Determination of thermal process schedule for emulsion type buffalo meat block in retort pouch

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Abstract The process temperature for buffalo met blocks processed in retort pouches calculated based on the heat resistance of *Clostridium sporogenes* PA 3679 in Phosphate buffer saline (PBS- Ph 7.0) as reference medium and in buffalo meat block (pH 6.28) was in the range of 110–121°C. The D values and Z values calculated for *C.sporogenes* PA 3679 confirmed that the suspension was best suited for conducting thermal resistance studies. The experiment for indirect confirmation of microbial safety of the products involving inoculating the buffalo meat emulsion filled in pouches with *C.sporogenes* PA 3679 and processed at F_0 12.13 min showed no growth of microorganisms.

Keywords Thermal processing · Buffalo meat block · Thermal death time · Retort pouch

Introduction

Thermal processing paved the way for developing shelf stable food products at ambient temperature. Nicholas Appert, a French scientist, developed a method in 1809 for preserving foods in ambient temperature called canning. The principle behind canning is making the products sterile by means of thermal treatment (Lopez 1981; Footit and Lewis 1995). Cylindrical cans made of metals are the most widely used containers for thermal processing. However use

of cans has several disadvantages, such as high cost, large space requirement, longer processing time and difficulty in disposal. New packaging material called Retort pouch was developed in the 1950s by the US Army Natick Research and Development laboratories, Natick in the United States (Tuomy and Young 1982). The retort pouch, a flexible laminated food package, can withstand thermal processing of metal can and the boil in bag. Retort pouches are perhaps the most significant advance in food packaging since the advent of the metal can. It is made from a laminate of outer-polyester for strength, middle-aluminum foil for moisture, light and gas barrier and inner polypropylene layer for heat seal and food contact material (Mermelstein, 1978). When compare to can, retort pouch takes 30–50% less time to reach sterilizing temperature, does not require refrigeration or freezing, can be consumed without heating, easily opened and takes less storage space. This pouch can withstand a temperature up to 115–145°C (Lampi 1977). It's use for all kinds of meat products was approved by USDA in 1974 (Lopez 1981).

Meat is categorized as low acid (pH>4.6) food. It can act as a good medium for the growth of the most hazardous spore forming anaerobe, *Clostridium botulinum*. The assurance of a thermally processed food is based on establishing the sterility value, which is determined by using the most heat resistance organism, *C.botulinum* in low acid foods. As this organism is highly toxic, *Clostridium.sporogenes* PA 3679 that is physiologically similar to *C.botulinum* is used for thermal resistant studies. The advantages of using *C.sporogenes* PA 3679 for such studies in foods are it's non toxigenic characteristics and thermal process behavior i.e a 5 log reduction (5D) of this organism is equivalent to a 12 log reduct ion (12 D) of *C.botulinum* (NCA 1968). Therefore it has a built in safety factor in standardization of thermal process schedules in food processing. The main objective of this study was to calculate thermal process schedules the for buffalo meat blocks in retort pouches by determining the

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D value, Z value and F_0 value and confirmation of product's safety by conducting inoculated pack studies.

Materials and methods

Preparation of buffalo meat emulsion

The buffalo meat emulsion for conducting thermal resistance study using *C.sporogenes* PA 3679 was prepared based on the procedure outlined by Anjaneyulu et al. (1990).

Thermal Death Time (TDT) determination

In order to obtain a process time for emulsion type buffalo meat blocks processed in retort pouches, the heat resistance of *C.sporogenes* PA 3679 in phosphate buffer (pH 7.0) as a reference medium and in buffalo meat emulsion (pH 6.28) was determined in the range of 110–121°C.

Test organism

C.sporogenes PA 3679 was procured from Institute of Microbial Technology (IMTEC), Chandigarh, MTCC-1349 in freeze dried form.

Medias used

Robertson Cooked Meat (RCM) medium, Tryptose sulfite cycloserine agar (TSC), Fluid thioglycollate medium (FTG) and Ellner's sporulation medium were used (APHA 1984).

Preparation of spore suspension of *C.sporogenes* PA3679

The freeze dried form of *C.sporogenes* PA 3679 was thawed and inoculated in to 20 ml tube containing RCM-Medium and incubated for a period of 24 h. The development of cloudiness after the incubation and foul smelling confirmed that the organism received was *C.sporogenes* PA 3679. The second confirmation of this organism was done by Gram's staining (Tortora et al. 1994). It showed that typical sub terminal spores of *C.sporogenes* PA 3679 under oil immersion (100 X magnification). After the preliminary screening of the organism for its purity it was further used in heat resistance studies to determine the required thermal process.

The spore suspension of *C.sporogenes* PA 3679 was prepared as per the procedure outlined by Armouni et al (1994). The freeze dried form of *C.sporogenes* PA 3679 was diluted with Robertson Cooked Meat medium (RCM) broth and was transferred to sterile RCM medium (pH 7.2±0.2). The tubes were incubated for 24 h. After incubation a loopful of the suspension was streaked on to Tryptose sulfite cycloserine (TSC) agar plates. TSC agar plates were placed

in an anaerobic jar and incubated at 37 °C for 18–20 h following incubation the typical round, black, smooth and 0.5 to 1 mm diameter colony was transferred to 5 tubes containing 10 ml fresh cooked meat medium. These tubes were incubated anaerobically at 37 °C for 18–20 h, then the tubes were removed and kept in refrigerator at 4±1 °C as stock culture. The stock culture was added to fluid thioglycollate (FTG) medium, heated, cooled and then incubated overnight. The FTG medium was then transferred into sporulation medium and incubated again for 10 days. The spores were harvested by a series of centrifugation and resuspension steps conducted 4 times and kept in freezer.

Determination of spore count

After spore harvesting, the spore count was determined by using 5-tube MPN method (APHA 1984). Liver broth was utilized as liquid media for spore count (Saikia and Ranganna 1992) and it was prepared as per (APHA 1984).

Heat resistance of *C.sporogenes* PA 3679 in PBS (pH 7.0)

The heat resistance of *C.sporogenes* PA 3679 in PBS (pH 7.0) as a reference medium was conducted to find out whether the spores are heat resistant enough to conduct the thermal resistance studies. The reference medium was a phosphate buffer which was prepared by mixing 0.15M Na_2HPO_4 and M/15 KH_2PO_4 to give a pH of 7.0. Highly purified salts were used. The buffer was autoclaved in flasks for 20 min at 121°C. The thermal death time (TDT) of *C.sporogenes* PA 3679 spores in PBS was determined as per TDT tube technique described by Esty and Williams (1924). Tubes were made using pyrex tube with 9 mm outside diameter and 1mm wall which was cut into approximately 12 cm length and sealed at one end in an oxygen flame. These tubes were wrapped in paper in bundles of 10 and dry sterilized. These were used as TDT tubes which were aseptically filled with 1 ml of PBS containing approximately 10,000 spores as per MPN method and sealed as close to the end as possible in an oxygen flame. For each time and temperature intervals six tubes were used. The following time-temperature combinations were studied “by” Temperature - Time (range with incremental value) schedules were (i) 110 °C, 5–50 min (5 min up to 35 min and 10 min up to 50 min), (ii) 115 °C, 3–27 min (3 min), (iii) 118 °C, 2–20 min (2 min up to 16 min and 4 min up to 20 min) and (iv) 121 °C, 0.5–4.5 min (0.5 min).

The heat resistance of spores in TDT tubes were determined by using thermostatically controlled oil bath. The tubes in a bundle of six for each time interval were fully immersed in the heating medium. The oil bath temperature was set a few degrees high in temperature before the tubes were put to compensate the drop in temperature which

occurs when a large number of tubes were immersed. After heating, the tubes were immersed in chilled water immediately. The survivors in the heated TDT tubes were determined by using liver broth as the growing medium. The positive tubes in each of the three rows were recorded after incubation at 27–30 °C for a period of 8 weeks and results were interpreted using 3 test tube MPN-Method (APHA 1984).

Heat resistance of C.sporogenes PA 3679 in buffalo meat emulsion (pH 6.28)

The TDT of *C.sporogenes* PA 3679 in buffalo meat emulsion (pH 6.28) was determined using the same procedure adopted for heat resistance studies in PBS. In this study approximately 2 gm of buffalo meat emulsion was distributed in TDT tubes using sterile syringe followed by 1 ml of suspension containing approximately 10,000 spores and sealed aseptically as close to the end as possible in an oxygen flame. The subsequent procedures of heating was similar to heat resistance studies in PBS. After heating the TDT tubes were incubated at 27–30 °C directly for period of 8 weeks, since meat emulsion it self act as a good medium for growth. After incubation the survivors were determined by growth -no- growth method (NCA 1968).

D,Z and Fo values determination

D value was determined by plotting the number of survivors on semilog paper. From the points plotted on semilog paper a best straight line was drawn using linear regression called TDT curve. The D value is the time in min required for the TDT curve to traverse one logarithmic cycle. For determination of D values of buffalo meat emulsion in TDT tubes without subculturing, the following equation was utilized for calculation.

$$D = \frac{U}{\log_a - \log_b}$$

D Decimal reduction time

U Heating time

a Initial number of spores

b Number of microorganism which survived the heating time.

Z value was determined by plotting the D values on logarithmic scale against temperatures(°C) on the linear scale. The best straight line drawn through these points is known as the “Phantom”TDT curve. The Z value represents the number of °C required for the phantom TDT curve to traverse one log cycle. F_0 value was calculated using the 5D concept of *C.sporogenes* PA 3679. A 5X decimal reduction

time (D- value) calculated will give the F_0 value, while using *C.sporogenes* PA 3679 as the test organism. The value is more or equivalent to the 12 D concept of *C.botulinum* (NCA 1968).

Heat penetration studies: coldest point determination

The coldest point that takes longest time to sterilize the retort pouch filled with buffalo meat emulsion (200 g) was determined using a still retort. It was fabricated by transforming a laboratory type autoclave of 25 L capacity with a safety valve and a pressure gauge fitted with six Cu-Constantan needle type (2 mm) thermocouple with a six channel digital temperature monitor (T-80. Century Instruments, Chandigarh). In order to locate the slowest heating point. Five thermocouples were placed in different locations including geometric center of the pouch filled with 200 g of buffalo meat emulsion labeled as A,B,C,D and E. Thermocouple E was utilized to measure the retort temperature. The time needed to reach a target temperature of 121 °C was recorded using the multichannel digital temperature monitor. The experiment was repeated three times and the coldest point was identified by locating the thermocouple which showed the longest time needed to reach the target temperature.

Heat penetration data

The heat penetration studies were made with Cu-Constantan thermocouple (type T,0.1mm diameter, Ellab ,Denmark)-placed in the coldest point of the retort pouch identified from the previous study. Three thermocouples were placed in the coldest point of three different pouches. The thermocouple among these three which showed the slowest heating time has been selected for process evaluation. Forty pouches each filled with 200 g emulsion were vacuum sealed using Roschermatic Packaging unit and thermally processed using Rotomat-Stock sterilizer, Germany at Chatha Foods,Chandigarh. Three replicates were made. All batches were processed to a lethality of 9D of *C.sporogenes* PA 3679 equivalent to a process lethality F_0 value of 12.13 min.

Process time determination

The process lethality and time was calculated as per the Numerical method developed by Patashnik (1953).

Inoculated pack studies

In order to validate the process, thirty packs inoculated using sterile syringe with approximately 10,000 spores of *C.sporogenes* PA 3679 were processed along with regular runs. Another inoculated 30 packs were unprocessed and

kept in room temperature as control. At monthly intervals six inoculated processed and six inoculated unprocessed pouches were examined for any change in the physical appearance and gas production up to 3 months. They were cut open to recognize foul smelling. The data were recorded.

Statistical analysis

The statistical analyses were made based on the procedure outlined by Snedecor and Cochran (1989). The experiments were conducted in three replicates.

Results and discussion

Heat resistance of *C. Sporogenes* PA 3679 in PBS

The heat resistance of *C.sporogenes* PA 3679 spores in PBS (pH7.0) showed the D-values of 12.90, 5.16, 2.66 and 1.60 min at 110 °C, 115 °C, 118 °C and 121 °C respectively. (Table 1) The D values had declined with increase in temperature. The Z value was found to be 12.30 °C in PBS. The D value at 110 °C (12.90 min) was more than the value stated by Wallace *et al* (1978) who reported a D value of 10.90 min at 110 °C value while Pang *et al* (1983) estimated a D value at 110 °C as 10 min. However Rodrigo and Martinez (1988) reported that D value at 115 °C (5.0 min) in PBS was found to be best suitable to perform an inoculated experimental pack test. The spores used in this study showed a D value at 115 °C as 5.16 min which is 0.16 min more than the values of Rodrigo and Martinez (1988) and hence these spores are also best suitable for heat resistance studies. The higher heat resistance shown by spores harvested using beef particles in the medium is in accordance with Goldoni *et al* (1980) who reported a D value at 115 °C for spores of PA 3679 harvested from medium with and without beef particles were 2.76 and 3.50 min, respectively.

The PA 3679 spores showed a D value at 121 °C of 1.60 min which was more than the D value at 121 °C for *C.botulinum*. Stumbo (1973) reported a D value at 121 °C

between 0.2 and 0.1 min and Z values between 8 and 10 °C for *C.botulinum* in phosphate buffer. The D values obtained at different temperature were higher than the D values reported by Stumbo (1973), Rodrigo and Martinez (1988) as well as Sillasantos and Torreszarzo (1995) and the spores were more heat resistant and best suited for heat resistant study in any low acid food substrate. The estimated Z value was 12.30 °C which was more than the classical Z value of 10 °C for *C.botulinum*.

Rodrigo and Martinez (1988) also reported a Z value of 12.6 °C, while using spores of PA 3679 for heat resistance studies, which was almost nearer to the Z value obtained in this study in PBS.

Heat resistance of *C.sporogenes* PA 3679 spores in buffalo meat emulsion

The heat resistance of spores of *C.sporogenes* PA 3679 in buffalo meat block (pH 6.28) were determined by growth-no-growth method outlined by NCA (1968). The D values of PA 3679 spores were 12.25, 4.41, 2.45 and 1.35 min at 110, 115, 118 and 121 °C respectively (Table 1) All the D values were lesser than that of corresponding D values obtained in PBS. The reason for lower D values in emulsion than in PBS might be due to lower pH of emulsion (6.28) in comparison of PBS (7.0). Xezones and Hutchings (1965) stated that the heat resistance (D values) of *C.botulinum* spores, decreased with a decline in pH (4–7). The acidification is intended to increase product safety by inhibiting growth of *C.botulinum* (Ito and Chen 1978; Lund *et al*. 1987) and reduce the heat resistance of spores in the product (Reynold *et al* 1951; Cameron *et al*. 1980). Obviously decline in D values with increase in temperature was in agreement with the findings of Sillasantos and Torreszarzo (1995) who stated that D values decreased as the temperature enhanced.

Table 2 Coldest point (expressed as time in required to reach 121.1 °C) in buffalo meat blocks. (n=3)

Location of the thermocouple	
A	65.5±.26 ^a
B	65.0±.24 ^b
C	65.5±.30 ^a
D	64.6±.29 ^c
E	64.1±.26 ^d

C- Geometrical center of the pouch

A,B,D,E- Equi distance from the geometrical center of the pouch

Mean ± SE with different superscripts differ significantly ($P<0.05$) (n=3)

Table 1 Thermal death time (D value, °C) of *C. sporogenes* PA 3679 in PBS and buffalo meat block at different temperatures

	D values at different temperatures and Z value	
	PBS-pH7.0 Z Value=12.30	Buffalo meat block Z Value=11.40
	13.0±.04	12.2±.04
	5.2±.04	4.4±.04
	2.7±.04	2.5±.04
PBS phosphate buffer saline	1.6±.04	1.4±.04

Heat Penetration studies

The coldest point of the retort pouch was found to be the geometric center of the pouch labeled as “C” which showed the longest time (65.50 min), when compared to other thermocouples positioned laterally to the geometric center. The other thermocouples labeled as A, B, D and E taken a heating time of 65.48, 65.02, 64.55 and 64.05 min (Table 2). The slight differences in heating times between the locations of thermocouples might be due to small size of the retort pouches (200 g) processed. For small sized cans of conduction heating foods, the critical point will be nearer to the center of the food mass (Kopelman *et al* 1982). The geometrical center will always be the slowest point during heating (Hurwicz and Tischer 1955).

The heat penetration studies using Ellab thermocouples indicated that the sterilizer was showing an initial retort temperature of 33.1 °C and a product temperature of 28.5 °C. The product temperature reached the first lethality rate of 0.001 when the product temperature was 91.7 °C and a retort temperature of 107 °C. The come-up-time (CUT) was 13 min. After heating time (HT) of 20 min the product has received a lethality 8.31. From 34 min of processing, the cooling time (CT) has started. Cooling time of 8 min gave a product lethality of 3.82. The total lethality received by the products was 12.13 or a F_0 value of 12.13 min which is equivalent to 9D of *C.sporogenes* PA 3679 (The D value of *C. sporogenes* PA 3679 was determined as 1.35 min).

Normally in canned low acid foods a process lethality equivalent to 5D of *C.sporogenes* PA 3679 will be selected (NCA 1968). Handling of meat in India is still not very satisfactory due several complicating factors such as primitive slaughtering practices that are still prevalent in meat trading, tropical weather condition, lack of refrigeration facilities for handling and transport. As a result the meat sold in the market in general carry a higher bacterial load than those reported in developed countries. Considering the above facts, to make the products microbiologically more safe and to give a built-in safety equivalent to 9 D of *C.sporogenes* PA 3679 was given to the product. The product have taken a total process time of only 41 min (CUT=13 min, HT=20 min, CT=8 min) to achieve the target F_0 value of 12.13 min. The shorter processing time might be due to the thin contour and the increased surface area of the retort pouches.

Inoculated pack studies

As the products were treated to a very high lethality (9D), the products were microbiologically safe. The inoculated pack studies indicated that there was no growth of *C.sporogenes* PA 3679 during the entire 90 days of storage. It validated the safety of the process. But the pouches inoculated and

unprocessed (control) showed physical deformities, ballooning due to gas production and foul smelling.

Conclusion

The heat resistance of *C. Sporogenes* PA 3679 spores in PBS (pH 7.0) and in buffalo meat emulsion was conducted to determine the thermal resistance studies. The study showed that the organism used (*C.sporogenes* PA 3679) for thermal resistance studies were highly heat resistant and showed a F_0 value of 6.75 min. Based on that the heat resistance studies, the process time was arrived as 41 min. The inoculated pack studies indicated that the products were microbially safe over a period of 90 days with out any hazard.

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