 Conclusions

This study showed that the heating values (F_c – values) recommended for the production of tropical safe and stable meat products are largely effective. Values 16 and above are excessive and should be applied only if the raw material is highly contaminated. The addition of potassium sorbate in the product had no effect on the heat sensitivity of *B. stearothermophilius*. Although acetic, citric and benzoic acids were particularly effective in reducing the heating values, their application is limited because the product sensory quality is adversely affected at levels exceeding 0.5% by weight.

Acknowledgements

We wish to acknowledge the financial assistance provided by Otto Benecke Foundation, Bonn, Germany. The FAO/WHO Collaborating Center for Research and Training in Food Hygiene and Zoonoses, Berlin and Germany Center for Collection of Microorganisms (DSM), Goettingen is acknowledged for providing the reference strains.

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