

**STERILIZATION OF THERMALLY STABLE
CURED MEAT BLOCKS IN RETORT POUCHES**



Thesis

**SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE
OF**

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IN

LIVESTOCK PRODUCTS TECHNOLOGY

BY

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TO

DEEMED UNIVERSITY

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Dedicated to

my beloved

Father **Shri G. IMMANUEL NADAR**

&

Mother **Shrimathi DAISY IMMANUEL**



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Dated : 25-8-2008

CERTIFICATE

Certified that the research work embodied in this thesis entitled, "**Thermal Processing of Shelf Stable Buffalo Meat Blocks in Retort Pouches**" submitted by **Dr. I. Prince Devadason**, for the award of degree of **Doctor of Philosophy** of Indian Veterinary Research Institute, is the original work carried out by the candidate himself under my supervision and guidance.

It is further certified that **Dr. I. Prince Devadason** has worked for more than 30 months in this Institute and has put in more than 23 months attendance under me from the date of registration for the degree of **Doctor of Philosophy** of the University, as required under the relevant ordinance.

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Certified that the thesis entitled, "Thermal Processing of Shelf Stable Buffalo Meat Blocks in Retort Pouches", submitted by Dr. I. Prince Devadason, in partial fulfilment for the requirements of the degree of Doctor of Philosophy of Indian Veterinary Research Institute, embodies the original work done by the candidate. The candidate has carried out his work sincerely and methodically.

We have carefully gone through the contents of the thesis and are fully satisfied with work carried out by the candidate, which is being presented by him for the award of degree of Doctor of Philosophy of Indian Veterinary Research Institute.

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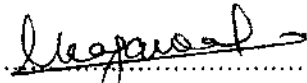
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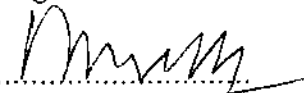
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(I. PRINCE DEVADASAON)

ABBREVIATIONS

Al foil	:	Aluminium foil
AOAC	:	Association of Analytical Chemists
<i>C. botulinum</i>	:	<i>Clostridium botulinum</i>
5D	:	5 log reduction value
12D	:	12 log reduction value
D-value	:	Decimal reduction time
FAA	:	Free amino acids
F ₀ value	:	F value when Z equals 18°F/10°C
FTG	:	Fluid thioglycollate medium
g	:	Gram
IMTEC	:	Institute of Microbial Technology
kg	:	Kilograms
L	:	Litre
mg	:	Milligram
ml	:	Millilitre
MPN	:	Most probable number
NCA	:	National Canners Association
NED	:	Nathyl ethylene diamine
PA-3679	:	Putrefactive anaerobe - 3679
PAGE	:	Polyacrylamide gel electrophoresis
PBS	:	Phosphate buffer solution
RCM	:	Robertson cooked meat medium
<i>C. sporogenes</i>	:	<i>Clostridium sporogenes</i>
SDS	:	Sodium dodecyl sulphite
SE	:	Standard error
TBARS	:	Thio barbituric acid reacting substances
TDT	:	Thermal death time
TPC	:	Total plate count
TSC	:	Tryptose sulfite cycloserine agar
TV	:	Tyrosine value
Z-value	:	It is the interval in temperature in degrees celcius, required for the line to pass though one log cycle on semilog paper

C O N T E N T S

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INTRODUCTION

India ranks first in the world in buffalo population (91.8 million) and possesses 56.5% of the total population. The production of buffalo meat is 1403 thousand MT (FAO, 1998) and it accounts for 35.7% of the total red meat production in our country. In the export meat trade, the major contribution (85%) is by buffalo meat. It is produced primarily from old and spent animals and it is therefore, very coarse and fibrous. This kind of meat can be profitably utilized by means of comminution. Some of the popular comminuted products developed from buffalo meat are sausages (Kocharli *et al.*, 1984; Krishnan and Sharma, 1990), kababs (Salahuddin *et al.*, 1991), patties (Anjaneyulu *et al.*, 1990b; Pati *et al.*, 1992) as well as nuggets and rolls (Anjaneyulu *et al.*, 1995; Sahoo and Anjaneyulu, 1997). The shelf life of these products is limited at refrigerated storage for domestic and overseas marketing.

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. Use of cans has several disadvantages such as high cost, large space requirement, longer processing time and difficulty in disposal.

A new packaging material called "Retort Pouches" 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 pouch is 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-aluminium foil for moisture, light and gas barrier and an inner polypropylene layer for heat seal and food contact material

(Mermelstein, 1978). The benefits are versatile. It 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 upto 115-145°C (Lampi, 1971). Its use for all kinds meat products was approved by USDA in 1974 (Lopez, 1981).

Meat is categorized as a low acid (pH above 4.6) food. It can act as a good medium for the growth of the most hazardous spore forming anaerobe, *C. botulinum*. The assurance of safety of a thermally processed food is based on establishing the sterility value which is determined by using the most heat resistant organism *C. botulinum* in low acid foods. As this organism is highly toxic, *C. sporogenes* PA 3679 which 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 its non-toxicogenic characteristics and thermal process behaviour i.e. a 5 log reduction (5D) of this organism is equivalent to a 12 log reduction (12D) of *C. botulinum* (NCA, 1968). Therefore, it has a built-in safety factor in standardisation of thermal process schedules in food processing.

In thermal processing, the products are subjected to a higher temperature (usually around 121°C). This leads to lot of physical, textural and sensory changes in the products while storage. To a limited extent, such adverse effects can be countered by use of cereal binders which have the ability to hold more water and improve the textural and sensory properties (Skrede, 1989).

Retort pouches have been successfully utilized for some meat products like meat biryani, meat chunks in curry, meat gravy etc. at Central Food Technological Research Institute (CFTRI) and Defence Food Research Laboratories (DFRL), Mysore (Mahadeviah and Gouramma, 1995), beef curries (Koo *et al.*, 1993), beef stew (Cremer and Pizzimonti, 1992) and chicken meat (Lyon and Klose, 1981).

Information is not available on buffalo meat products in retort pouches. In this study, therefore, retort pouch technology has been attempted for the very first time for development of buffalo meat products. Value added products from

buffalo meat capable of storage at ambient temperature using frontline retort pouch technology have strong future potential. Findings of this research are poised to tremendously increase the trade potential and improve the earnings of buffalo meat industry in India.

This study was conducted with following objectives :

1. **Determination of a thermal process for buffalo meat blocks in retort pouches.**
2. **Optimization of product quality.**
3. **Evaluation of shelf life of the products at ambient temperature.**

REVIEW OF LITERATURE

2.1 BUFFALO MEAT

A detailed study on the chemical constituents of edible carcass, tissue protein and depot fats between river buffaloes and cattle revealed that there were no significant difference between these two species (Ognjanovic, 1974). Physical, chemical and structural characteristics of buffalo meat and meat from cattle showed no significant difference and that the two meats were basically similar in chemical and physical properties (Anjaneyulu *et al.*, 1990c).

Market studies have indicated that corned beef produced from buffalo meat and beef were indistinguishable in their organoleptic quality characteristics while the former was found to have better appearance due to white color of the fat (Karvir, 1985).

Development of a variety of products from buffalo meat is important to create demand for all types of buffalo meat and efficient utilization of the meat and edible by-products. The products developed from buffalo meat include sausages (Kocharli *et al.*, 1984; Krishnan and Sharma, 1990); patties (Pati *et al.*, 1992) corned buffalo meat (Karvir, 1985), Kababs (Salahuddin *et al.*, 1991), nuggets (Sahoo and Anjaneyulu, 1997) and restructured buffalo meat steaks (Keerti, 1998). Production of minced or emulsion type products is important for better utilization of tough meat from spent buffaloes. The colour of sausages made from buffalo meat could be improved by incorporation of 100 or 200 ppm NaNO_2 or strawberry color (Sharma *et al.*, 1982). Chinese style salami sausages made from buffalo meat were as acceptable as beef sausages and acceptability could be further enhanced by improving the colour (Almira and Sison, 1975). Buffalo meat patties containing hydrogenated vegetable oil at 15 and 20% level or combination of 10% vegetable oil and 5% buffalo fat had better organoleptic acceptability, but incorporation of 15 or 20% buffalo fat alone resulted in mouth coating (Padda *et al.*, 1986).

2.2 THERMAL PROCESSING

Thermal processing in general sense relates to determination of heating conditions required to produce microbiologically safe products of acceptable eating quality. The products known originally as canned or bottled products are now referred to as heat preserved foods or thermally processed foods (Holdsworth, 1997). Canning arose in the midst of a political and social ferment in Europe and the man who had the most to do with its development was Nicholas Appert, who developed foods in wide-mouth glass bottle in 1809. Canning as we know it developed over a period of 190 years. The three milestones in the development of canning are the practical development of Appert in 1809, the scientific discoveries of Pasteur in 1864 and the technological developments of Underwood and Prescott in 1890 (Goldblith, 1971).

2.2.1 Containers for thermally processed foods

The two most common containers for canned foods today are basically the same as those used in Appert's day. They are tinfoil cans and glass containers. However, the container's design and characteristics have changed dramatically, making them superior to their ancestors. In 1810, Peter Durand introduced tin cans which replaced glass containers dramatically. In the United Kingdom, large amounts of food were preserved in tin canisters, with large quantities manufactured by the firm of Donkin and Hall and taken on Arctic expeditions. Durand patented tin-plate metal containers called "canisters". By 1823, a can with a hole in the top was invented allowing the cans to be heated in boiling water with a loose lid on the hole. The lid was soldered on the hole after the heat treatment. In 1900 the first open top "Sanitary" style double seamed, three piece can was used. An important development was made in 1937 was electrolytic tinning of tinplate. In the mid 70's the "Half tray" or "Retortable tray" was introduced. As recently as 1977, a historical development took place in the U.S. canned food industry. The "retortable" or "sterilizable" pouch was approved in 1974 for use for low acid canned foods by both U.S.F.D.A. and U.S.D.A. (Lopez, 1981).

2.3 RETORT POUCH TECHNOLOGY

The retort pouch - a flexible laminated food package that can withstand thermal processing and that combines the advantages of the metal can and the boil-in bag - has earned the Institute of Food Technologists 1978 Food Technology Industrial achievement award for the U.S. Army Natick R & D Laboratories, Continental Flexible packaging and Reynolds Metals Company. The award was presented in recognition of the outstanding contribution in the application of food technology (Mermelstein, 1978).

The retort pouch, perhaps the most significant advance in food packaging since the development of the metal can, is a flexible package made from a laminate of three materials : an outer layer of polyester for strength and printing; a middle aluminium foil as a moisture, light and gas barrier; and an inner layer of polypropylene as the heat seal and food contact material. It has been shown to be a feasible and desirable alternative to the metal can, the glass jar and the aluminium foil frozen food tray (Steffe *et al.*, 1980).

2.3.1 Combination of laminates for retortable pouch

The following are the most commonly used combination of laminates for retortable pouch (Mahadeviah and Gowramma, 1995).

Polyester 12 μ /Nylon 15 μ /aluminium foil 9 μ /cast polypropylene 70 μ .

Nylon 15 μ /cast polypropylene 60 μ

Polyester 16 μ /aluminium foil 9 μ /cast polypropylene 60 μ

Nylon 15 μ /cast polypropylene 70 μ

Nylon 25 μ /cast polypropylene 70 μ

2.3.2 Requirements for a retort pouch

The retort pouch for thermal processing should meet the following requirements (Duxbury *et al.*, 1970).

Sterilization temperature	:	116 - 145°C
Oxygen permeability	:	O _{cc} /M ² /24 h/atmosphere
Moisture vapour transmission rate	:	O _g /M ² /24 h

Seal strength	:	2 - 3.5 kg/100 mm
Bond strength	:	150 - 500 g/10 ml
Heat seal range	:	±2 μ (inner ply only) 10% of value ± 7.0 g/m ² / inner ply only
Burst test	:	7.5 kg/15 mm seal
Residual solvent	:	30 mg/m ² 0.5 ppm as toluol.

2.3.3 Filling and sealing systems

Systems used for filling retort pouches range from manual to fully automatic with variations and combination of both. A Bartelt Model D conical auger filler was used with highly viscous products like beef loaf, chicken loaf and ham. For sealing retort pouches, the four seal type design is always preferred over finseal design, since the later features multiple seal junction and prone to leakages. In the retort pouches a seal width of 5 to 10 mm is desired for good strength (Lampi, 1977). The two common heat sealing techniques for retort pouches have been the hot-sealer and impulse sealer. Variations in terms of bar configurations, supports etc. are available.

2.3.4 Retorts

Different kinds of retorts are used for pouches processing. Non-agitating or still retort is the most widely used retort. These retorts are closed pressure vessels that operate in excess of atmospheric pressure and use pure steam or super heated water as the heating medium for cooking. These are either vertical or horizontal. Vertical retorts are more efficient than horizontal ones in terms of number of pouches per unit of retort volume and less floor space. In continuous agitating, the pouches/ cans are agitated while in the retort. This results a shorter processing schedule. Hydrostatic sterilization is so named because steam pressure is maintained by water pressure. The main disadvantage in hydrostatic sterilizer is high initial investment (Pearson and Gillet, 1996). The better known systems for continuous sterilization of retort pouches are Rexham-hydrolock; Stork-storklane; stock-rotomat and FMC-convenience food sterilization (Lopez, 1981).

2.3.5 Heating medium

The three commonly used heating medium for retort pouch processing are saturated steam, steam air mixture and water with air pressure cooling (Lopez, 1981). Saturated steam is the most commonly used heating medium for commercial sterilization of packaged foods due to several advantages. During the heating period, steam condenses on the surface of the package resulting in very large values of surface thermal conductance. The rate of heat transfer from the heating medium (steam) through the package wall into the outer layer of food is high. From this point, penetration of heat into the coldest region of the food is controlled by the thermal properties of the food itself. Retortable pouches, because of their flexible nature and limited seal strength are unable to support internal pressure developed by expansion of headspace gases at thermal processing temperatures. Therefore the pouches are sterilized in an environment where the external pressure in the retort is equal to or greater than the internal pressure of the pouches during heating and cooling cycles of the process (Bhowmik and Tandon, 1987). In most cases, this is achieved by processing the pouches using hot water (heating medium) under overriding air pressure to equalize the internal and the external pressure of the pouches undergoing sterilization (Mermelstein, 1978).

2.3.6 Advantages

The most important benefits of using retort pouches are enumerated as follows. The retort pouch has a thinner profile than cans or jars. It takes about 30-50% less time to reach sterilizing temperature at the centre of the food in the pouch than in cans or jars. There is a significant reduction in the processing time required to achieve an equal lethality for retortable pouched fishery products when compared to cans containing equal weight of product (Chia *et al.*, 1983). Processing of late-run chum salmon in retortable pouches resulted in 48% reduction in processing time for equivalent lethality (Durance and Collins, 1991).

Because the product near the surface is not overcooked, as it may be with cans and jars, the product quality is maintained - the product is truer in colour, firmer in texture, and fresher in flavour. There is most likely less nutrient loss. The

pouch is especially beneficial for such products as delicate sauces, seafoods, and entrees when color and texture are important. The pouched product is commercially sterile. It does not need refrigeration or freezing, and is stable at room temperature at least as long as canned foods. The pouched product can be eaten without heating or it can be heated quickly by placing the pouch in boiling water for 3-5 minutes. Frozen foods, in contrast, require heating for about half an hour. Thus, less energy is required for heating the retort pouch. There is no need for a can opener. The pouch can be easily and safely opened by tearing across the top at a notch in the side seal or by cutting with scissors. The product can be eaten directly from the pouch, or served on dishes. The pouches, empty and full, weigh less than comparable cans and jars, thus reducing the distribution costs. They need less storage space compare to cans (Mermelstein, 1978).

2.3.7 Limitations

The disadvantages of the pouch are as follows : Major capital investment; filling is slower and more complex; thermal processing of retort pouches is more complex and processes have to be established for each product in the particular type and size of container. Since the pouch is a flexible container, the detection of leakage is more difficult. Getting the trade and consumers used to properly handling the pouched products may be difficult (Lopez, 1981).

2.3.8 Processed products

The products that may be processed in retort pouches are meat balls and gravy or tomato sauce, chicken - A-La-king, chicken stew, beef stew, ravioli, spaghetti and meat, Hungarian goulash, beef stronganoff, barbeque-chicken, Sukiyaki and others (Lopez, 1981). Research has been carried out at CFTRI and DFRL, Mysore on the packaging of vegetables, meat products like meat biryani, meat chunks in curry, meat gravy etc. and rice preparation like plain rice, bisibele bath (mixture of rice and dhal) etc. in retortable pouches.

2.3.9 Quality of products

The physical, chemical and sensory properties of rainbow trout, pollock and shrimp processed at equal lethalties in cans and retortable pouches were

compared at certain storage periods. Pouched rainbow trout, pollock and shrimp required respectively 34%, 32% and 37% less thermal processing time than the canned product. The pouched products had a firmer texture and lighter color than the canned products. In sensory evaluation, the pouched products were scored higher in most cases, for color, flavour and overall acceptability (Chia *et al.*, 1983). Fowl meat processed in cans and flexible pouches was compared for heat effects on sensory properties and concluded that the retort pouch process offered a method for improving the texture of processed chicken meat from spent hen by adequately cooking to tenderize the meat but not overcooking it to the extent that the meat chunks were reduced to fibrous, shredded or stringy pieces (Lyon and Klose, 1981). Sexually mature salmon muscle was packaged in thin-profile retort pouches and in conventional metal cans. The results indicated that pouch product was firmer, more fibrous, drier and chewier than the canned product. The overall acceptability of pouch processed sample was greater than canned chum. Histological examination revealed a more compact structure in chum muscle processed in retort pouches, possibly due to air over pressure applied to flexible pouches during processing (Durance and Collins, 1991).

2.3.10 Critical factors

The major critical factors that affect thermal processing of retort pouches are the following : minimum head-space, product consistency; maximum filling or drained weight; initial temperature (IT); processing temperature (RT); processing time (t); temperature distribution; container orientation; residual gas in headspace and in food; processing and racking systems; processing medium, confinement, pouch thickness and overpressure (Beverly *et al.*, 1980; Lopez, 1981).

2.4 THERMAL DEATH TIME (TDT) DETERMINATION

Safety from botulism in canned low-acid foods stems the pioneering research done by scientific of the National Food Processors Association (NFPA, USA) in the early 1920's. It was W.D. Bigelow, who first used the term thermal death time in connection with the minimum time required for the total destruction of a microbial population. (Bigelow and Esty, 1920). They determined the thermal resistance of

spores harvested from the most heat resistant clostridium botulinum strain known to them. As *C. botulinum* produces a highly fatal neurotoxin, they are not suitable for experimental inoculated pack studies. An alternate for *C. botulinum* was identified by NFPA, USA and was named as *C. sporogenes* PA 3679.

2.4.1 Description of *C. sporogenes* PA. 3679

C. Sporogenes PA. 3679 is a gram positive, obligate anaerobe. It produces subterminal spores. These organism is similar in physiological requirements to *C. botulinum*, but they are non-toxic and produces spores with a higher heat resistance than *C. botulinum* spores. It is gelatinolytic and can form acid with glucose and gas (Willis, 1977).

In experimental heat resistance studies in food products *C. sporogenes* PA. 3679 is preferred than *C. botulinum* as this organism is non-toxic and thermal process that produces a 5 log reduction (5 D) of this organism equivalent to a 12 log reduction (12 D) of *C. botulinum* thereby it provides a built-in safety factor (NCA, 1968).

Different varieties of medias are used for sporulating *C. sprogenes* PA 3679. Beef heart infusion broth (Goldoni *et al.*, 1980). Pork broth medium (Reed *et al.*, 1952); liver broth (Stumb, 1973); 10% egg meat medium (Reynolds *et al.*, 1952) and liver or beef heart broth (NCA, 1968) are some of the commonly used medias for growth of *C. sprogenes* PA 3679.

2.4.2 Factors influencing thermal resistance of bacterial spores

Many factors influence the resistance of bacteria to heat. A number of different techniques have been used to measure thermal resistance. Medias for growing organisms for thermal resistance determinations are many and varied as are the menstrua in which bacteria are suspended during heating. Inherent resistance of the bacterial spores, environmental influences active during the growth and formation of cells or spores and environmental influences active during the time of heating of the cells or spores (Schmidt, 1957). The following are some of the factors that influence the resistance of bacterial spores.

2.4.2.1 Age

It is no doubt related to the environmental factors active during growth of the bacteria and during storage of cell suspension. However, some workers under the conditions of their experiments, could find no correlation between age and resistance of spores of some species. Vinton (1964) reported reduced resistance of PA 3679 spores stored for 20 years at 4.4°C (40°F).

2.4.2.2 Growth temperature

The temperature at which spores are produced has been shown by various workers to influence heat resistance. Generally the heat resistance of microorganisms tend to increase as the temperature of incubation increases and this is especially true for thermophilic spore formers. Though exception have been reported. Most resistant spores of mesophilic obligate anaerobes (*C. sporogenes* PA 3679) are usually produced at or near the lowest temperature at which good vegetative growth occurs (Sugiyama, 1951).

2.4.2.3 Concentration

The heat resistance of spore suspension increases with the increase in the number of organisms present as can be seen from the following example for spores of *C. botulinum* (Esty and Meyer, 1922).

No. of spores of <i>C. botulinum</i> strain-90	Resistance in min. at 220°F	
	+	-
900,000,000	41	48
9,000,000	34	36
90,000	18	20
900	12	14
9	-	2

Carpenter (1967) also found that the heat resistance of bacterial spores are depending on the number of spores. It took 240 min at 100°C to destroy 72,000,000,000 spores of *C. botulinum* where as it took only 40 min to destroy 328 spores of *C. botulinum*

2.4.2.4 Nature of medium in which spores are produced

A number of studies suggest that resistance to heat in spores that occur naturally may be different from that shown by spores produced in laboratory media (Vinton *et al.*, 1964). Many nutrient conditions have been found to increase or decrease resistance when compared to one nutrient condition taken as a standard. Sugiyama (1951) found that, for *C. botulinum*, reduction of Fe⁺⁺ and Ca⁺⁺ concentrations in the sporulating medium below certain levels reduced heat resistance of the spores.

2.4.2.5 Effect of nature of medium in which bacteria are suspended

The chemical environment of the bacterial cell at the time it is subjected to heat has a marked effect on its resistance (Stumbo, 1973; Hersom and Hulland, 1980). Some of the major factors are pH, NaCl concentration, concentration of sugars and other carbohydrates, concentration of fats, agents used in curing meats particularly sodium nitrite and water content.

Acidity usually lowers the resistance of bacterial spores to heat, although it has also been observed that its effect depends on the treatment temperature and heating substrate (Ocio *et al.*, 1994). Maximum resistance is obtained in substrates in the range pH 6-7. There is evidence that the change in pH of a product which occurs during heating may progressively change the slope of the death rate curve (Reed *et al.*, 1952).

Sugar added to food material increases the resistance of organisms suspended therein. The greater the concentration of sugar present the greater the resistance. However, the concentration of sugar in pure sugar solutions appears to have little effect on heat resistance. Sugar in concentrations of above 50% will inhibit the growth of *C. botulinum*.

Salt (NaCl) has been found protective upto 2-4% but at a higher levels it lowers the heat resistance of bacteria. To prevent bacterial growth in vegetables from 20-25% salt is required. Ten percent salt is generally considered necessary to inhibit growth of *C. botulinum* although no toxin production has been observed

at 8%. Microorganisms can survive a much more severe heat treatment in a dry environment than when in contact with water or saturated steam. If bacteria are trapped in the fat or oil of a product, they are protected from contact with water or steam and consequently are difficult to destroy (NCA, 1968).

Nitrite inhibits *C. botulinum* by interfering with iron-sulfur enzymes such as ferredoxin and thus preventing the synthesis of adenosine-triphosphate (ATP) from pyruvate. The first direct finding in this regard was that of Woods *et al.* (1981) who showed that the phosphoroclastic system of *C. sporogenes* is inhibited by nitric oxide and later that the same occurs in *C. botulinum* resulting in the accumulation of pyruvic acid in the medium (Woods *et al.*, 1981). The antibotulinal effect requires atleast 120 ppm of nitrite for shelf stable luncheon meat (Chang *et al.*, 1974).

Sodium tetra pyrophosphate (STPP), trisodium polyphosphate and sodium hexameta phosphate had no significant inhibitory effect on mesophilic and psychrotrophic organisms. However, sodium acid pyrophosphate significantly inhibited aerobic and anaerobic bacteria including *C. sporogenes* PA 3679 upon temperature abuse storage (24°C of cooked bratwurst followed by TSPP and STPP. Cooking of 65.5°C helped to retain antimicrobial properties of phosphates to some extent. Enzymatic hydrolysis of phosphates is postulated as a major factor in loss of antimicrobial activity of phosphates in processed meats (Molins *et al.*, 1985). Actual mechanism of antimicrobial activity of polyphosphates (PP) is still not well known. However, proposed modes of action of PP include sequestration of metal ions, reaction with cell membranes to hinder transport functions and inhibition of enzyme systems (Sofos, 1986). Salt and phosphate preblending did not significantly inhibit bacterial growth of ground buffalo meat during refrigerated storage (Anjaneyulu *et al.*, 1990a). The addition of selected single or blended phosphates may offer some degree of protection against clostridial growth and toxin production (Wagner and Busta, 1983).

2.4.3 Methods to determine TDT

The most common methods now in use for measuring thermal resistance of bacteria are TDT-tube method, TDT-can method, Tank method, Flask method,

Thermoresistometer method, Unsealed TDT-tube method, Capillary tube method (Stumbo, 1973).

TDT-tube method needs simple, inexpensive equipment available to most laboratories. In this method, bacterial growth in clear media and spoilage changes in some food products may be observed visually without opening the tubes. Tubes may be easily opened for subculture with little danger of contamination space required for incubation of unopened TDT-tubes is small (Stumbo, 1973).

2.4.4 D values in thermal process

Gillespy (1948) first used 'D' as an abbreviation of decimal reduction time to represent the slope of the survivor curve, based on the heat resistance of thermophillic bacteria. This is the time in minutes required to kill 90 percent of the bacteria cells at a given temperature. This value is numerically equal to the number of minutes required for the survivors to represent the slope of the survivor curve. Wallace *et al.* (1978) reported 'D' values of 12.9 min, 2.9 min and 1.3 min at 110, 115 and 121°C for *C. sporogenes* PA 3679. Rodrigo and Martinez (1988) reported a 'D' value of 5 min. at 118°C for *C. sporogenes* PA 3679 spores in phosphate buffer saline. A 'D'₁₂₁ value of 0.2 - 0.1 min was reported for *C. botulinum*, which is much more less than D₁₂₁ value of 1.5 min for *C. sporogenes* PA 3679 (Stumbo, 1973). PA 3679 spores harvested using beef particles in the growth media showed higher 'D' values at 115°C (3.5 min) when compared to spores harvested without meat particles in the media (2.76 min) (Goldoni *et al.*, 1980).

2.4.5 'Z' values in thermal process

Z value is the slope of thermal death time curve or 'phantom' thermal death time curve. The Z value represnets the number of degrees F required for the curve to traverse one logarithmic cycle, and measure the change in thermal death time or death rate with changing temperature (NCA, 1968). The classical 'Z' value of *C. botulinum* in phosphate buffer saline (pH 7.0) is 10°C (or) 18°F. However, several authors recorded higher 'Z' values also. This depends on the variation in heat resistance of the experimental organism. Wallace *et al.* (1978)

recorded a 'Z' value of 21.0°F, which is 3°F more than the classical 'Z' value of 10°C. Stumbo (1973) determined the 'Z' value of 8 - 10°C for *C. botulinum* in phosphate buffer saline (pH 7.0). Several other authors recorded different 'Z' values. Sillasantos *et al.* (1995) recorded a Z value of 8.99°C, where as Goldoni *et al.* (1980) obtained a 'Z' value of 9.6 - 15.1°C depending on the medium in which the spore suspension is harvested. Spores harvested using beef particles showed higher 'Z' values (15.1°C) when compared to spores harvested in medium with no meat particle (9.6°C). Reynolds *et al.* (1952) obtained a 'Z' value 21°F, where as Pang *et al.* (1983) and Reed *et al.* (1952) obtained a 'Z' value of 18°F and 21.4°F respectively.

2.4.6 F₀ values in thermal process calculations

F₀ value is the number of minutes required to destroy a specified number of spores at 250°F/121°C when Z = 18°F (or) 10°C F₂₅₀¹⁸ (or) F₁₂₁¹⁰. The F value at a given temperature is a heating time which will result in a definite proportion of survivors. This is usually one surviving spore in 1,00,000 (5D) for PA-3679 and one in 1 x 10¹² (12D) for *C. botulinum* spores (NCA, 1968).

2.5 THERMAL PROCESS EVALUATION

Process evaluation, also called process determination, is the science of determining the F-value for a given process time or the process time required for a given F-value (Holdsworth, 1997).

2.5.1 Different methods for process determination

The simplest of all methods known as the "general method", is universally used in experimental work, precisely because of its simplicity and involves graphical and numerical methods.

In "graphical method" the time -temperature history graph is plotted into a lethal rate graph using appropriate data and then to determine the area under the curve, by counting the squares or using a planimeter (Patashnik, 1953; Hayakawa, 1973 and Leonhardt, 1978). Lethal rate paper was developed for Z = 18°F by using general method (Schultz and Olson, 1940).

In "numerical method" the temperatures are listed at equal time intervals. Usually one minute, the corresponding lethal rates are listed in an adjoining column and the F-value obtained by simple addition of the lethalties (Patashnik, 1953).

The nomogram method is a quick and simple procedure for use when the heat penetration curve on semilog paper is a straight line and Z value is 10°C (18°F). This method is not convenient for broken heat penetration curves (Olson and Stevens, 1939). The formula method is adopted to any problem in the determination of process or lethalties (Ball, 1928). But it is time consuming and need only be used when the heat penetration curve is broken (NCA, 1968).

2.6 HEAT PENETRATION STUDIES

In thermal process evaluation, the lethalties are determined by recording the product temperature at the coldest point of the container, using a thermocouple (NCA, 1968; Stumbo, 1973). The validated process schedule is determined by heat penetration measurements. The earliest work on heat penetration into cans of food was carried out by Bigelow *et al.* (1920), at NCA in Washington. The heat transfer in canned foods is based on its rheological properties. Such as convection or conduction. Meat products are classified under conduction heated products (Jackson and Olson, 1940).

2.6.1 Thermocouples for heat penetration studies

In 1917, Scientists of the National Food Processors Association were first utilized thermocouples for measuring the temperature profile of the canned product. In 1949, Ecklund of the American Can Company designed the non-projecting plug-in thermocouple. This is the most widely used thermocouple in industry. It is made of copper-constantan (Copper 45%, nickel alloy) with a standard size is 1.6 mm in diameter. For very accurate work thin wires are used to reduce the conduction errors (Ecklund, 1956).

In Europe, an alternative system Ellab heat penetration system is widely used (Ellab A/s Copenhagen, Denmark). This has type T thermocouples and has

a dedicated system for fn and j parameters. The system uses self threading receptacles which are placed in an opening in the filled and sealed container. A packing gland with a compression fitting allows for the location of the thermocouple sensor at the appropriate point (Eisner, 1976). More recently a device with encapsulated data logging facilities, the Ball Data Trace Unit has become available (Data trace Division, Mesa Medical Inc., Wheat Ridge Co., USA). The performance of this unit has been thoroughly examined and shown to be reliable for temperature measurements in canned foods (May, 1992; Mark Seiboth, 1999).

For retort pouches special methods of sealing wires into the package and for locating the thermocouple sensor have been devised (Bhowmik and Tandon, 1987; Spinak and Wiley, 1982). These use a folded strip welded to the pouch sides which straightens up across the pouch when filled, so that the thermocouple sensor is correctly centred. PTFE blocks has been used to support the thermocouple (Peterson and Adams, 1983). The nylon cross spacer across the pouch to locate the thermocouple has been recommended (Thorpe and Atherton, 1972).

2.7 INOCULATED PACK STUDIES

The inoculated pack method, also known as the count reduction technique is the simplest method and involves inoculating the food product with organisms of known heat resistance, preferably with a high D-value, 12-16 min at 115.5°C (Hersom and Hulland, 1980). The extent of survival of the spores is determined by culture or incubation tests. The spores should be of appropriate heat resistance for the particular type of product being dealt with. For low acid canned foods heat resistant spores of PA 3679 are generally used. The level of inoculum is usually between 10^3 and 10^5 spores per can or pouch. In the case of more solid materials a syringe can be used to deliver the inoculum as near to the centre of the food mass as possible. The inoculated cans are then treated in a series of different processes such that the lowest level of sterilization a 100% spoilage should be achieved and at the highest level 0%. In the case of gas-producing spoilage it is possible to estimate the degree of spoilage from the number of blown cans (Holdsworth, 1997).

2.8 BINDERS IN MEAT PRODUCTS

Binding of water and fat are the two most important processes in the stabilization of complex system of ground meat products. Incorporation of suitable non-meat ingredients can improve the formation and stabilization of meat emulsion (Mittal and Usborne, 1985; Bawa *et al.*, 1988; Rao *et al.*, 1997).

Starch products have been used as binders in comminuted meat products since time immemorial. The amount of starch used in processed meat varies depending upon the type of product, manufacturing procedure and government regulations. Starches are used as binders in meat processing to absorb moisture which is released from meat proteins during heating. A satisfactory binder must hold moisture throughout processing, cooking, chilling and storage of the meat product. Preferably the binder should also assist in binding the fat and maintaining its dispersion throughout the mix.

Starches are commonly added to emulsion type meat products and are popular not only for their functional properties and also to extend the more expensive lean meat portion of the product (Hedrick *et al.*, 1994). The effect is based on the ability of starch to gelatinize when heated in a water containing medium, thereby binding relatively large amounts of water (Hodge and Osman, 1976). The process involving the transformation of starch into a starch paste is termed gelatinization. It causes swelling of starch granules and may result in gels when starch concentration are optimal. Starch exerts minimum water holding capacity prior to heating. Several types of starch are available for use in meat products from various plant sources : cereal grains (wheat, corn, rice), tubers (potatoes) and roots (tapioca). Each starch has its specific characteristics which are reflected in size of native and swollen granules, gelation temperature. 55 to 80°C depending upon type of starch.

The functionality of a starch is obtained upon gelatinization. At this point, the granule swells and loses birefringence with an increase in both clarity and viscosity. The amylose molecules dissolve and will reassociate to form a gel. Starches with high amounts of amylopectin such as waxy corn starch, will not gel

or weep after gelatinization. Tapioca, with a small amount of amylose will form a soft gel when gelatinized. High amylose starch will set to a very stiff gel (Lamkey, 1998). The ability of starch products to produce a viscous paste when heated in water is most important practical property.

Use of carbohydrates especially hydrocolloids modifies both texture and sensory attributes of meat products (Skrede, 1989). Dry-addition of tapioca starch had a positive effect on physical and organoleptic parameters of low-fat pork sausages when used alone, and in combination with preformed gel blends (Lyons *et al.*, 1999). Decrease in cook losses of restructured meat products was reported on dry-addition of tapioca starch (Knight and Perkin, 1991). They reported enhanced flavour release during mastication of sausages containing tapioca starch. Further they concluded that this effect was possibly due to the ability of starch to slowly release bound water during physical breakdown, allowing for a more effective flavour release and juicy final product. Skrede (1989) had rated potato flour as the best suited starch followed by corn and wheat starch while tapioca was rated least suited for use in meat sausage.

In summary, starches have been used in meat products for the following reasons.

(i) Starches are used as water absorbants and binding agents (ii) improve the texture (firmness, cohesion) and juiciness of meat products, (iii) serve as relatively low cost filler and reduce the cost of meat products, (iv) decrease the cooking loss and shrinkage of meat products and (iv) starches are also used to replace the fat in the formulations of health foods.

2.9 FACTORS AFFECTING THE QUALITY OF MEAT PRODUCTS

2.9.1 pH

The influences of pH on the various physico-chemical and functional properties of meat such as water holding capacity (WHL), emulsifying capacity, emulsion stability, cooking loss, meat color, odour, texture, juiciness, microbial growth, driploss etc. have been very well documented. Higher pH values resulted

in greater bind, higher cooking yield and improved juiciness, in fresh pork patties (Keeton, 1983) and decreased TBARS values in fresh pork products (Drerup *et al.*, 1981). The increase of pH was caused by microbial production of alkalinizing substances especially ammonia was released as a consequence of amino acids degradation (Jay, 1972). pH values decreased when meat spoilage was caused by Gram positive bacteria especially lactic acid bacteria, (Shelef, 1975).

2.9.2 Emulsion stability

The binding properties of structural proteins which form a network around fat globules are responsible for emulsion stability of sausages. Two mechanisms are involved in the stabilization of fat in comminuted batters : emulsification due to the formation of an interfacial salt soluble protein film during chopping at low temperature and mechanical entrapment within the protein matrix formed primarily by protein-protein interactions upon cooking (Deng *et al.*, 1981). The factors which influence emulsion stability are, salt concentration in the product formulation, emulsion pH, type of meat used, level of added water, chopping temperature and processing procedure (Townsend *et al.*, 1968). The major factors affecting the emulsion stability appeared to be the physical properties of protein fat matrix, the fat protein interaction and the size of fat droplets (Lee *et al.*, 1981). Chopping temperature also plays an important role in emulsion stability. American Meat Institute (1953) recommended a maximum temperature of 12.7°C for use in commercial establishments for meat emulsion production. Ambrosiadis and Wirth (1984) recommended that the final emulsion temperature of the finished mixture should not be more than 10-12°C where no phosphate is used or about 15-18°C when phosphate is added.

2.9.3 Product yield

The product yield is based on the cooking loss of the product. When muscle is subjected to heating, coagulation of proteins and thermal shrinkage of meat takes place resulting in the release of meat juices. Cooking losses are dependent on the type of meat, time, temperature and method of cooking (Lawrie, 1991). It was observed that Biceps femoris and Supra spinetus muscles of old animals

showed cooking losses of 51.86 and 52.86% respectively; whereas, in young animals the corresponding losses were 46.94 and 47.30 (Syed Ziauddin *et al.*, 1994). They also found that cooking losses were significantly higher in muscles of old animals. Use of polyphosphates in the formulations significantly improves the emulsion stability and yield of meat products (Kondaiah *et al.*, 1985; Anjaneyulu *et al.*, 1989).

2.9.4 TBARS number

Thiobarbituric acid reacting substances (TBARS) number is reported to be the most popular method for measuring oxidative deterioration of lipids in muscle foods. It measures the amount of malonaldehyde, a water soluble secondary product obtained mainly from the oxidation of polyunsaturated fatty acids in food samples (Pearson *et al.*, 1983). TBARS number is used as an indicator of food quality and is highly correlated with taste panel scores for oxidised and warmed over flavour (WOF) in muscle foods (Wilson *et al.*, 1976; Poste *et al.*, 1986). The process of lipid oxidation results in the formation of many compounds such as n-hexanal which contribute to the undesirable odour and flavours associated with rancidity. Addition of nitrite during chopping may play an important role in preventing WOF (Pearson *et al.*, 1977). Incorporation of phosphates protected cooked meat from autooxidation (Tims and Watts, 1958). The threshold value of TBARS was found to be 1-2 mg/kg for rancidity in meat Watts (1962). pH and TBA number were found to be inversely related (Tichivangona and Morrissey, 1985). A TBA range of 0.6 to 2.0 was considered to be minimum detectable level for oxidised flavour in ground beef by an inexperienced panel (Greene and Cumuze, 1982). However, trained taste panels could detect oxidised flavour in a 0.5 to 1.0 TBA range (Tarladgis *et al.*, 1960). Off odours generally become apparent when TBARS numbers are greater than 1.0 (Love and Pearson, 1974). Retort pouched products showed a lower degree of oxidation measured by the thiobarbituric acid test (TBA) and peroxide values Chia *et al.* (1983).

It is well known that retorted uncured canned meats do not develop WOF Zipser and Watts (1961) stated that the production of antioxidants in meat itself

at high temperature was responsible for stability against oxidative rancidity. However, the chemical nature of the antioxidant and its specific location in the meat were not determined. The experiments of Sato and Hegarty (1971) showed that cooked meat loaves containing reducing type substances which would act as inhibitors of WOF development.

2.9.5 Tyrosine value

Tyrosine complex has been used as one of the methods for detecting microbial spoilage in meats, poultry and sea foods (Jay, 1996). Minced beef had the tyrosine value in the range of 26-85 mg/100 g meat. Tyrosine value is a most effective monitor of bacterial contamination of intact meat among seven analytical tests evaluated by Strange *et al.* (1977). Increase in the concentration of tyrosine, ammonia and NPN could occur due to microbial activity only when spoilage was in a very advanced stage (Dainty *et al.*, 1975). The reason attributed was that bacteria usually produce proteolytic enzymes in the late logarithmic phase of growth. Changes were not significant in amino acids and NPN until spoilage become evident. No significant correlation was observed between tyrosine value and proteolysis at different days of storage. However, there was a highly significant correlation between tyrosine value and length of storage period in untreated meat samples was reported (Morrissey *et al.*, 1980). Fresh buffalo meat had a tyrosine value of 0.19 mg/kg of meat (Kulkarni, 1989).

2.9.6 Free amino acids (FAA)

FAA are important precursors of flavour compounds of meat and other foods (Macy *et al.*, 1964). Free amino acids are released by the action of catheptic enzymes released from lysosomes during ageing or conditioning of meat following slaughter. The actual levels in meat are governed by time and temperature of ageing and storage. The levels of FAA in cooked meat are governed not only by initial levels of raw meat but also by formation of FAA through protein hydrolysis during cooking, by heat degradation of protein or by a combination of these processes. During cooking FAA reacts with sugars and possibly lipid oxidation products to produce heterocyclic compounds that contribute to the flavour and aroma profiles of cooked meats (Shibamoto, 1980).

2.9.7 Residual nitrite

Nitrite produces a characteristic pink colour and specific texture and flavour and provides a preservative effect, especially against out growth of spores of *C. botulinum* (Cassens, 1990). Nitrite is also a potent antioxidant. When nitrite is added to the biologically complex system of meat, it reacts with or is bound to various naturally occurring chemical components such as protein. The heating conditions normally used in the curing process speeds up these reactions, and when the manufacturing process is complete, only about 10-20% of the originally added nitrite is detectable. The residual nitrite level declines further during storage and distribution, as the product moves to the consumer for final preparation and consumption (Cassens, 1995).

2.9.8 Texture profiles

The textural integrity of a meat product are affected by pH, emulsion stability, protein content, addition of different kinds of binders, method of cooking etc. The formation of gels while heating with cereal binders influences the textural properties. Binders containing small amount of amylose will form a soft gel when gelatinized (Lamkey, 1998). During the period of storage oxidative effects on proteins, peptides and amino acids can impair, texture, flavour and nutritive value of the products (Spanier *et al.*, 1992).

2.9.9 Sensory attributes

The fishery products processed in retort pouches were rated high for sensory attributes when compared to products processed in cans. In general, statistically significant differences between the can and pouch were seen most often in flavour followed by color. The texture of the pouched products were firmer than the same products in cans (Chia *et al.*, 1983). The addition of phosphate significantly improved the sensory properties of buffalo meat patties and kababs (Anjaneyulu *et al.*, 1990a; Salahuddin *et al.*, 1991).

MATERIALS AND METHODS

3.1 PROCESSING OF BUFFALO MEAT BLOCKS

3.1.1 Raw materials

Buffalo meat

Buffalo meat samples from the round portion comprising semi membranous, semitendinosus, biceps femoris and quadriceps muscles of carcasses of similar conformation of spent adult female Murrah buffaloes, slaughtered by halal method were procured from local retail market of Bareilly. The fat, tendons and loose connective tissues adjoining the deboned meat chunks were trimmed off and packed in low density polyethylene bags and brought to the laboratory within 20 minutes. The meat chunks were kept for about 20 h in refrigerator ($4\pm 1^\circ\text{C}$).

Refined sunflower oil (Dhara), food grade sodium chloride (salt) and sugar, laboratory grade sodium nitrite (Merck) and sodium tripolyphosphate (CDH) were utilized in the product formulation.

Onions and garlic paste

Onions and garlic were used as condiments. The external coverings of onions and garlic were peeled off and cut into small pieces. Onions and garlic were blended in the ratio of 4:1 in Sumeet grinder with suitable blade to the consistency of a fine paste.

Spice mix

The spice ingredients were cleaned to remove the extraneous matter and dried in hot air oven at 50°C for 2 h. The ingredients were ground in Sumeet grinder with suitable blade and sieved through a fine mesh. The powders were mixed in the proportions mentioned in Table 3.1 to get the spice mix for use in preparation of buffalo meat emulsion (Anjaneyulu, 1988).

Table 3.1 Composition of spice mix

Spice ingredients	Percent in mix
Aniseed (Soanf)	10
Black pepper (Kali mirch)	10
Capsicum (Mirch powder)	8
Caraway seed (Ajowan)	10
Cardamom (Bada elaichi)	5
Cinnamom (Dal chini)	5
Cloves (Laung)	2
Coriander powder (Dhania)	15
Cumin seeds (Zeera)	15
Ginger dried (Sonth)	15
Turmeric (Haldi)	5
Total	100

Retort pouches

Three ply laminate retort pouches consisting of external layer-polyester (16 μ); middle layer-aluminium foil (9 μ); inner layer - cast polypropylene (60 μ), procured from M.H. Packaging, Ahmadabad were utilized in this study.

3.1.2 Product formulation

The formulation for emulsion based buffalo meat blocks was standardised by conducting several trials. Preliminary studies indicated that corn flour was superior as a binder in the meat blocks. The standardized formulation was used in the entire studies (Table 3.2).

Table 3.2 Formula for buffalo meat emulsion

Ingredients	Percent used
Ground buffalo meat	70.0
Refined sunflower oil	10.0
Salt	1.8
Sodium nitrite	0.015
Sodium tripolyphosphate	0.4
Sugar	0.3
Spice mix	1.5
Onions and garlic paste	3.5
Corn starch	2.5
Ice flakes	10.0

3.1.3 Preparation of emulsion

The buffalo meat emulsion was made as per the procedure outlined by Anjaneyulu *et al.* (1990a). Salt, sodium nitrite and sodium tripolyphosphate were added and mixed thoroughly with ground buffalo meat and chopped for 2 min using Sedelmann Food Cutter (Model K20 Ras, Germany). Ice flakes were added and chopping continued for another 1-2 min. Refined sunflower oil was incorporated slowly while chopping and was continued till oil was completely dispersed in the batter. Sugar, spice mix, condiments and corn starch were added at regular intervals and chopping continued for another 2 minutes to get a fine viscous emulsion. The temperature was maintained between 10-12°C while chopping. The quality of meat emulsion was evaluated by determining pH and emulsion stability.

3.1.4 Product processing

About 200 g of buffalo meat emulsion was carefully filled in each retort pouch. The sealing area was swabbed with absorbant cotton smeared with emulsion. The pouches were vacuum sealed using Roschermatic packaging machine (Model VS 19S, Germany). These pouches were thermally processed either in the fabricated retort in the laboratory or in the Stöck Sterilizer, Germany at M/s Chatha Foods, Chandigarh as per the experimental requirement.

3.2 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.

3.2.1 Description of the test organism

Meat is a low acid food (pH > 4.5). The preferred organism for heat resistance studies in low acid foods is *C. botulinum*. It produces a highly fatal neurotoxin and is unsuitable for thermal process experiments. An alternate organism *C. sporogenes* PA 3679 which is similar to *C. botulinum* in physiological characteristics, but nontoxic was identified by National Food Processors Association (NFPA). It is the organism of choice for heat resistance studies in low acid products like meat. It is a gram positive, rod shaped, sub-terminally spore forming and gas producing obligate anaerobe (Willis, 1977).

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

3.2.2 Media

The following media were used to test the purity of the organism and sporulation.

3.2.2.1 Robertson cooked meat medium (RCM)

RCM-medium was prepared as per the procedure outlined by APHA (1984).

Beef heart	454.0 g
Peptone	20.0 g
Dextrose	2.0 g
Sodium chloride	5.0 g

1.25 g of commercial RCM preparation was distributed into test tubes and 10 ml of distilled water was added to each tube to wet particles and soaked for 15

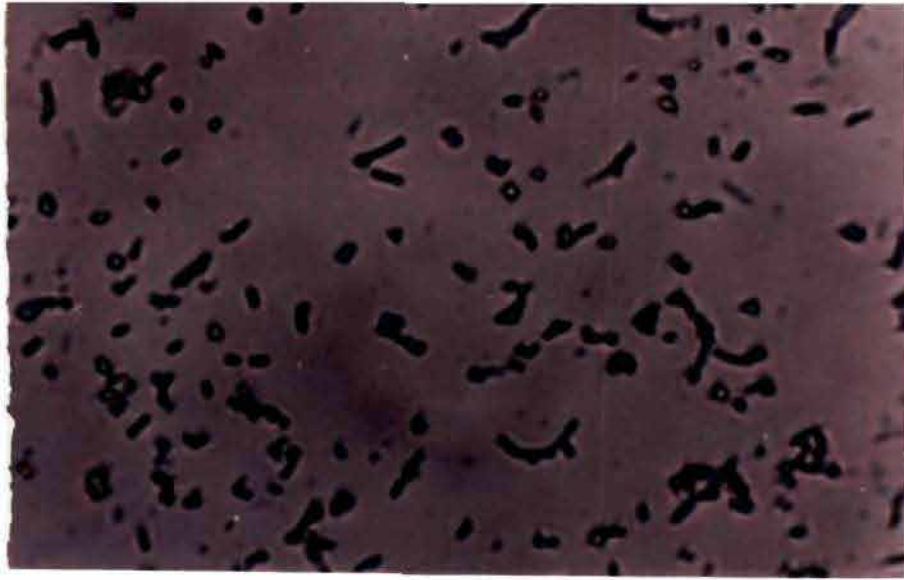


Plate 1: Subterminal spores of *C. sporogenes* PA. 3679 (x 1000 magnification) by Gram's staining



Plate 2: Fluid thioglycolate medium showing the growth of *C. sporogenes* PA. 3679

min. Final pH was adjusted to 7.2 ± 0.2 . Tubes were sterilized at 121°C for 15 min and autoclave was cooled slowly to less than 75°C before the door was opened.

3.2.2.2 Tryptose sulfite cycloserine agar (TSC)

TSC agar plates were prepared as per the procedure outlined by APHA (1984).

Tryptose	15.0 g
Soytone	5.0 g
Yeast extract	5.0 g
Sodium bisulfite (meta)	1.0 g
Ferric ammonium citrate	1.0 g
Agar	20.0 g
Distilled water	1.0 L

All ingredients were dissolved in distilled water and pH was adjusted to 7.6 ± 0.2 and autoclaved for 10 minutes at 121°C . To each liter of autoclaved medium, 10 ml of 4.0% filter sterilized solution of D-cycloserine was added to give a final concentration of approximately $400 \mu\text{g}$ per ml. The medium was then cooled to 50°C and 40 ml of 50% sterile egg yolk in saline emulsion per 500 ml of medium was added. The medium was dispensed in standard petridishes for surface plating. Plates were air dried for 24 hours at room temperature in laminar flow. Plates were prepared fresh each time.

3.2.2.3 Fluid thioglycollate medium (FTG) and Ellner's sporulation medium were made from commercial preparations as per the manufacturer's instructions (Hi-Media Laboratories, Bombay).

3.2.3 Preparation of spore suspension of *C. sporogenes* PA 3679

The freeze dried form of *C. sporogenes* PA 3679 received from IMTEC, Chandigarh was thawed and inoculated into a 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 staining (Tortora *et al.*, 1994). It showed the typical sub-terminal spores of

C. sporogenes under oil immersion (100X magnification) (Plate 1). After the preliminary screening of the organism for its purity, it was used for further heat resistance studies to determine the thermal schedules (D, Z and F₀ values).

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 aseptically into sterile RCM medium (pH 7.2 ± 0.2). The tubes were incubated for 24 h. After incubation a loopful of the suspension was streaked onto tryptose sulfite cycloserine (TSC) agar plates. TSC agar plates were placed in an anaerobic jar and incubated at 37°C for 18 to 20 h. Following incubation, the typical round, black, smooth and 0.5 to 1 mm diameter colony was transferred to five tubes containing 10 ml fresh cooked meat medium. These tubes were incubated anaerobically at 37°C for 18 to 20 h; then the tubes were removed and kept in refrigerator at 4°C as stock culture. The stock culture was added to fluid thioglycollate (FTG) medium, heated, cooled and then incubated overnight (Plate 2). The FTG medium was then transferred into sporulation medium and incubated again for 10 days. Spores were harvested by a series of centrifugation and resuspension steps conducted four times and kept in freezer.

3.2.4 Determination of spore count

After spore harvesting the spore count was determined by using 5-tube MPN method (APHA, 1984) and results were interpreted from MPN table. Liver broth was utilized as liquid media for spore count (Saikia and Ranganna, 1972) and it was prepared as per APHA, 1984.

Liver broth :

Fresh beef liver	454.0 g
Distilled water	1.0 L
Tryptone	10.0 g
Soluble starch	1.0 g
Dipotassium phosphate	1.0 g

Seperable fat from 454 g of fresh beef liver was removed and ground. Then mixed with 1 L of distilled water and boiled slowly for 1 h. The pH was adjusted to 7.6 and the liver particles were removed by staining through cheese cloth. The volume of the broth was made to 1L with distilled water. Tryptone, dipotassium phosphate and soluble starch were added and refiltered. 15 ml of the broth was dispensed into 20X 150 mm tubes and liver particles were added to a depth of 2 cm in each tube. The tubes were autoclaved at 121°C for 20 min.

For spore count determination, 15 tubes containing sterile liver broth was taken. These tubes were arranged in 3-rows and labelled as I, II and III. In each row 5 tubes were kept. 100 µl, 10 µl and 1 µl of spore suspension which has to be determined was inoculated into I, II and III rows of tubes respectively. After inoculation tubes were kept for incubation in an anaerobic condition for 8 weeks at 27-30°C. The survivors were determined by recording positive growth in each row and interpretation were made from the 5-tube MPN-table.

3.2.5 Heat resistance of *C. sporogenes* PA 3679 in PBS

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 is a phosphate buffer, and is prepared by mixing M/15 Na₂HPO₄ and M/15 KH₂PO₄ 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. sporognes* PA 3679 spores in PBS was determined as per TDT tube technique described by Esty and Williams (1924).

Pyrex tubes with 9 mm outside diameter and 1 mm wall which is cut into approximately 12 cm lengths and sealed at one end in an oxygen flame were made. These tubes were wrapped in paper in bundles of ten and dry sterilized. These were used as TDT tubes. 1 ml of PBS containing approximately 10,000 spores was aseptically filled into TDT tubes. After filling, the tubes were sealed as close to the end as possible in an oxygen flame. For each time, temperature intervals six tubes were used. The following time-temperature combination were studied.

Temperature °C		Time (min)								
110°C	–	5	10	15	20	25	30	35	40	50
115°C	–	3	6	9	12	15	18	21	24	33
118°C	–	2	4	6	8	10	12	14	16	20
121°C	–	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	5

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 are put to compensate the drop in temperature which occurs when a large number of tubes are 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 3 rows, were recorded after incubation at 27-30°C for a period of 8 weeks and results were interpreted using 3-tube MPN-method (APHA, 1984).

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

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 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 a period of 8 weeks, since meat emulsion itself acts as a good medium for growth. After incubation the survivors were determined by Growth - no-growth method (NCA, 1968).

3.2.7 Determination of D, Z and F₀ values

D value was determined by plotting the number of survivors on logarithmic scale against time in minutes on linear scale of a four logarithmic cycle semilog

paper. From the points plotted on semilog a best straight line was drawn using linear regression called TDT curve. The 'D' value is the time in minutes 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 microorganisms which survived the heating time

'Z' value was determined by plotting the 'D' values on logarithmic scale against temperature (°C) on the linear scale. The best straight line drawn through these points is known as "phantom" TDT curve. The Z value represents the number of °C required for the phantom TDT curve to traverse one log cycle.

F₀-value was calculated using the 5D concept of *Clostridium sporogenes* PA 3679. A 5 x decimal reduction time (D-value) calculated will give the F₀ value, while using *C. sporogene* PA-3679 as the test organism. The value is more or equivalent to the 12D-concept of *C. botulinum* (NCA, 1968).

3.3 HEAT PENETRATION STUDIES

3.3.1 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 copper-constantan needle type (2 mm) thermocouples and with a six channel digital temperature monitor (T-80, Century Instruments, Chandigarh) (Plate 3). In order to locate the slowest heating



Plate 3: Fabricated laboratory type still retort with pressure gauge and six channel digital temperature indicator

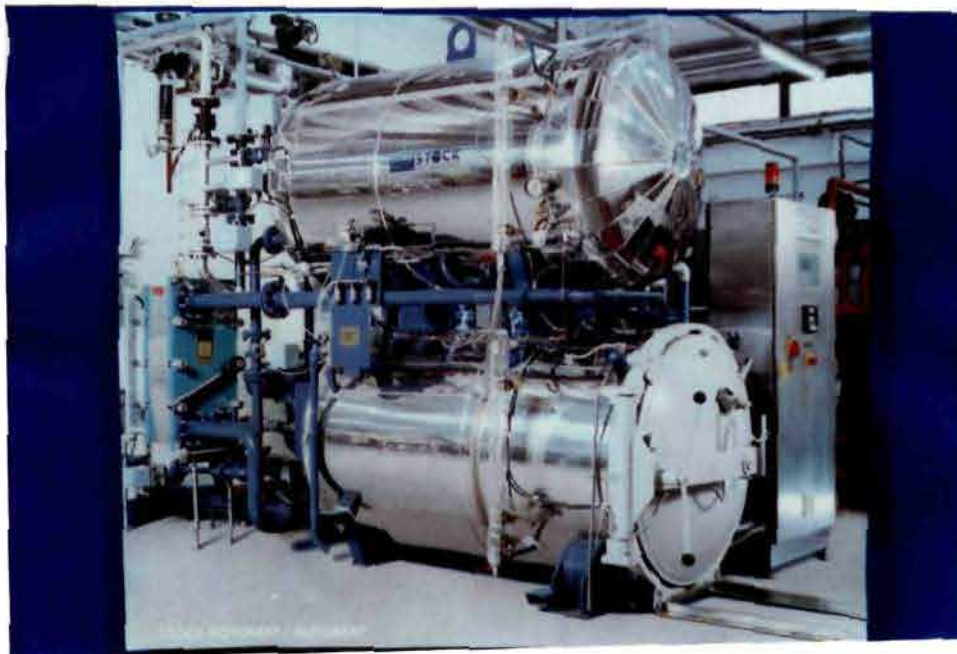


Plate 4: Industrial type Rotomat Stock sterilizer, Germany

point five thermocouples were placed in different locations including geometric center of the pouch filled with 200 gm of buffalo meat emulsion labelled as A, B, C, D and E. Thermocouple F 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.

3.3.2 Heat penetration data

The heat penetration studies were made with copper constantan thermocouples (Type T, 0.1 mm diameter, Ellab, Denmark) placed in the coldest point of the retort pouch identified from the previous study (3.3.1). 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 Röschermatic packaging unit and thermally processed using Rotomat Stock Sterilizer, Germany at Chatha Foods, Chandigarh (Plate 4). 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.

3.4 PROCESS TIME DETERMINATION

The process time was determined as per the numerical method (A classification of General method) developed by Patashnik (1953). In this method, the temperatures are listed at an equal time intervals of 1 min., the corresponding lethal rates were listed in an adjoining column and the F_0 value was obtained by simple addition of the lethalties.

3.5 INOCULATED PACK STUDIES

In order to validate the process, inoculated pack studies were conducted. Thirty packs inoculated with approximately 10,000 spores of *C. sporogene* PA 3679 were processed along with regular runs. Another 30 packs were inoculated

with the same number of spores (10,000) but unprocessed and kept in room temperature as control. At monthly intervals three inoculated processed and three inoculated unprocessed pouches were examined for any change in the physical appearance and gas production. They were cut open to recognize foul smelling. The data were recorded.

3.6 OPTIMIZATION OF PRODUCT QUALITY

Drip or exudation in thermally processed products is an important problem which leads to loss of product yield, poor acceptability and shortens the shelf life of products. The product was optimized based on physical, chemical, textural and sensory properties using four different binders. Corn starch, refined wheat flour (maida), tapioca starch (cassava), and wheat semolina at 2.5% were incorporated in the formulations (Table 4.3). The processing of the meat blocks in retort pouches were carried out as per the procedure followed in 3.1.

Table 4.3 Formulation of buffalo meat blocks containing different binders

INGREDIENTS (%)	FORMULATIONS			
	Corn starch	Refined wheat flour	Tapioca starch	Wheat semolina
Ground buffalo meat	70.0	70.0	70.0	70.0
Refined sunflower oil	10.0	10.0	10.0	10.0
Salt	1.8	1.8	1.8	1.8
Sodium nitrite	0.015	0.015	0.015	0.015
Sodium tripolyphosphate	0.4	0.4	0.4	0.4
Sugar	0.3	0.3	0.3	0.3
Spice mix	1.5	1.5	1.5	1.5
Onions and garlic	3.5	3.5	3.5	3.5
Binders				
Corn starch	2.5	–	–	–
Refined Wheat flour	–	2.5	–	–
Tapioca starch	–	–	2.5	–
Wheat semolina	–	–	–	2.5
Ice flakes	10.0	10.0	10.0	10.0

3.6.1 Physico-chemical, textural and sensory studies

The following parameters viz., emulsion stability, product yield, driploss, pH, shear force value, frying loss, moisture, protein, fat, texture profiles and sensory attributes were studied as per the procedure described in 3.7.

3.6.2 Driploss

The pouch was cut open and exudate was drained out and measured with a measuring cylinder and expressed as ml.

3.6.3 Histological study

The histological structure of buffalo meat blocks were studied as per procedure outlined by Lillie (1979). Samples of blocks were fixed in 10% formal saline and then embedded in paraffin blocks. Sections of 5-6 microns thickness were stained with haematoxylin and eosin stain and examined under microscope to observe the dispersion of fat globules in the continuous phase of protein matrix.

3.7 SHELF LIFE STUDIES

Shelf life of the products stored in a oven at $30\pm 1^{\circ}\text{C}$ have been studied from 0 to 135 days at 15 days interval. One retort pouch containing the product was used for physico-chemical and microbiological evaluation and another pouch was utilized for the assessment of sensory attributes. The following parameters were studied.

3.7.1 Physico-chemical properties

3.7.1.1 pH

pH of ground buffalo meat emulsion/blocks was determined (Trout *et al.*, 1992) by blending 10 g of the sample with 50 ml distilled water for 1 min using ultraturrex tissue homogenizer (KA, model T-25, Germany). The pH of the suspension was recorded by dipping combined glass electrode of digital pH meter (Century, Model CP 901).

3.7.1.2 Emulsion stability

Emulsion stability was determined (Anjaneyulu *et al.*, 1989) by heating emulsion samples (25 g), in polypropylene bags at 80°C in a thermostatically

controlled water bath for 20 min. After draining out the exudate, the cooked mass was cooled, weighed and weight was expressed in percent as emulsion stability.

3.7.1.3 Product yield

The weights of meat blocks before and after cooking were recorded. Product yield was expressed in percentage.

3.7.1.4 Frying loss

The buffalo meat nuggets prepared from blocks were fried with refined oil following shallow pan frying method till they get attractive golden brown colour and served to the taste panel members for organoleptic evaluation. The weight of nuggets before and after frying was recorded. Frying loss was expressed in percent.

3.7.1.5 W-B shear force

The nuggets of 15 mm thickness were made and sheared at different points of each nugget using Warner Bratzler shear press (model No.81031307, G.R. Elect. Mfg. Co., USA). The shear force was recorded (in kg) as per the method of Berry and Stiffler (1981). Ten observations were recorded for each group of nuggets to get the average value.

3.7.1.6 Proximate composition

The moisture, protein and fat contents of buffalo meat blocks were determined by following the methods laid down by A.O.A.C. (1995).

3.7.1.7 2-thiobarbituric acid reacting substances (TBARS) number

The distillation method of Tarladgis *et al.* (1960) was followed to determine TBARS number. Meat sample was (10 g) blended with 50 ml of distilled water in pestle and mortar for 2 min. The slurry was quantitatively transferred to a 500 ml : Kjeldahl flask. They were rinsed with 45 ml of distilled water and washings were transferred to the flask to which 5 ml of 6N HCl was added. Few drops of liquid paraffin were added to prevent frothing and few glass beads to prevent bumping during heating. The flask was heated on high heat and 50 ml distillate was collected in a graduated stoppered glass cylinder. The distillate was thoroughly mixed and 5 ml of distillate was pipetted in duplicate into 20 ml glass stoppered test tubes. 5 ml

of TBA reagent (0.02 M 2-thiobarbituric acid in 90% glacial acetic acid) was added to each test tube. The contents were mixed well and immersed in boiling water both for 35 min. The blank consisting of 5 ml distilled water and 5 ml TBA reagent was similarly prepared. The tubes were cooled for 10 min, under tap water and optical density (O.D.) was recorded using Beckman Spectrophotometer (Model DU-640) at 538 nm. The O.D. was multiplied by the factor 7.8 and TBARS value was expressed as mg malonaldehyde/kg of sample as suggested by Koniecko (1979).

3.7.1.8 Tyrosine value

The procedure of Strange *et al.* (1977) was used with slight modification. Trichloroacetic acid (TCA) extract was prepared by blending 20 g of buffalo meat sample with 50 ml of precooled 20% TCA solution for 2 min. After homogenisation, the contents were transferred quantitatively to a beaker by rinsing with 50 ml cold distilled water, mixed and filtered through a Whatman filter paper No.42. To estimate tyrosine value, 2.5 ml of TCA extract was mixed with equal amount of distilled water. The mixture was then blended with 10 ml of 0.5 N sodium hydroxide. Then 3 ml of diluted Folin and Ciocalteu reagent (1 volume concentrated F&C reagent + 2 volumes distilled water) added to it and shaken. The mixture was allowed to stand in a dark place at room temperature for 15 min for proper colour development. The optical density was measured at 700 nm using Beckman Spectrophotometer (DU-640). Tyrosine value was calculated as mg tyrosine per g of meat by referring to a standard graph which was prepared as per the procedure described by Pearson (1968).

3.7.1.9 Free amino acids

Free amino acids were determined by the method of Rosen (1957) in non-protein nitrogen fraction by ninhydrin reagent.

To 10 ml of sarcoplasmic (water soluble) fraction 5 ml of 10% TCA was added for precipitation of true proteins. The contents were centrifuged for 15 min at 3000 rpm and the supernatant was decanted. 2 ml of supernatant was diluted to 100 ml in a volumetric flask. From this 2 ml of solution was taken in a test tube and 1 ml of ninhydrin reagent was added and heated in a water bath for about 10

minutes and cooled in running tap water. The absorbance was measured at 530 nm using Beckman Spectrophotometer. The concentration was read by regression equation constructed from the reference values.

Reference values : 65.5 mg of Leucine was dissolved in 100 ml of distilled water. From this solution 0.5, 1.0, 2.5, 2.0 and 2.5 ml were taken in a volumetric flasks and the volume was made up to 100 ml with water. Two ml of this concentration was treated as above to measure the colour absorbance at 530 nm.

3.7.1.10 Nitrite

Nitrite content was determined as per the procedure outlined by AOAC (1995). 5 gm of finely comminuted and thoroughly mixed sample was taken in a 50 ml beaker. To this 40 ml distilled H₂O was added and heated to 80°C. While heating entire content was mixed thoroughly with a glass rod to break up all lumps and transferred to 500 ml volumetric flask. Added enough hot water to bring volume to 300 ml. The flask was then transferred to steam bath for 2 h with occasional shaking. After this, the flask was cooled to room temperature and diluted with distilled water to 500 ml. It was filtered through Whatman No.1 filter paper. 45 ml of filtrate was added with 2.5 ml sulfanilamide reagent and after 5 min 2.5 ml NED reagent was added and kept in a dark place for 15 min. Absorbance at 540 nm has been taken using Beckman Spectrophotometer (Model DU-640) against a blank of 45 ml H₂O, 2.5 ml sulfanilamide reagent and 2.5 ml NED.

The concentration in ppm was determined comparing with standard curve. Standard curve is straight line to 1 ppm NaNO₂ in final solution.

3.7.2 Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE of meat extractable proteins was carried out as per the method of Laemmli (1970) with slight modification using 1 mm slab gel using Bio-Rad electrophoresis equipment and power supply apparatus.

3.7.2.1 Preparation of sample

Meat proteins were extracted following the procedure of Ishikawa *et al.* (1987). 500 mg of buffalo meat block was homogenized in 5 ml of ice cold 0.1M

ammonium bicarbonate solution (pH 8.6) by 3 or 4 strokes using tissue homogeniser (Remi). The homogenate was centrifuged using micro centrifuge (RM 12C Remi) at 3000 rpm for 15 min. The supernatant containing extractable protein was collected. The protein content was determined (Bradford, 1976) and kept at -20°C for use in SDS-PAGE.

3.7.2.2 SDS-PAGE reagents

- a) **4x lower buffer (1.5M Tris/HCl, pH 8.8)** : 18.17 g Tris base was dissolved in 90 ml distilled water and adjusted pH with concentrated HCl to 8.8. Then 0.4 g SDS was dissolved in it. Final volume was made to 100 ml with distilled water.
- b) **Acrylamide 30%/bisacrylamide 0.8%** : 30 g acrylamide and 0.8 g N,N'-methylene bisacrylamide were taken in a 150 ml conical flask and 60 ml distilled water was added to it. Stirring was continued till the chemicals dissolved and volume was made to 100 ml. 2 teaspoonfuls of activated charcoal was added, stirred for 5 min and filtered to get clear solution.
- c) **Sodium dodecyl sulphate (SDS) 10%** : Dissolved 5 g SDS in 45 ml distilled water and volume was made to 50 ml.
- d) **Upper buffer (0.5 M Tris/HCl, pH 6.8)** : 6.05 g Tris base was dissolved in 80 ml distilled water and pH was adjusted to 6.8 with concentrated HCl. Volume was made to 100 ml.
- e) **Ammonium persulphate (APS) 10%** : It was prepared freshly by dissolving 100 mg APS in 1 ml distilled water.
- f) **2x sample buffer** : 2 ml glycerol, 1 ml 2-mercaptoethanol, 4.5 ml 10% SDS, 1.7 ml upper buffer and 0.2 ml 0.1% bromophenol blue were taken and 0.6 ml distilled water was added to them. The solution was mixed properly and stored at -20°C.
- g) **Electrode buffer (pH 8.3)** : 6 g Tris base and 28.8 g glycine were dissolved in 1500 ml distilled water. The pH was checked, if not 8.3, adjusted with 1N HCl, 2 g SDS (or 20 ml 10% SDS) was added to it and the final volume was made to 2 litre.

- h) **SDS-PAGE gel stain** : 1.25 g Coomassie blue (R 250), 500 ml methanol, 100 ml acetic acid and 400 ml distilled water were mixed stirred for 30 min and filtered to get 1 litre solution.
- i) **SDS-PAGE gel destain** : 300 ml methanol, 70 ml glacial acetic acid and 630 ml distilled water were mixed properly to get 1 litre solution.

3.7.2.3 Separating gel (10%)

For 10 ml acrylamide/bisacrylamide solution, 7.5 ml deaerated 4x lower buffer and 12.4 ml distilled water were mixed gently in a beaker. Then freshly prepared 74 μ l of 10% ammonium persulphate and 10 μ l N,N,N', N' tetramethylenediamine (TEMED) were added immediately and mixed.

3.7.2.4 Casting of gel

2% melted agar was poured from side through spacers to seal the bottom and side of the glass plates. Then 10% separating gel was poured carefully into the gel casting space between the glass plates until about 75% of the space was filled. Water saturated n-butanol was layered over the gel and after polymerization of the separating gel; n-butanol was drained off by tilting the gel cast assembly. The gel upper surface was then washed with distilled water to remove n-butanol, if any. 5% stacker gel solution was layered over the separating gel after washing the upper surface by the same gel solution. Slot forming comb was carefully inserted into the top of the gel casting area until both ends of the comb were stopped at tops of the side spacers. Water saturated n-butanol was overlaid. After polymerization of stacker gel, the comb was removed slowly and carefully.

3.7.2.5 Running of gel

Protein samples and molecular weight marker (MW-SDS-200 kit) were diluted with equal volume of 2x sample buffer and boiled in hot water bath for 5 min. First molecular weight marker protein of about 10 μ l was applied. Then samples were applied to each slot so that amount of protein was 200 μ g in each case. The upper chamber of the electrophoresis apparatus was filled with about 600 ml electrode buffer and about 1400 ml buffer was poured into the lower chamber. **Electrophoresis was performed at a constant voltage mode of 50 V/**

slab at 13 mA for first 1 hr and increased to 100 V/slab subsequently and maintained at same voltage for about 5 to 6 hrs or till the tracking dye reached the lower end of the gel. Electrophoresis was carried out at room temperature.

3.7.2.6 Removal of gel

Power supply was turned off and the electrophoresis unit was disassembled. Removed clamps from each gel sandwich. The spacer bars were slid out and the glass plates were separated with syringe handle. Nicked the gel at appropriate place (lower corner opposite molecular weight marker, dye front edge of molecular weight strip). Then stacking gel was cut off.

3.7.2.7 Fixing, staining and destaining of gels

The gels after electrophoresis were fixed for overnight with fixing solution (10% acetic acid and 50% methanol in distilled water) and stained with the gel stain solution for 4 to 5 hrs. The gels were then destained with several changes of gel destain solution. After thorough destaining, the gels were stored in 7% acetic acid till photographed.

3.7.3 Microbiological quality

All the microbiological parameters were determined following the methods of ICMSF (1978).

3.7.3.1 Preparation of serial dilution

10 g of sample buffalo meat block was taken near flame in a sterile mortar with the help of sterilized forceps and scissors. 90 ml of sterile 0.1% peptone water was added to it and homogenised using a sterile pestle for 2 min for uniform dispersion to get 10^{-1} dilution. Serial dilutions were made as per requirement.

3.7.3.2 Aerobic mesophiles count

Plate count agar 23.5 g was suspended in 1 litre distilled water, boiled to dissolve completely and sterilised by autoclaving at 15 lbs pressure (121°C) for 15 mintues. Final pH was 7.0 ± 0.2 . Duplicate sets of petridishes were inoculated aseptically with 1 ml aliquots from appropriate dilutions. About 10-15 ml of plate count agar melted and maintained at $44-46^{\circ}\text{C}$ was poured gently. Plates were incubated at 37°C for 48 h. The number of colonies were multiplied by the reciprocal of the dilution and expressed as \log_{10} CFU/g.

3.7.3.3 Total anaerobes count

Suspended 58 g of anaerobic agar in 1 litre distilled water, boiled to dissolve the medium completely and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 min. Final pH of the medium was 7.2 ± 0.2 . Serial dilutions were made as per 3.7.3.2. The petridishes were put into the anaerobic jar and incubated at 35°C for 48 hrs. White colonies on the surface of the medium were counted and expressed as \log_{10} CFU/g.

3.7.4 Texture profile analysis

The texture profile was analysed as per Bourne (1978) and Brady *et al.* (1985), using Instron, Universal Testing Machine (Model-1000) at Dept. of Animal Products Technology, Marathwada Agricultural University, Parbhani, Maharashtra.

The buffalo meat blocks were cut into 1 cm squares and compressed to 50% of original height. A 50 N load cell was used with a load range of 0-50 N at cross head and chart speed of 50 mm/min. Two compression cycles per cubes were generated to form a "two bite" work force compression curve. The following texture parameters were derived from the two successive compression curves: (a) Firmness, (b) Cohesiveness (c) Springiness (d) Gumminess and (e) Chewiness.

The detailed procedure is as follows : The instrument was calibrated by using 50 Newton load transducer and the weight of Anvil was balanced out. Gauge length was set at 15 mm by driving the moving crosshead up and down using push buttons on crosshead control panel. The distance between Anvil and specimen table was measured using a ruler. The upper and lower crosshead limits were set accurately by tightening the screws.

The conditions set for analysis were :

Load	50 N
Cross head speed	50 mm/min
Chart magnification	100 mm ²
Chart magnification	1:5
Compression	50%
Sample size	100 MM X 10 MM

The sample of buffalo meat blocks having cross sectional area of 100 mm² were subjected to 50 per cent compression twice for obtaining force deformation curve from which various textural attributes were measured (Bourne, 1978).

The results were interpreted as follows (Brady *et al.*, 1985; Chen and Trout, 1991).

- | | |
|-----------------|---|
| 1. Firmness | Maximum force of first compression cycle (N) |
| 2. Cohesiveness | Area of curve 2 / Area of curve 1 |
| 3. Springiness | Width between curves or width down stroke curve 2 |
| 4. Gumminess | Firmness x cohesiveness |
| 5. Chewiness | Gumminess x springiness |

3.7.5 Sensory evaluation of buffalo meat blocks

Experienced taste panel members consisting of scientists and postgraduate students of the division and the institute evaluated the sensory attributes viz., colour and appearance, flavour, juiciness, texture, mouth coating and overall palatability of the products, using 8 point hedonic scale (Keeton 1983) as shown in Annexure I. The pouched buffalo meat blocks were examined for physical deformity and ballooning. They were out open to observe the colour and odour of the products. The meat blocks were cut into nuggets, shallow pan fried till they get attractive golden brown colour and served warm to the panelists.

3.8 STATISTICAL ANALYSIS

The data obtained from the experiments were subjected to analysis of variance and critical difference was calculated (Snedecor and Cochran, 1989) to compare the means for finding the treatment effect on various parameters. The correlation coefficient of you was also determined for relevent parameters.

**DIVISION OF LIVESTOCK PRODUCTS TECHNOLOGY, IVRI, IZATNAGAR
PROFORMA FOR ORGANOLEPTIC EVALUATION OF MEAT PRODUCTS**

Dated :

Name _____
Product _____
Scoring Guide _____

Sensory attributes

8-point scale for descriptive attributes of product

	8	7	6	5	4	3	2	1
Appearance	Excellent	V. good	Good	Fair	Sli. Poor	Mod. Poor	V. Poor	Extr. Poor
Flavour	Extremely desirable	V. desirable	Mod. desirable	Sli. desirable	Sli. undesirable	Mod. undesirable	V. undesirable	Extr. undesirable
Juiciness	Extremely juicy	V. juicy	Mod. juicy	Sli. juicy	Sli. dry	Mod. dry	V. dry	Extr. dry
Texture	Extremely desirable	V. desirable	Mod. desirable	Sli. desirable	Sli. undesirable	Mod. undesirable	V. undesirable	Extr. undesirable
Overall palatability	Extremely palatable	V. Palatable	Mod. Palatable	Sli. Palatable	Sli. Unpalatable	Mod. Unpalatable	V. Unpalatable	Extr. Unpalatable
Sample	Appearance	Flavour	Juiciness	Texture	Overall palatability			

- 1.
- 2.
- 3.
- 4.

Remarks

RESULTS AND DISCUSSION

4.1 HEAT RESISTANCE OF *C. sporogenes* PA 3679 SPORES IN PBS

The heat resistance of *C. sporogenes* PA 3679 spores in PBS (pH 7.0) showed the D-values of 12.90, 5.16, 2.66 and 1.60 min at 110°, 115°, 118° and 121°C respectively. The 'D' values had declined with increase in temperature. These 'D' values were determined by linear regression of the survivors (Table 4.1 to 4.4 and Fig. 4.1 to 4.2). The 'Z' value was calculated from the plots of D values at 110, 115, 118 and 121°C in PBS (Table 4.5 and Fig. 4.5). The Z value was found to be 12.30°C in PBS.

The D value at 110°C (12.9 min) was more than the value stated by other workers. Wallace *et al.* (1978) reported a D value at 110°C value (10.9 min) while Pang *et al.* (1983) estimated a D value of at 110°C as 10 min. D value at 115°C (5 min) in PBS was found to be best suitable to perform an inoculated experimental pack test by Rodrigo and Martinez (1988). 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. As the spores were harvested using beef particles in the medium they showed more heat resistance (D value at 115°C = 5.16 min) than the spores obtained by Goldoni *et al.* (1980). They reported a D value at 115°C for spores of PA 3679 with a range of 2.76 and 3.50 min based on the absence or presence of beef particles in the medium respectively.

The PA 3679 spores showed a D value at 121°C of 1.60 min which is more than the D value at 121°C for *C. botulinum*. Stumbo (1973) reported a D value at 121°C between 0.2 - 0.1 min and Z values between 8 and 10°C for *C. botulinum* in phosphate buffer. The D values obtained at different temperatures (110, 115, 118 and 121°C) were higher than the 'D' values reported by other workers (Stumbo, 1973; Rodrigo and Martinez, 1988; Sillasantos and Torreszarzo, 1995) and the spores are most heat resistant and best suited for heat resistance study in any low acid food substrate.

Table 4.1. Thermal Death Time (TDT) record of *C. sporogenes* PA. 3679 in PBS at 110° C.

THERMAL DEATH TIME RECORD				
Medium	: Phosphate Buffer			
pH	: 7.00			
Organism	: <i>C. sporogenes</i> PA. 3679			
Initial Number of spores	: 10,000 per tube			
Incubation period	: 8 weeks			
Spore count	: Most Probable Number Method			
Heating time (min)	MPN	No. of spores survived	No. of spores as per linear regression	'D' value min
0	0-0-0	10000	8629	
5	3-0-1	3900	3655	
10	2-1-1	1500	1548	
15	1-1-0	700	656	12.9
20	0-1-0	300	277	
25	0-0-0	---	--	
30	0-0-0	---	--	

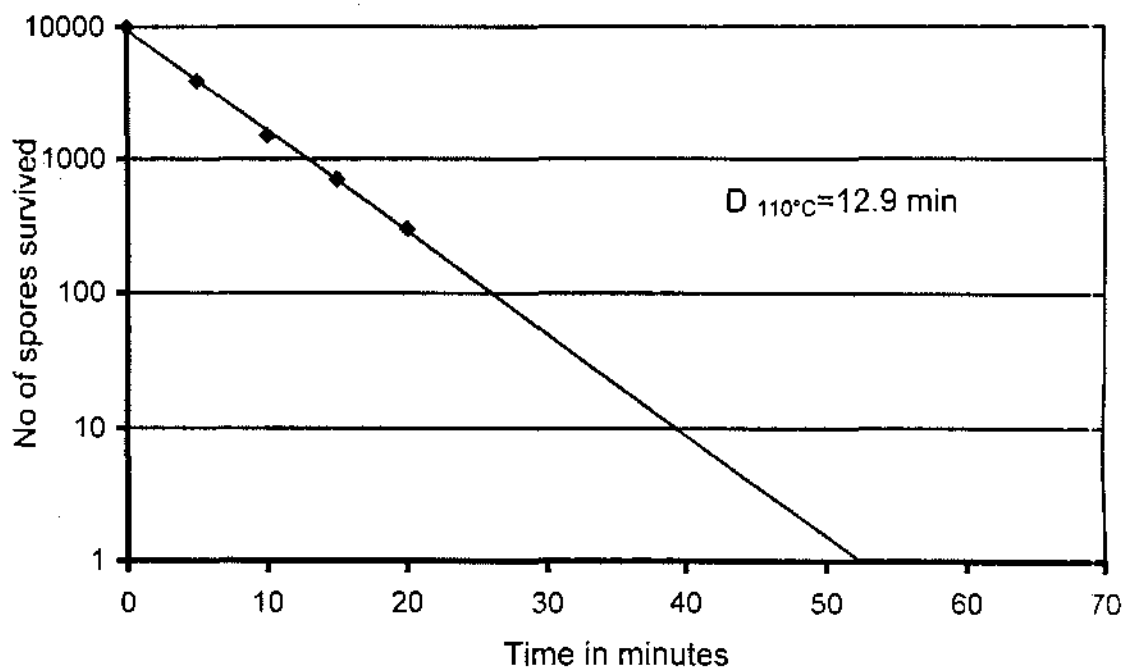


Fig 4.1 D_{110°C} of *C. sporogenes* PA.3679 in PBS

Table 4.2. Thermal Death Time (TDT) record of *C. sporogenes* PA. 3679 in PBS at 115° C.

THERMAL DEATH TIME RECORD				
Medium	: Phosphate Buffer			
pH	: 7.00			
Organism	: <i>C. sporogenes</i> PA. 3679			
Initial Number of spores	: 10,000 per tube			
Incubation period	: 8 weeks			
Spore count	: Most Probable Number Method			
Heating time (min)	MPN	No. of spores survived	No. of spores as per linear regression	'D' value min
0	0-0-0	10000	9817	
3	3-0-0	2300	2546	
6	3-1-1	750	660	
9	2-1-0	200	173	5.16
12	1-0-0	40	12	
15	0-0-0	---	--	
18	0-0-0	---	--	

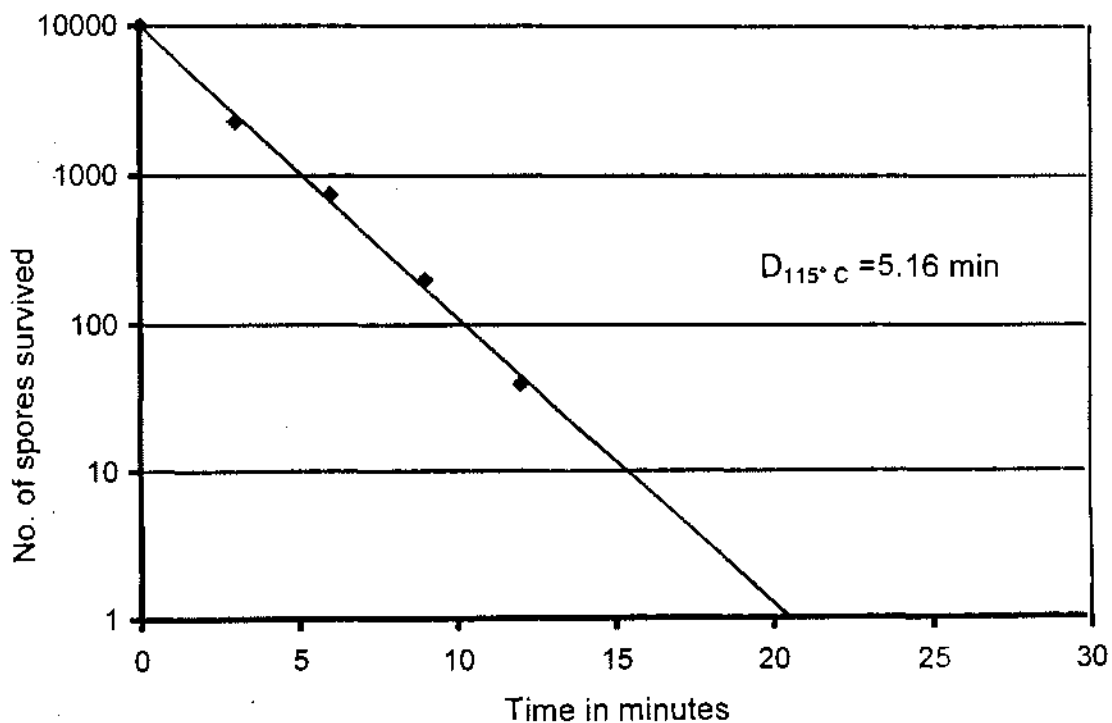


Fig 4.2 $D_{115^{\circ}C}$ of *C. sporogenes* PA.3679 in PBS

Table 4.3. Thermal Death Time (TDT) record of *C. sporogenes* PA. 3679 in PBS at 118° C.

THERMAL DEATH TIME RECORD				
Medium	: Phosphate Buffer			
PH	: 7.00			
Organism	: <i>C. sporogenes</i> PA. 3679			
Initial Number of spores	: 10,000 per tube			
Incubation period	: 8 weeks			
Spore count	: Most Probable Number Method			
Heating time (min)	MPN	No. of spores survived	No. of spores as per linear regression	'D' value min
0	0-0-0	10000	11428	
2	3-2-2	2100	1845	
4	3-0-1	390	297	
6	1-0-0	40	46	2.66
8	0-0-0	--	--	
10	0-0-0	---	--	

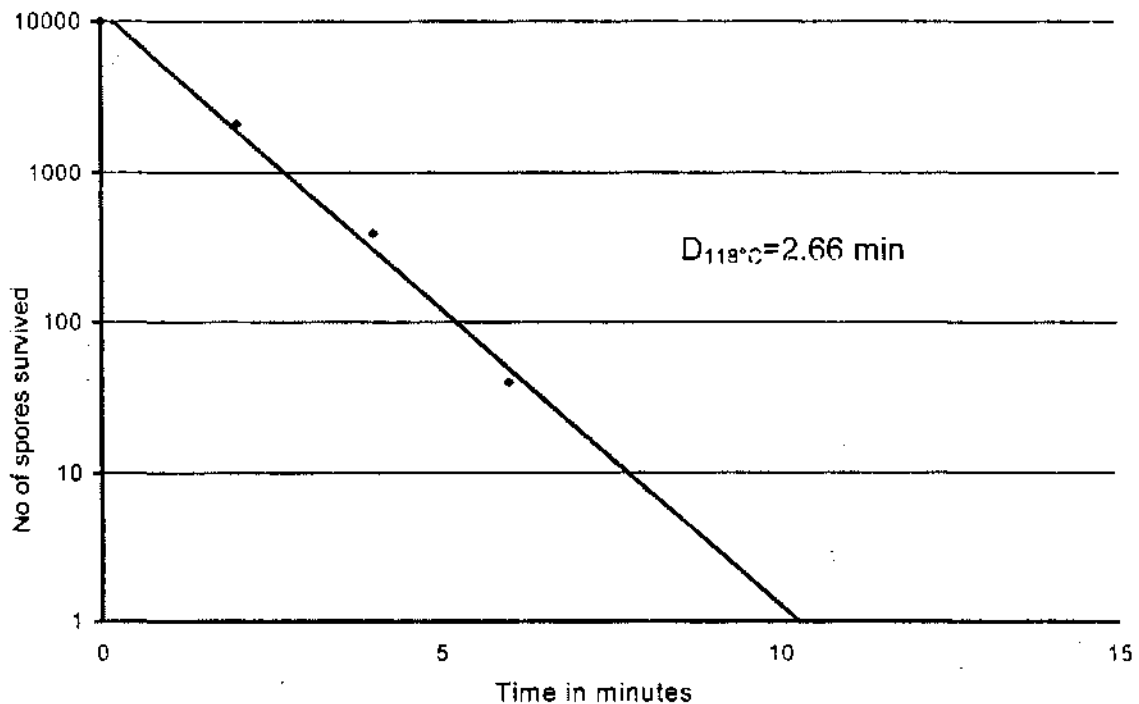


Fig 4.3 $D_{118^{\circ}\text{C}}$ of *C.sporogenes* PA.3679 in PBS

Table 4.4. Thermal Death Time (TDT) record of *C. sporogenes* PA. 3679 in PBS at 121° C.

THERMAL DEATH TIME RECORD				
Medium	: Phosphate Buffer			
pH	: 7.00			
Organism	: <i>C. sporogenes</i> PA. 3679			
Initial Number of spores	: 10,000 per tube			
Incubation period	: 8 weeks			
Spore count	: Most Probable Number Method			
Heating time (min)	MPN	No. of spores survived	No. of spores as per linear regression	'D' value min
0	0-0-0	10000	9332	
.5	3-3-1	4600	4677	
1.0	3-3-0	2400	2344	
1.5	3-1-2	1200	1174	1.60
2.0	3-0-2	640	588	
2.5	3-2-1	280	295	
3.0	2-1-0	150	147	
3.5	1-1-0	70	74	
4.0	1-1-0	30	37	
4.5	0-0-0	--	--	
5.0	0-0-0	--	--	

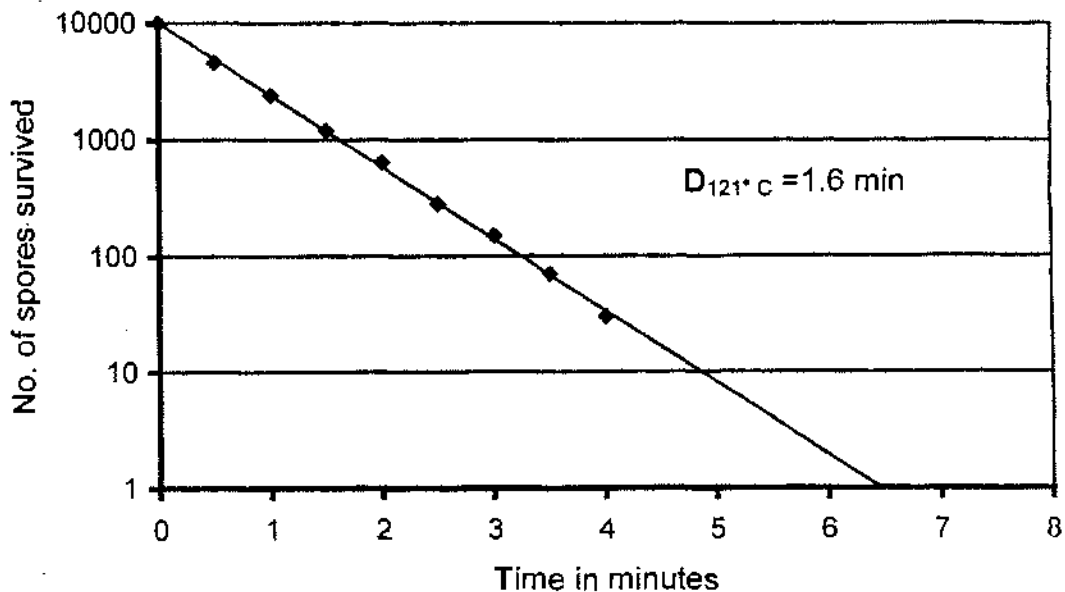


Fig 4.4 $D_{121^{\circ}\text{C}}$ - of *C. sporogenes* PA. 3679 in PBS

Table 4.5 'Z' Values of PA. 3679 in PBS

Temperature °C	'D' Value (minutes)	'Z' Value (° C)
110	12.90	12.30
115	5.16	
118	2.66	
121	1.60	

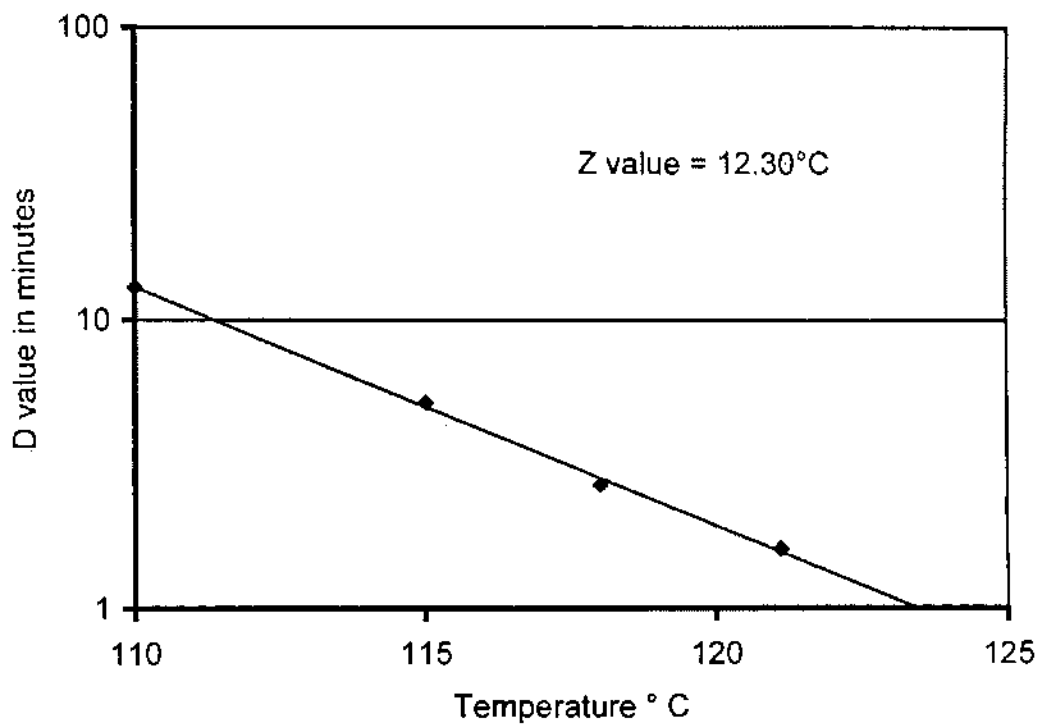


Fig 4.5 'Z' value of *C. sporogenes* PA 3679 in PBS (pH 7.0)

49346

664-902842
D49T

50



The estimated Z value was 12.30°C which is more than the classical Z value of 10°C for *C. botulinum*. Goldoni *et al.* (1980) obtained Z values between 9.6 to 15.1°C, Rodrigo and Martinez (1988) reported a 'Z' value of 12.6°C, while using spores of PA 3679 for heat resistance studies, which is almost nearer to the Z value obtained in this study in PBS.

4.2 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 emulsion (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 4.6 to 4.10 and Fig. 4.6 to 4.10). 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 to 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 to 7). Wallace *et al.* (1978) as well as Brown and Thorpe (1978) working with other micro-organisms and substrates, also found a reduction in thermal resistance with decreasing pH, upto a temperature of 121°C. The findings of the above authors clearly indicate, a reduction in pH would be expected to yield a corresponding decline in the D-values of spores of PA 3679. 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 (Reynolds *et al.*, 1952; 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 diminished as the temperature increased.

4.3 HEAT PENETRATION STUDIES

The coldest point of the retort pouch was identified and the results are recorded in the table 4.11. It was the geometric centre of the pouch labelled as 'C' which showed the longest time (65.50 min), when compared to other

Table 4.6. Thermal Death Time (TDT) record of *C. sporogenes* PA. 3679 in buffalo meat emulsion at 110° C.

THERMAL DEATH TIME RECORD			
Medium	: Buffalo meat emulsion		
pH	: 6.28		
Organism	: <i>C. sporogenes</i> PA. 3679		
Initial Number of spores	: 10,000 per tube		
Incubation period	: 8 weeks		
Spore count	: Growth-No growth method		
Heating time (min)	No. of samples Heated	Positive	'D' value min
5	6	6	U
10	6	6	D= -----
15	6	6	Log _a -log _b
20	6	6	
25	6	6	D= Death rate in minutes
30	6	6	U= Heating time in minutes
35	6	6	a= Initial number of microorganisms
40	6	6	b= No. of microorganisms which survived the heating time 'U'
45	6	6	50
50	6	5	= ----- = 12.25
55	6	0	
60	6	0	log ₆₀₀₀₀ -log ₅

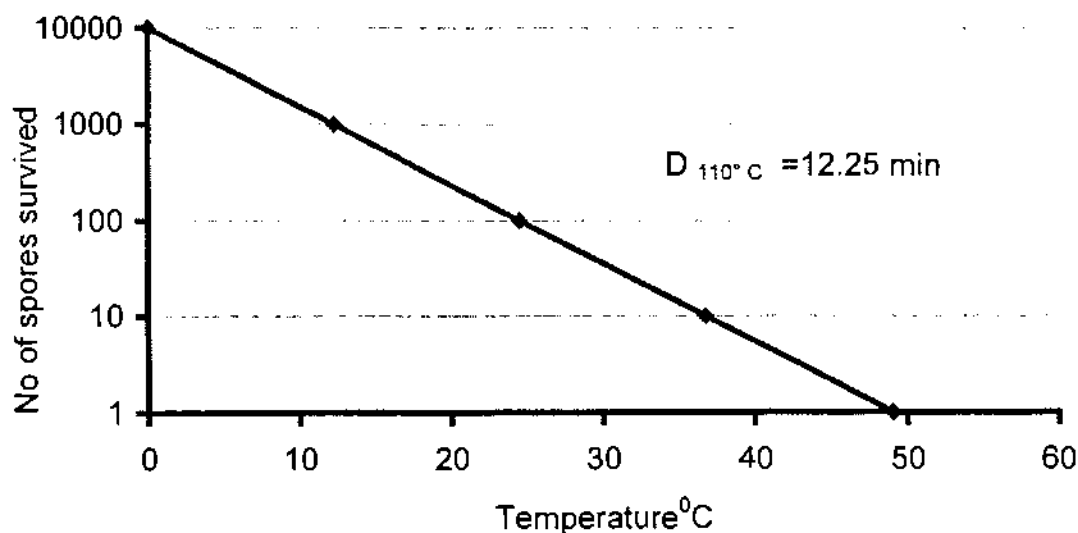


Fig 4.6 $D_{110^{\circ}\text{C}}$ of *C. sporogenes* PA.3679 in Buffalo meat emulsion

Table 4.7. Thermal Death Time (TDT) record of *C. sporogenes* PA. 3679 in buffalo meat emulsion at 115° C.

THERMAL DEATH TIME RECORD			
Medium	: Buffalo meat emulsion		
PH	: 6.28		
Organism	: <i>C. sporogenes</i> PA. 3679		
Initial Number of spores	: 10,000 per tube		
Incubation period	: 8 weeks		
Spore count	: Growth-No growth method		
Heating time (min)	No. of samples Heated	Positive	'D' value min
3	6	6	U
6	6	6	D= ----- Log _a -log _b
9	6	6	
12	6	6	D= Death rate in minutes U= Heating time in minutes
15	6	6	a= Initial number of microorganisms
18	6	5	b= No. of microorganisms which survived the heating time 'U'
21	6	0	18
24	6	0	= ----- = 4.412 log ₆₀₀₀₀ -log ₅

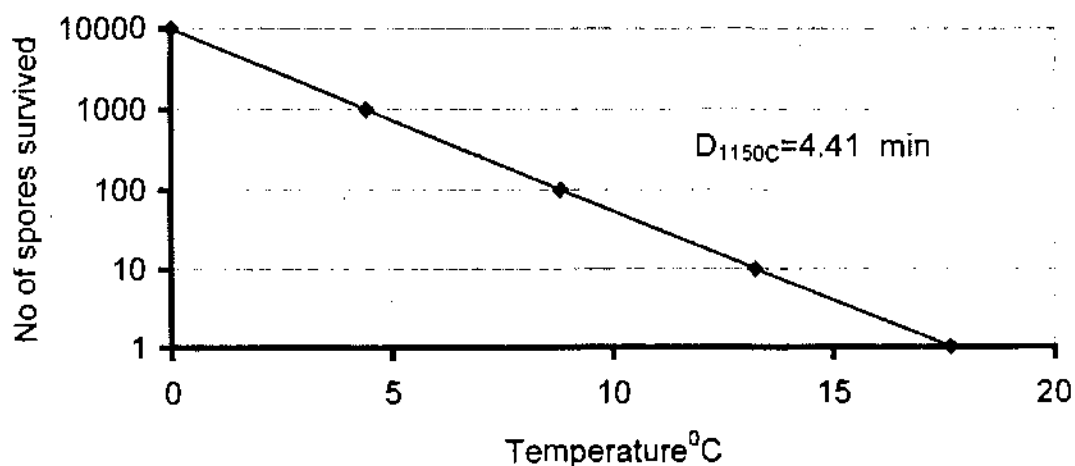


Fig 4.7 $D_{115^{\circ}C}$ of *C. sporogenes* PA.3679 in Buffalo meat emulsion

Table 4.8. Thermal Death Time (TDT) record of *C. sporogenes* PA. 3679 in buffalo meat emulsion at 118° C.

THERMAL DEATH TIME RECORD			
Medium	: Buffalo meat emulsion		
pH	: 6.28		
Organism	: <i>C. sporogenes</i> PA. 3679		
Initial Number of spores	: 10,000 per tube		
Incubation period	: 8 weeks		
Spore count	: Growth-No growth method		
Heating time (min)	No. of samples		'D' value min
	Heated	Positive	
2	6	6	U
4	6	6	D= ----- Log _a -log _b
6	6	6	
8	6	6	D= Death rate in minutes U= Heating time in minutes
10	6	5	a= Initial number of microorganisms b= No. of microorganisms which survived the heating time 'U'
12	6	0	10
14	6	0	= ----- = 2.45 log ₆₀₀₀₀ -log ₅

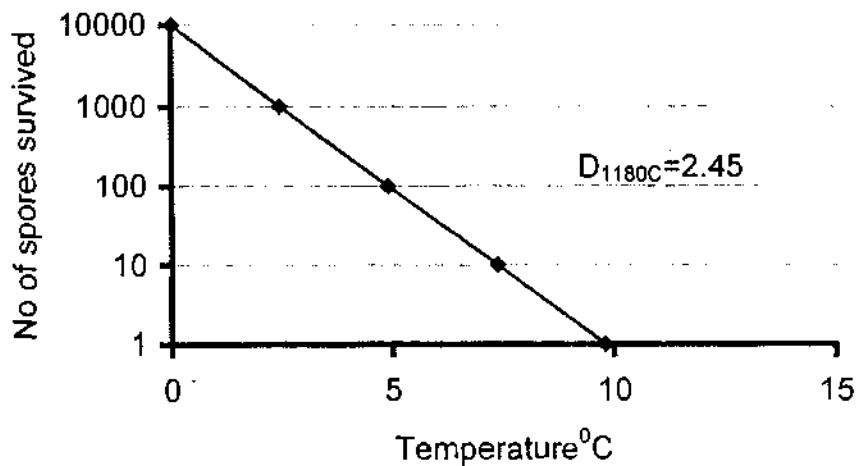


Fig 4.8 D_{118°C} of *C. sporogenes* PA.3679 in Buffalo meat emulsion

Table 4.9. Thermal Death Time (TDT) record of *C. sporogenes* PA. 3679 in buffalo meat emulsion at 121° C.

THERMAL DEATH TIME RECORD			
Medium	: Buffalo meat emulsion		
pH	: 6.28		
Organism	: <i>C. sporogenes</i> PA. 3679		
Initial Number of spores	: 10,000 per tube		
Incubation period	: 8 weeks		
Spore count	: Growth-No growth method		
Heating time (min)	No. of samples Heated	No. of samples Positive	'D' value min
0.5	6	6	U
1.0	6	6	D= -----
1.5	6	6	Log _a -log _b
2.0	6	6	
2.5	6	6	D= Death rate in minutes
3.0	6	6	U= Heating time in minutes
3.5	6	6	a= Initial number of microorganisms
4.0	6	6	b= No. of microorganisms which survived the heating time 'U'
4.5	6	6	5.5
5.0	6	5	= ----- = 1.35
5.5	6	5	
6.0	6	0	log ₆₀₀₀₀ -log ₅
6.5	6	0	

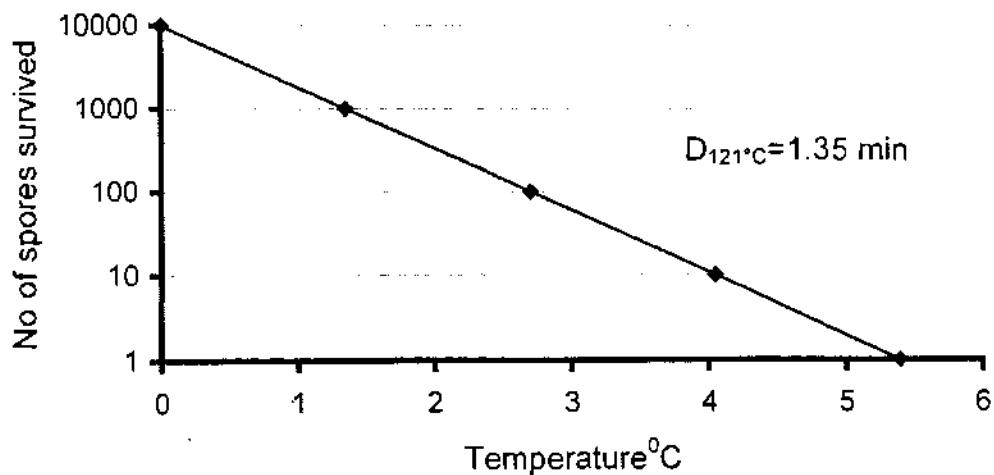


Fig 4.9 D_{121°C} of *C. sporogenes* PA.3679 in Buffalo meat emulsion

Table 4.10 'Z' Values of PA. 3679 in Buffalo meat emulsion

Temperature °C	'D' Value (minutes)	'Z' Value (° C)
110	12.25	
115	4.41	11.40
118	2.45	
121	1.35	

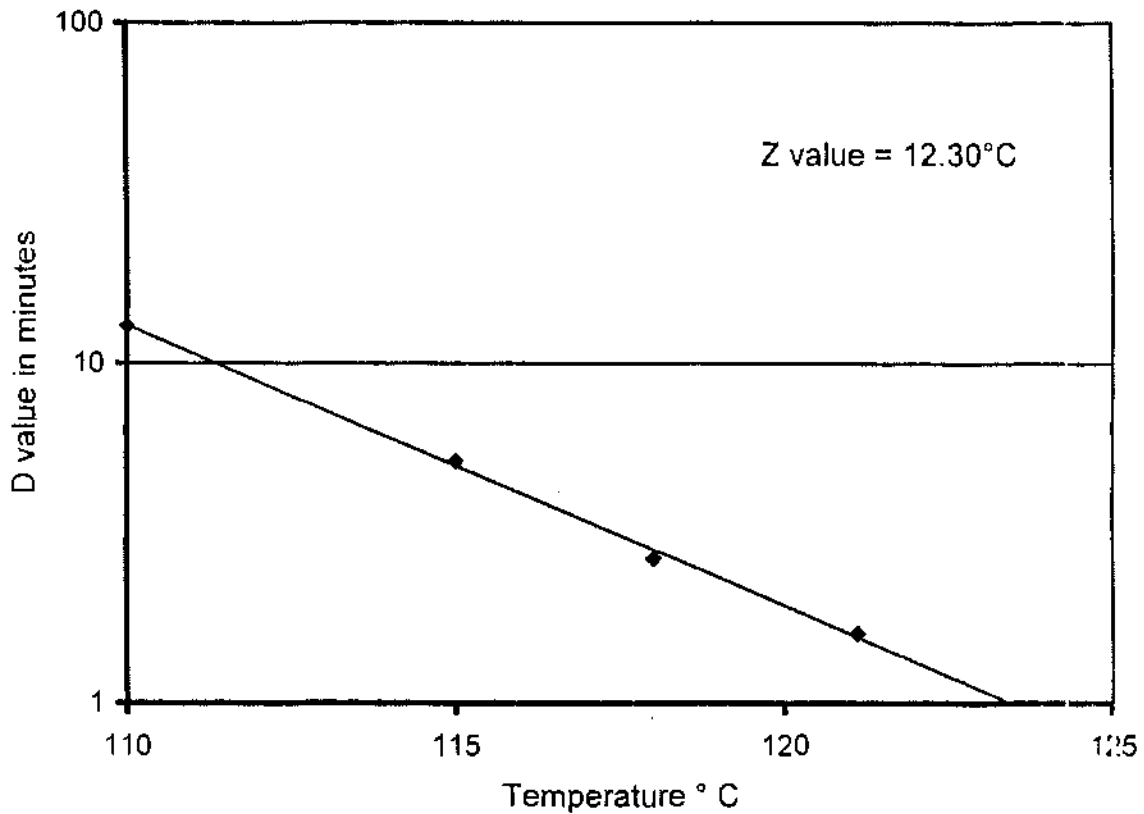


Fig 4.10 Z value of *C. sporogenes* PA.3679 in Buffalo meat emulsion

Table 4.11 Determination of coldest point in buffalo meat blocks processed in retort pouches using laboratory type fabricated still retort.

Location of thermocouple	Time required to reach 121 ⁰ c			Mean ± SE Min
	I	II	III	
A	65.30	66.00	65.15	65.48±0.26
B	64.55	65.20	65.30	65.02±0.24
C	64.16	65.10	64.25	65.50±0.30
D	64.40	64.15	65.10	64.55±0.29
E	64.20	64.40	63.55	64.05±0.26

C- Geometrical center of the pouch

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

thermocouples positioned laterally to the geometric centre. The longer heating time was probably due to cold temperature of the emulsion ($12\pm 1^{\circ}\text{C}$) and the fabricated still retort. The other thermocouples labelled as A, B, D and E taken a heating time of 65.48, 65.02, 64.55 and 64.05 min. 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 food, the critical point will be nearer to the centre of the food mass (Kopelman *et al.*, 1982). The geometrical centre 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 (Table 4.12). 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 . To reach this first lethality (0.001), that is the come-up-time (CUT) it took 13 min. The total heating time (HT) was noted as 20 min. After heating time of 20 min the product has received a lethality of 8.31. From 34 min of processing the cooling time (CT) has started. A 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 to several complicating factors, such as primitive slaughtering practices that are still prevalent in meat trading, tropical weather conditions, lack of refrigeration facilities for handling and transport. As a result the meats 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 or to give a built-in safety a lethality equivalent to 9D of *C. sporogenes* PA 3679 was given to the product. The products 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 (9D of *C. sporogenes* PA 3679). The thin

Table : 4.12 Heat penetration data of retort pouches with buffalo meat blocks processed for a targeted $F_0 = 12.13$ (9 D of *C. sporogenes* PA. 3679)

Heating Time Interval (min)	Retort temperature ° C	Product Temperature ° C	Lethal Rate	Summing up of Lethal Rate	Processed Time (min)
0	33.1	28.5			
1	58.2	29.4			
2	77.4	34.3			
3	87.3	42.1			
4	87.6	50.4			
5	89.1	57.6			
6	91.3	63.6			
7	93.3	68.9			
8	95.6	73.6			
9	97.8	77.8			
10	100.4	81.7			
11	102.5	85.3			
12	104.9	88.6			CUT=13 min
13	107.0	91.7	0.001	0.001	
14	109.6	94.7	0.002	0.003	
15	111.7	97.5	0.004	0.007	
16	114.2	100.2	0.008	0.015	
17	116.4	102.9	0.015	0.030	
18	118.6	105.4	0.027	0.057	
19	121.1	108.0	0.049	0.106	
20	122.4	110.6	0.089	0.195	
21	122.3	112.9	0.151	0.346	
22	122.1	114.8	0.234	0.580	
23	122.0	116.4	0.339	0.919	
24	121.9	117.6	0.447	1.366	
25	121.9	118.5	0.550	1.916	
26	121.9	119.3	0.661	2.577	
27	121.9	119.9	0.759	3.336	
28	122.0	120.4	0.851	4.187	
29	122.0	120.8	0.933	5.120	
30	122.0	121.1	1.000	6.120	
31	122.0	121.4	1.072	7.192	
32	122.0	121.6	1.122	8.314	HT=20 min
33	119.3	121.7	1.148	9.462	
34	113.1	121.4	1.072	10.534	
35	106.7	120.1	0.794	11.328	
36	98.6	117.6	0.447	11.775	
37	92.6	114.3	0.209	11.984	
38	87.7	112.4	0.134	12.118	
39	79.2	103.7	0.018	12.128	
40	75.4	98.0	0.005	12.133	
41	71.9	95.11	0.003	12.136	CT=8 min
Total process Time= CUT + HT+ CT					=41 min

Table:4.13 Inoculated pack studies- Products processed at $F_0 = 12.13$ min

Storage period (Days)	No. of pouches inoculated and processed	No. of positive pouches	No. of pouches inoculated and unprocessed	No. of positive pouches
0	6	Nil	6	6
30	6	Nil	6	6
60	6	Nil	6	6
90	6	Nil	6	6
120	6	Nil	6	6
150	6	Nil	6	6

profile and the increased surface area of the retort pouches are responsible for the reduction in the heating time (Chia *et al.*, 1983).

4.4 INOCULATED PACK STUDIES

The inoculated pack studies indicated that there was no growth of *C. sporogenes* PA 3679 during the entire 135 days of storage (Table 4.13). It validates the safety of the process. But the pouches inoculated and unprocessed (control) showed physical deformities, ballooning due to gas production and foul smelling. As the products were treated to a very high lethality (9D), the products were microbiologically safe.

4.5 EFFECT OF BINDERS ON THE QUALITY OF BUFFALO MEAT BLOCKS PROCESSED IN RETORT POUCHES

The functional performance of 4 binders was assessed in order to improve the quality of buffalo meat blocks. Incorporation of corn and tapioca starch had significantly increased the stability of emulsion than refined wheat flour and wheat semolina (Table 4.14). However corn starch had contributed better stability to the emulsion than tapioca starch. It might be due to better binding properties of corn starch over others. Addition of tapioca starch had significantly improved the emulsion stability in comparison to refined wheat flour possibly due to its greater peak viscosity, the thickening power of starch. However, the product yield, drip loss and pH did not vary significantly due to addition of refined wheat flour, corn and tapioca starch.

Use of wheat semolina had significantly decreased the emulsion stability resulting in significant lower product yield and higher drip loss, as a consequence of its lower pH in comparison to other binders. However, Madhwaraj *et al.* (1983) reported that addition of wheat semolina 3% and sodium pyrophosphate 1% prevented the water separation in corn buffalo meat. Drip loss was markedly lower for the products containing corn starch in comparison to ones with refined wheat flour and tapioca starch.



Plate 5: Shelf stable buffalo meat blocks processed in retort pouches

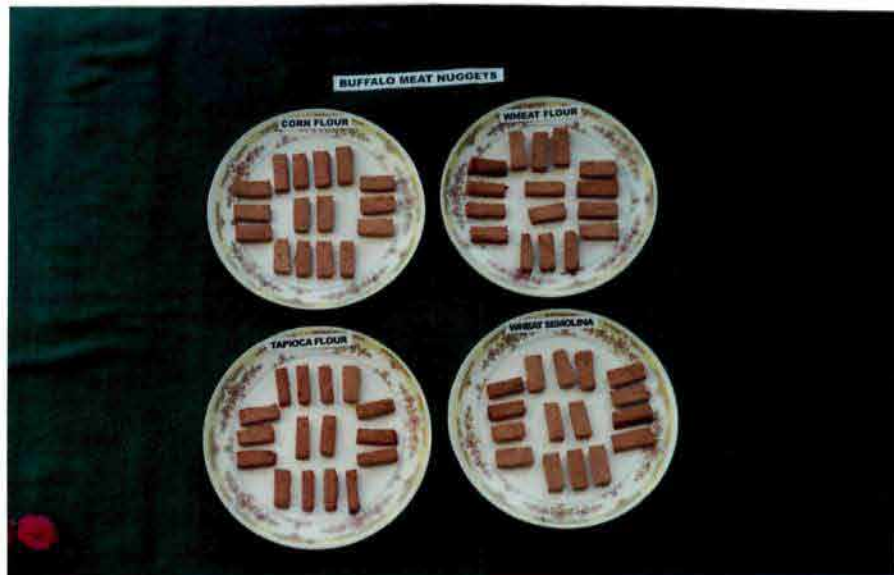


Plate 6: Nuggets made from buffalo meat blocks processed in retort pouches

Table 4.14. Effect of different binders on the physicochemical properties of buffalo meat blocks processed in retort pouches.

Parameters	Formulations			
	Corn starch	Refined wheat flour	Tapioca starch	Wheat semolina
Emulsion stability (%)	89.58 ± 0.36 ^a	85.03 ± 0.36 ^b	88.67 ± 0.44 ^a	83.13 ± 0.19 ^c
Product yield (%)	99.14 ± 0.23 ^a	98.48 ± 0.55 ^a	99.09 ± 0.27 ^a	97.16 ± 0.47 ^b
Drip loss ml	0.62 ± 0.07 ^a	0.92 ± 0.22 ^a	0.90 ± 0.24 ^a	2.80 ± 0.58 ^b
Product pH	6.28 ± 0.01	6.26 ± 0.02	6.23 ± 0.01	5.89 ± 0.33
Shear force value (kg/cm ³)*	0.42 ± 0.01 ^a	0.36 ± 0.01 ^b	0.32 ± 0.01 ^c	0.32 ± 0.01 ^c
Frying loss (%)*	14.46 ± 0.91	15.09 ± 0.29	16.81 ± 0.63	14.96 ± 0.42
Moisture (%)	65.57 ± 1.24	64.89 ± 0.11	64.43 ± 0.34	63.97 ± 0.03
Protein (%)	19.54 ± 0.32	19.74 ± 0.12	20.08 ± 0.01	20.08 ± 0.01
Fat (%)	10.47 ± 0.09 ^a	10.03 ± 0.09 ^b	9.97 ± 0.09 ^b	10.15 ± 0.01 ^b

Means with common superscripts in each row do not differ significantly.

n = 8

* = 8 observations

Table 4.15. Effect of different binders on the texture profiles and sensory attributes of buffalo meat blocks processed in retort pouches.

Parameters	Formulations			
	Corn starch	Refined wheat flour	Tapioca starch	Wheat semolina
Texture profiles^A				
Firmness (N)	22.17 ± 2.55	21.50 ± 0.75	17.41 ± 0.46	16.52 ± 2.12
Cohesiveness	0.77 ± 0.05	0.69 ± 0.01	0.74 ± 0.05	0.73 ± 0.02
Springiness(mm)	4.80 ± 0.11	4.60 ± 0.01	4.43 ± 0.37	4.27 ± 0.29
Gumminess (N)	17.36 ± 2.71	14.99 ± 0.52	12.87 ± 1.19	11.90 ± 1.17
Chewiness (Nmm)	83.8 ± 12.51	75.66 ± 8.09	56.47 ± 9.36	51.34 ± 8.42
Sensory attributes^B				
Appearance	7.38 ± 0.13 ^a	6.86 ± 0.14 ^b	6.81 ± 0.11 ^b	6.76 ± 0.19 ^b
Flavour	7.19 ± 0.08	6.42 ± 0.13 ^b	6.35 ± 0.11 ^b	6.35 ± 0.13 ^b
Juiciness	7.17 ± 0.08 ^a	6.47 ± 0.13 ^b	6.23 ± 0.15 ^b	6.43 ± 0.18 ^b
Texture	7.12 ± 0.07 ^a	6.50 ± 0.15 ^b	6.45 ± 0.14 ^b	6.56 ± 0.14 ^b
Overall acceptability	7.23 ± 0.09 ^a	6.48 ± 0.13 ^b	6.45 ± 0.14 ^b	6.35 ± 0.13 ^b

*Based on 8-point hedonic scale, wherein 8 = extremely desirable and 1 is extremely undesirable

Means with common superscripts in each row do not differ significantly.

A=3 observations; B= 24 observations.

Table 4.16: Analysis of variance for the effect of different binders on the quality of buffalo meat blocks processed in retort pouches.

Quality Characters	Treatments @			Error
	MSS	F	DF	MSS
Emulsion stability	27.6435	79.07**	8	0.3496
Product yield	2.5321	5.13*	8	0.4938
Product pH	0.1025	1.24	8	0.0823
Drip loss	3.8275	11.27**	8	0.3396
Shear force value	0.01705	34.10**	28	0.0005
Moisture	3.8757	3.07	8	1.2603
Proteins	1.2036	13.68**	8	0.0879
Fat	0.6487	32.04**	8	0.0202
Frying loss	8.3493	2.79	28	2.9859
Texture Profiles				
Firmness	2431.167	2.75	8	885.4375
Cohesiveness	0.0027	0.65	8	0.0042
Springiness	0.1822	1.03	8	0.1767
Gumminess	17.6248	2.25	8	7.8062
Chewiness	713.6128	2.49	8	285.5284
Sensory Attributes				
Appearance	1.7460	3.82*	80	0.4571
Flavour	3.4643	12.04**	80	0.2878
Juiciness	3.4633	8.43**	80	0.4107
Texture	2.0436	5.86**	80	0.3482
Overall acceptability	3.6111	11.12**	80	0.3247

*=P<0.05; **=P<0.01 ; @ DF=3

Texture of the products as indicated by shear force value was significantly higher for the products with corn starch, followed by refined wheat flour than that of others. However, the shear force value did not vary between the products containing tapioca starch and wheat semolina. Tapioca starch with small amount of amylose will form soft gel when gelatinized (Lamkey, 1998) causing less firm (soft) texture to the meat product. Type of binder used had no significant effect on frying loss, moisture and protein content of products. Addition of corn starch had significantly increased the fat content as well as markedly moisture content, in comparison to other binders. The physico-chemical properties indicated that the corn starch has better functional properties.

Texture profiles such as firmness, cohesiveness springness, gumminess and chewiness of the product containing different binders did not differ significantly (Table 4.15). However, corn starch and refined wheat flour had contributed firmer texture to the product whereas tapioca starch and wheat semolina had imparted relatively soft texture which was also indicated by lower shear force value. Corn starch also contributed greater gumminess and chewiness to the products resulting higher sensory scores for texture and overall acceptability. Part of the differences in the texture profiles could be due to variation in gelatinization properties of starches (Skrede, 1989).

The product containing corn starch had significantly higher sensory scores for all attributes in comparison to the products with other binders which are inconsonance with the findings of texture profiles. Significantly better texture of the products with corn starch might be due to its ability to form firm gel. Although sensory attributes did not differ significantly for the products with refined wheat flour, tapioca starch and wheat semolina, the products containing refined wheat flour had slightly higher sensory scores for appearance, flavour, juiciness and overall acceptability. Products with wheat semolina had least overall acceptability which might be due to loss of functional properties during its processing. Skrede (1989) also rated potato flour as the best suited starch followed by corn and wheat starch, while tapioca as least suited for use in meat sausages.

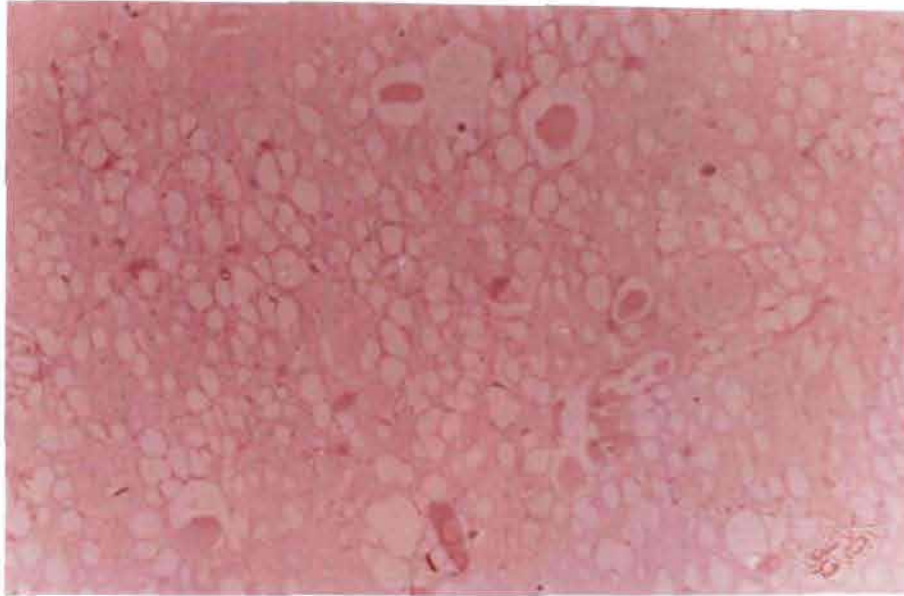


Plate 7: Histological structure of buffalo meat blocks prepared with corn starch as binder under light microscope (x 100 magnification)

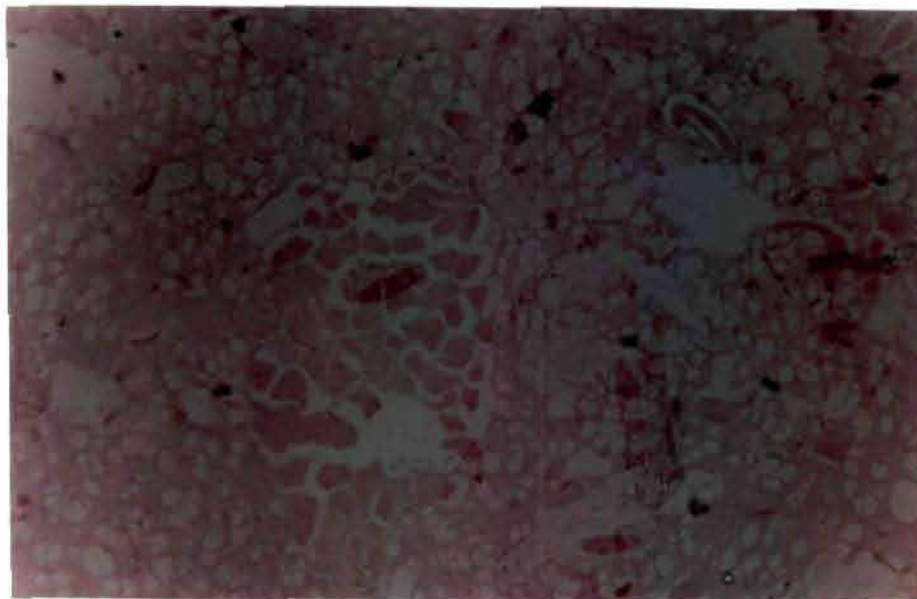


Plate 8: Histological structure of buffalo meat blocks prepared with refined wheat flour as binder under light microscope (x 100 magnification)

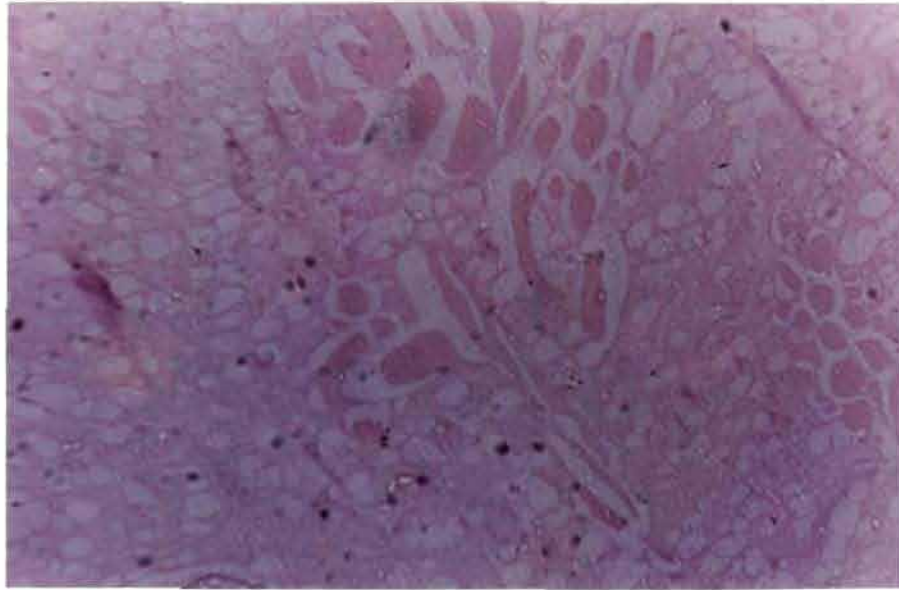


Plate 9: Histological structure of buffalo meat blocks prepared with tapioca starch as binder under light microscope (x 100 magnification)

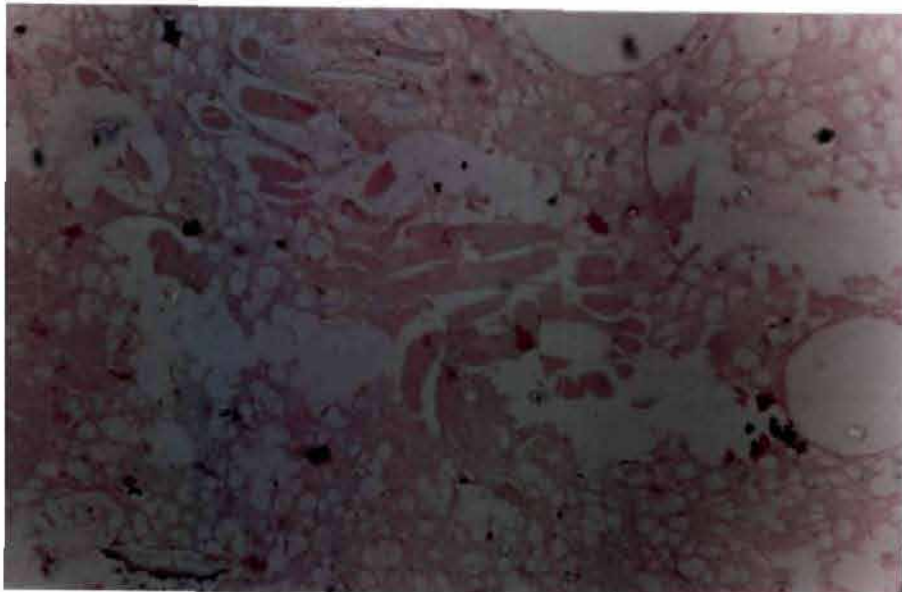


Plate 10: Histological structure of buffalo meat blocks prepared with wheat semolina as binder under light microscope (x 100 magnification)

Panelists preferred the products containing different binders in the order of corn starch > refined wheat flour > tapioca starch > wheat semolina.

Histological studies

Micrograph obtained from histological study indicate that the corn starch containing product has a dense matrix (Plate 7). This dense matrix could be the protein matrix formed from the gelatin of salt soluble protein at the cooking which is existed as a continuous phase (Ray *et al.*, 1981). The fat globules are small, numerous and of irregular shape. The fat globules are seen covered with the meat protein matrix which might be the reason for better emulsion stability.

Micrograph of refined wheat flour incorporated sample shows that small irregular fat globules dispersed in meat protein matrix and coalescence of some fat globules (Plate 8). Micrograph of tapioca starch added product reveals that smaller fat globules dispersed in protein matrix with many fat channel (Plate 9).

Micrograph of wheat samolina containing product shows large clumps of fat not properly dispersed in the matrix (Plate 10) which might have resulted in lower stability to emulsion and higher drip loss.

Based on the physico-chemical histological structure, texture profiles and sensory characteristics of the products, the suitability of the binders are rated in the order of corn starch > refined wheat flour > tapioca starch > wheat semolina for use in the emulsion type buffalo meat blocks processed in retortable pouches at higher temperatures.

4.6 QUALITY AND SHELF LIFE OF BUFFALO MEAT BLOCKS PROCESSED IN RETORT POUCHES

4.6.1 Quality of meat blocks

Physico-chemical and sensory characteristics of processed meat products are very important for their acceptability and marketing. The initial quality attributes of buffalo meat blocks processed in retort pouches were presented in Table 4.17.

Table 4.17. Physico-chemical parameters, texture profiles and sensory attributes of buffalo meat blocks processed in retort pouches on day one.

Parameters	No.of observations	Mean \pm S. E.
Emulsion stability (%)	3	90.33 \pm 0.60
Yield (%)	6	98.47 \pm 0.59
pH	3	6.28 \pm 0.01
Shear force value kg/cm³	12	0.4475 \pm 0.01
Frying loss	3	13.25 \pm 0.68
Fat (%)	3	10.08 \pm 0.19
Texture profiles		
Firmness (N)	3	16.58 \pm 0.96
Cohesiveness	3	0.71 \pm 0.02
Springiness (mm)	3	4.33 \pm 0.33
Gumminess (N)	3	11.74 \pm 1.08
Chewiness (Nmm)	3	50.68 \pm 4.82
Sensory attributes		
Appearance	24	7.67 \pm 0.09
Flavour	24	7.37 \pm 0.10
Juiciness	24	7.29 \pm 0.13
Texture	24	7.62 \pm 0.12
Overall acceptability	24	7.58 \pm 0.10

Stability of buffalo meat emulsion 90.3% was markedly lower than the value 93.3% reported by Deenathyalan (1997) which might be due to variation in the formulation. It is also influenced by salt and polyphosphate level in the product formulation, emulsion pH, type of meat used, level of added water, chopping temperature and processing procedure (Townsend *et al.*, 1968; Kondaiah *et al.*, 1985). The major factors affecting the emulsion stability appeared to be the physical properties of protein fat matrix, the fat protein interaction and size of fat droplets (Lee *et al.*, 1981).

Product yield (98.4%) was markedly higher than the yield of buffalo meat blocks (96.3%) processed in aluminium moulds (Sahoo, 1995). The excellent yield of the product was due to its higher pH, good emulsion stability and addition of polyphosphates (Anjaneyulu *et al.*, 1989). Cooking yields are dependent on type of meat, time, temperature and cooking method (Lawrie, 1991).

Texture of the product as indicated by the shear force value (0.45 kg/cm³) was comparable with the buffalo meat nuggets incorporated with 5% refined mustard oil (Sahoo, 1995). The frying loss of the products was 13.2%. The higher frying loss might be due to greater moisture loss indicating the more of free water in it and also due to loose binding of water during emulsion formation. Moisture, protein and fat contents of products were comparable with the composition of buffalo meat nuggets incorporated with 10% oil (Sahoo, 1985). Proximate composition of the product depends upon the several factors such as lean and fat ratio, incorporation of food additives and processing temperature.

Texture profiles-firmness, cohesiveness, springiness gumminess and chewiness of the products were comparable to the values of smoked chicken sausages (Anjaneyulu *et al.*, 1996). These texture profiles were markedly higher in comparison to the values reported for chicken nuggets containing different emulsion stabilizers (Rao *et al.*, 1997). Texture of the products also depends on meat particle size, type of meat and its proteins, fat and added water content, method and duration of cooking (Anjaneyulu *et al.*, 1995).

Sensory scores for appearance, flavour, juiciness, texture and overall acceptability of the products ranged from 6.6 to 7.5 on 8 point hedonic scale which indicates the excellent palatability of the product. These sensory scores were comparable to the scores reported for other communitied buffalo meat products such as patties (Anjaneyulu *et al.*, 1994), smoked sausages (Deenathayalan, 1997) and nuggets (Sahoo and Anjaneyulu, 1997). Physico-chemical, textural and sensory characteristics had demonstrated the excellent palatability and acceptability of the buffalo meat blocks processed in retort pouches.

4.6.2 Shelf life of meat blocks

A major cause of deterioration in the quality of stored muscle is oxidative rancidity. Flavour stability and oxidative deterioration are important considerations for the consumer acceptability of precooked products. Changes in the quality characteristics of buffalo meat blocks processed in retort pouches during 135 days of storage in a oven at $30\pm 1^{\circ}\text{C}$ were presented in the Tables 4.18 - 4.21).

pH

The pH of the products had declined gradually and significantly from 6.28 to 6.07 with increasing period of storage (Fig. 4.11) which might be due to protein degradation and liberation of free amino acids. However, Persson and Sydow (1974) reported insignificant decrease in pH of canned beef during storage.

Shear force value

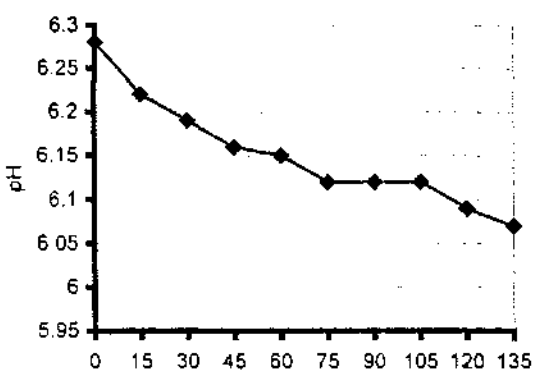
The texture of the products, as indicated by shear force values had decreased significantly from 0.46 to 0.40 kg/cm³ as the storage period advanced (Fig. 4.12). Decline of product texture could be ascribed to oxidative effects on proteins, peptides and amino acids (Spanier *et al.*, 1992).

Residual nitrite

Rapid and significant decrease of residual nitrite content of the products from 82.6 to 30 ppm was observed with the increase of storage (Fig. 4.13). The trend is in accordance with the findings of Hill *et al.* (1973), Mathew (1992),

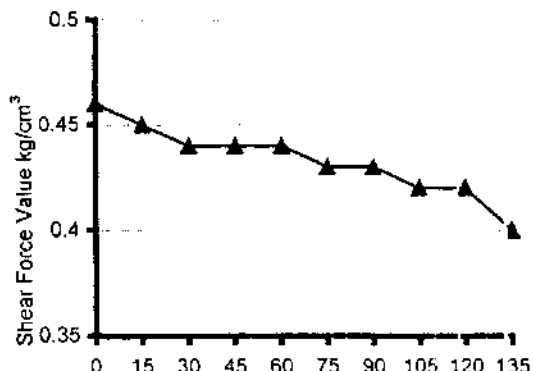
Table 4.18: Changes in pH, shear force value, nitrite of buffalo meat blocks processed in retort pouches during storage (30 ±1°C).

Storage (Days)	PH	Shear force value (kg/cm ³) (n=12)	Residual nitrite (ppm) (n=3)
0	6.28±0.01	0.46 ± 0.01	82.67 ± 2.33
15	6.22±0.01	0.45 ± 0.01	74.00 ± 1.1.5
30	6.19±0.01	0.44 ± 0.01	60.00 ± 1.15
45	6.16±0.01	0.44 ± 0.01	57.67 ± 1.45
60	6.15±0.01	0.44 ± 0.01	53.35 ± 1.20
75	6.12±0.01	0.43 ± 0.01	49.33 ± 1.76
90	6.12±0.01	0.43 ± 0.01	45.00 ± 1.73
105	6.12±0.01	0.42 ± 0.01	41.33 ± 1.20
120	6.09±0.01	0.42 ± 0.01	36.00 ± 1.53
135	6.07±0.01	0.40 ± 0.01	30.00 ± 2.08
CD5 %	0.02	0.03	4.75
CD1 %	0.03	-	6.47
n	3	12	3



Storage period in days

Fig.4.11 pH changes in buffalo meat block processed in retort pouche during storage.



Storage period in days

Fig 4.12 Shear Force Value of buffalo meat block processed in retort pouches during storage.

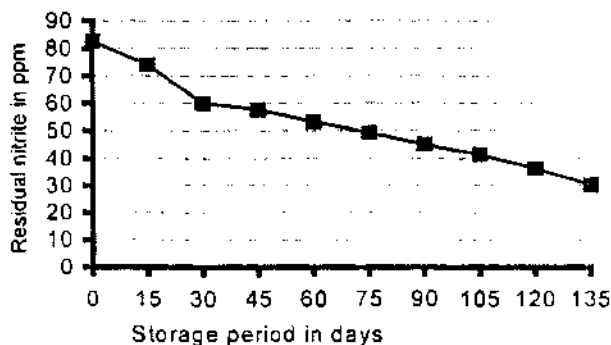


Fig 4.13 Residual nitrite levels in buffalo meat block processed in retort pouches during storage.

Cassens (1995). Amount of nitrite added to meat products are known to be rapidly depleted depending on initial nitrite level, temperature of storage, pH, salt concentration and presence of reducing agents (Bushway and Jen, 1984).

TBARS number

Significant and linear increase of TBARS number from 0.24 to 0.98 mg malonaldehyde/kg was found with increase in period of storage (fig. 4.15). It was below the minimum threshold value of TBARS number i.e. 0.5 - 1.0 mg malonaldehyde (Tarladgis *et al.*, 1960). They also reported that TBARS numbers were highly correlated with sensory scores of trained panelists for rancid odour in pork. As 3 ply laminate pouches were used which inhibit the permeability of oxygen coupled with vacuum sealing of the pouches might have caused slow oxidation. Similarly, a lower degree of oxidation was reported in retort pouched fish products (Chia *et al.*, 1983). Production of antioxidants in beef itself at high sterilization temperature was responsible for the stability against oxidative rancidity (Zipser and Watts, 1961). TBARS value of raw and cooked muscles of beef, chicken and pork had increased during storage (Rhee *et al.*, 1996).

Tyrosine value

There was linear and significant increase of tyrosine value from 0.35 to 0.74 mg/100 g with increase of storage period (Fig. 4.15). Tyrosine value of meat increased with storage time until deamination of amino acids limited the formation of free amino acids (Pearson, 1968). Similarly, an increase of tyrosine value was also reported in sterile and inoculated beef samples (Morrissey *et al.*, 1980) and buffalo meat samples (Agnihotri, 1988) during refrigerated storage. Tyrosine value is an indicator of proteolysis as it measures tyrosine and tryptophan in the extract of meat. An intrinsic changes of meat by autolysis had affected the protein degradation (Strange *et al.*, 1977).

Free amino acids

A significant and steady increase of free amino acids from 124.3 to 257.5 mg/100 g product was found with increase of storage period (Fig. 4.16). Thermal

Table 4.19 :Changes in TBARS number, tyrosine value, free aminoacids value of buffalo meat blocks during storage.(30±1°C)

Storage period (days)	TBARS Number(Malonaldehyde/kg)	Tyrosine value (mg/ kg)	FAA (mg/kg)
0	0.24 ± 0.03	0.37 ± 0.02	124.32 ± 2.26
15	0.31 ± 0.04	0.35 ± 0.01	147.00 ± 1.73
30	0.42 ± 0.02	0.49 ± 0.01	158.02 ± 2.76
45	0.43 ± 0.04	0.49 ± 0.01	177.38 ± 3.69
60	0.49 ± 0.03	0.52 ± 0.01	188.44 ± 2.59
75	0.53 ± 0.02	0.55 ± 0.01	209.95 ± 3.18
90	0.67 ± 0.02	0.58 ± 0.03	217.51 ± 0.80
105	0.75 ± 0.02	0.65 ± 0.02	222.89 ± 5.62
120	0.85 ± 0.14	0.66 ± 0.01	249.57 ± 1.73
135	0.98 ± 0.04	0.74 ± 0.01	257.50 ± 6.78
CD5%	0.09	0.08	12.21
CD1%	0.13	0.11	16.44
N	3	12	12

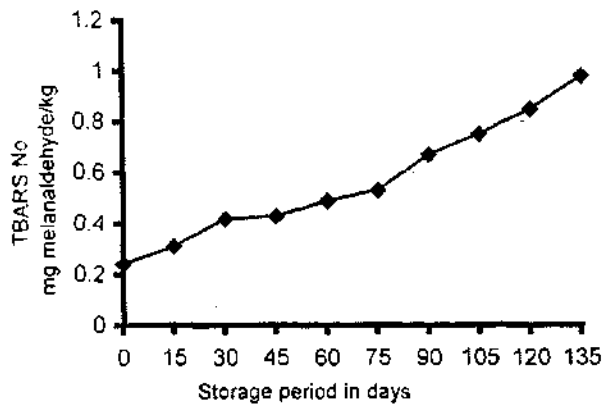


Fig 4.14 TBARS No of buffalo meat blocks processed in retort pouches during storage.

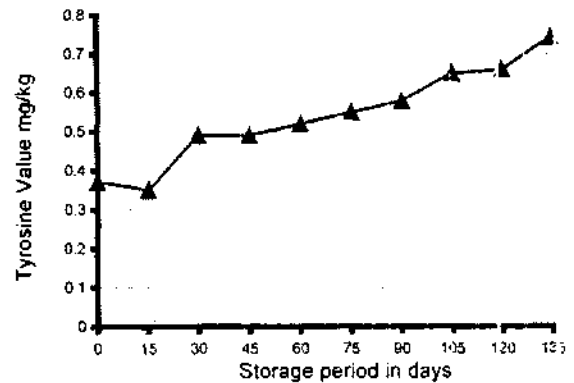


Fig 4.15 Tyrosine Value of buffalo meat blocks processed in retort pouches during storage.

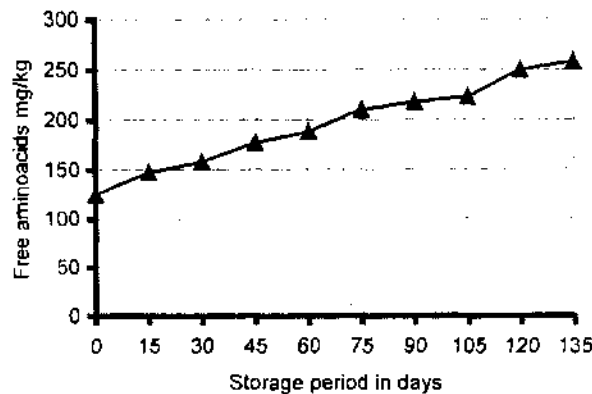


Fig 4.16 Free aminoacids level of buffalo meat blocks processed in retort pouches during storage.

processing at high temperature might have caused an in the rate of protein break down resulting more free amino acids during storage period.

Microbiological quality

Few colonies of aerobic mesophils were found occasionally. However, the anaerobes were totally absent which indicates the effectiveness of the thermal process.

SDS-PAGE

The protein samples were subjected to SDS-PAGE analysis from 0 day to 135 days of storage at 15 days intervals. The proteins hydrolysis pattern (Plate 11) showed barely discernible 205 KDa protein from 'O' day to 135 days of storage. This is mainly, because the products were processed at a very high temperature of 121°C. Higher molecular weight proteins were cleaved into lower molecular weight protein fragments. In the molecular range of 68 and 29 KDa, the protein concentration was more 68, 43 and 29 KDa protein bands were clearly visible upto a period of 135 days. This has indicated that storage period did not affect the hydrolysis of proteins significantly. There was no further cleavage of proteins and presence of new bands were noticed, as the autolysis was minimal in this retort pouch processed products. However, Sahoo and Anjaneyulu (1997) reported, increase in the concentration of lower molecular weight proteins as the storage period increases due to autolytic changes of meat proteins. This study indicated that increase in storage period did not influence significantly the break down of protein molecules.

Sensory attributes

A significant decrease in the intensities of sensory characteristics of the product was observed with the increase of storage (30±1°C) (Table 4.20, Fig. 4.17).

Appearance : Sensory scores for appearance of the product had declined slowly from 7.6 to 6.2 based on 8 point hedonic scale in 90 days and rapidly to 5.5 in 120 days of storage. Similarly, Persson and Sydow (1974) reported that the appearance of canned beef became lighter in colour and more reddish during storage.

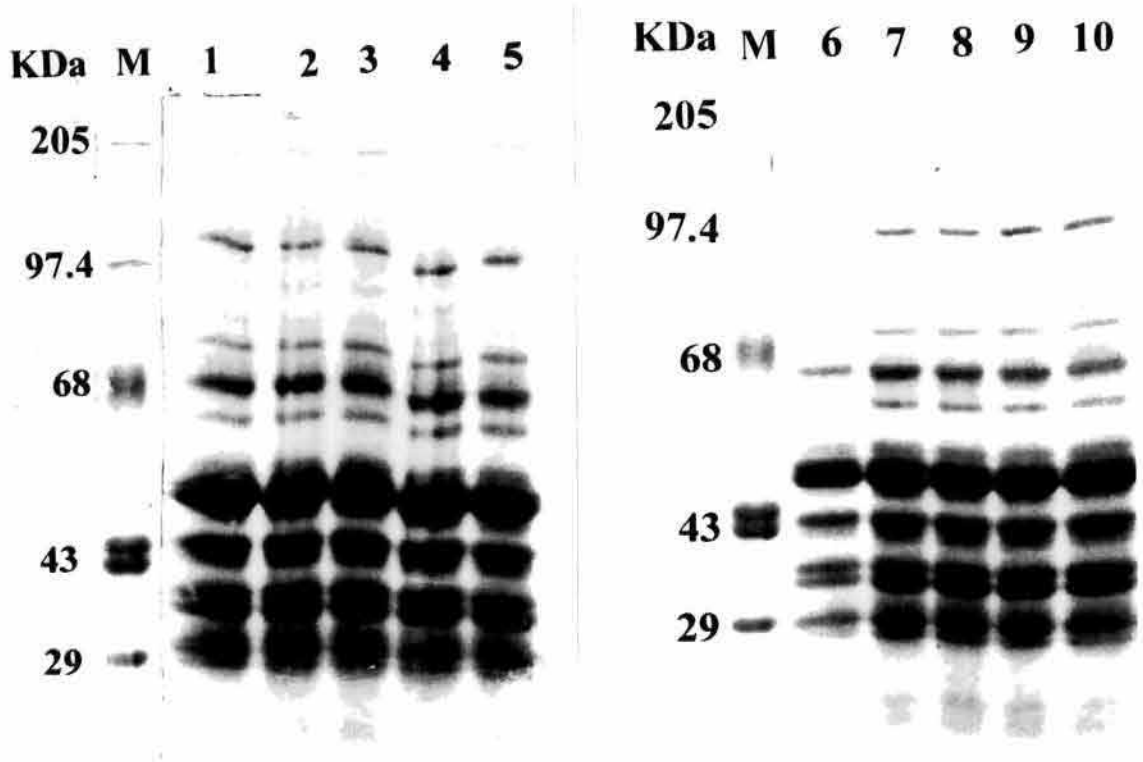


Plate H: Protein hydrolysis pattern of buffalo meat blocks stored up to 135 days
 M- Molecular weight marker- broad range
 Lane 1- 10: Different period of storage from 0-135 days

Table 4.20. Changes in sensory attributes of buffalo meat blocks processed in retort pouches during storage (30± 1°C).

Storage period days	Sensory attributes*					Overall acceptability
	Appearance	Flavour	Juiciness	Texture		
0	7.66 ± 0.09	7.37 ± .10	7.29 ± 0.15	7.62 ± 0.11		7.58 ± 0.10
15	7.50 ± 0.10	7.16 ± 0.15	6.79 ± 0.08	7.12 ± 0.12		7.29 ± 0.14
30	7.08 ± 0.15	7.12 ± 0.15	6.58 ± 0.10	7.08 ± 0.12		6.96 ± 0.11
45	6.91 ± 0.13	6.54 ± 0.10	6.45 ± 0.15	6.79 ± 0.15		6.66 ± 0.10
60	6.75 ± 0.14	6.37 ± 0.17	6.42 ± 0.10	6.71 ± 0.09		6.50 ± 0.10
75	6.50 ± 0.10	6.29 ± 0.16	6.37 ± 0.29	6.54 ± 0.10		6.37 ± 0.14
90	6.29 ± 0.09	6.12 ± 0.15	6.10 ± 0.13	6.19 ± 0.09		6.13 ± 0.13
105	5.62 ± 0.14	5.46 ± 0.13	5.46 ± 0.12	5.58 ± 0.16		5.41 ± 0.13
120	5.58 ± 0.13	5.33 ± 0.09	5.29 ± 0.11	5.41 ± 0.10		5.37 ± 0.12
CD 5%	0.33	0.38	0.42	0.35		0.34
CD 1%	0.44	0.51	0.55	0.46		0.44

n=24

*Based on 8-point hedonic scale wherein 8= extremely desirable, 1= extremely undesirable

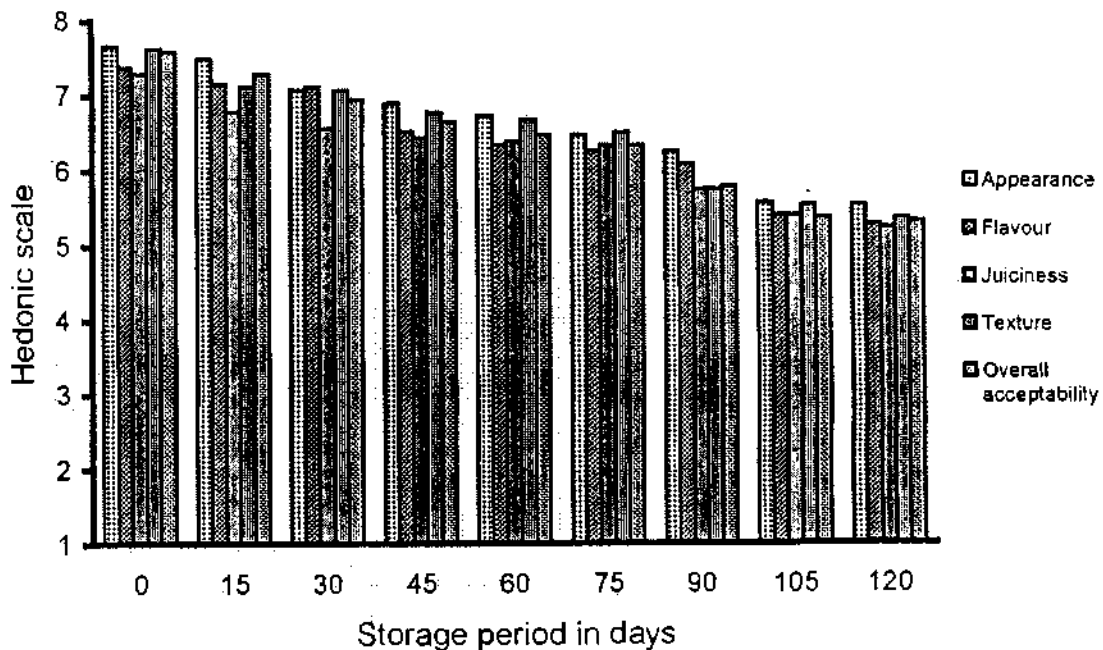


Fig 4.17 Sensory attributes of buffalo meat blocks processed in retort pouches during storage period

Table 4.21. Coefficient of correlation (r) between number of storage days and values of different quality parameters

Parameter	Coefficient of correlation (r)
PH	-0.961**
Nitrite	-0.978**
Shear force value	-0.786**
TBARS number	+0.985**
Free amino acids	+0.975**
Tyrosine value	+0.976**
Appearance	-0.986**
Flavour	-0.980**
Juiciness	-0.980**
Texture	-0.974**
Overall acceptability	-0.990**

** (P<0.01)

Table 4.22: Analysis of variance for the effect of storage period on certain quality parameters of buffalo meat blocks processed in retort pouches.

Quality Characters	Storage period (Days)			Error	
	DF	MSS	F	DF	MSS
PH	9	0.0116	86.74**	20	0.0027
Shearforce value	9	0.0036	2.52*	119	0.0014
Residual nitrite	9	631.3925	81.29*	20	7.7667
TBRAS value	9	0.1715	55.60**	20	0.0031
Free amino acids	9	5152.01791	96.07**	20	53.6264
Tyrosine value	9	0.0454	43.73**	20	0.0010
Appearance	8	13.5312	38.40**	207	0.3523
Flavour	8	12.5775	27.11**	207	0.4639
Juiciness	8	9.9803	18.27**	207	0.5461
Texture	8	13.9178	35.75*	207	0.3893
Overall acceptability	8	14.7292	41.48	207	0.3551

*P< 0.05 ; ** P< 0.01

Flavour : Significant and gradual decline of flavour scores from 7.3 to 6.1 in 90 days and further rapid decrease to 5.3 during 4 months storage was observed. The flavour of the products was found to be slightly unacceptable on 135th day of storage due to development of mild "acid, sour" taste. The significant decline in flavour might be due to oxidation products (Spanier *et al.*, 1992) and also evident from the increased TBARS number during storage. Persson and Sydow (1974) also reported rapid aroma changes of beef in flexible pouches in comparison to cans, attributed to oxygen penetration through the pouches. They also found the loss of typical meat aroma and increase in some of the negative odour qualities acid, sour and like whey butter in canned beef. Similar unpleasant odour qualities were also observed in the pouched buffalo meat blocks at 135 days of storage. These flavours were suspected to originate from volatile fatty acids which increase during storage. It is probable, that the odour from acids (mainly acetic, butyric, hexanoic and heptanoic acids) was masked during initial period of storage (Persson and Sydow, 1974). Further they stated that as the concentration of sulfur compounds and aldehydes decreased, the flavour from acids become of increasing importance.

Juiciness : There was significant decrease of juiciness scores from 7.2 to 6.1 in 90 days and further drastically declined to 5.2 in 120 days of storage. Similarly Mathew (1992) reported the decline of juiciness scores of cured and smoked buffalo meat chunks with the advance of storage period.

Texture : A significant decrease was observed in texture score, from 7.6 to 6.2 in 90 days and further reduced to 5.4 in 120 days of storage. Texture of the product might be affected by the oxidative changes in protein and amino acids (Spanier *et al.*, 1992). Decline of product texture was further evident from the decrease of shear force value during storage. Texture scores might have also affected by the degradative changes in proteins which was showed by the increase of the free amino acid content during storage.

Overall acceptability : There was a significant reduction of acceptability scores from 7.5 to 6.1 in 90 days and further rapidly decreased to 5.3 in 120 days of

storage. The products were well acceptable upto 90 days and were slightly palatable upto 120 days of storage. However, they were slightly unacceptable on 135 days of storage due to development of mild acid, sour flavour. This might be due to changes in the physico-chemical properties of the product during storage. Persson and Sydow (1974) also stated that the chemical changes had followed by analogous changes in sensory attributes. Chia *et al.* (1983) also observed a significant decline of colour, flavour and overall acceptability of pouched fish products after 90 days of storage. Similarly, Sahoo and Anjaneyulu, 1997) reported a decrease in sensory qualities of nuggets as the storage period increased.

Correlation coefficients

Relationship of various quality parametrs of buffalo meat blocks processed in retort pouches with storage period was established by calculating correlation coefficients (*r*) (Table 4.21).

The pH, shear force values, residual nitrite and all sensory attributes had significant inverse correlation with storage period. TBARS numbers, tyrosine value and free amino acid content had linear positive correlation with storage period. All the physico-chemical and sensory properties of pouched meat blocks were dependent on the period of storage. Similar correlation coefficients were reported for buffalo meat nuggets during refrigerated storage (Sahoo and Anjaneyulu, 1997).

CONCLUSIONS

The product was found to have good acceptability upto 90 days of storage based on the evaluation of physico-chemical microbiological and sensory parameters upto 135 days of product storage. The product was microbiologically safe and recommended a shelf life of 90 days at 30±1°C.

SUMMARY

Buffaloes play a very important role in Indian economy. India is number one in buffalo population (91.8 million) in the world and they contribute annually 1403 thousand MT of meat (FAO, 1998). About 85% of meat exported from our country is of buffalo origin. Buffalo meat from India, is having very good demand in international trade, especially from Middle East and South East Asian countries. Spent and unproductive animals are mostly slaughtered for meat purpose and the meat is tough due to coarse and fibrous nature. Comminution is a method of making tough meat into palatable meat products. Comminuted or emulsion based products such as sausages, patties, blocks, kababs and nuggets are some of the popular products. But the shelf life of these products is less due to lack of cold storage facilities, improper packaging technology and frequent power failures.

Thermal processing or heat sterilization is a method of preserving food products in ambient temperature. It was discovered in 1809 by a French Scientist, Nicholas Appert. He used glass containers for thermal processing of foods. Later on, metal cans replaced glass containers. Due to many disadvantages experienced with metal cans such as cost, longer process time, more storage space and difficulty in disposal etc., an alternate packaging material using flexible laminates was developed in late 1950s by US Army Natick R & D Laboratories along with Continental Can Company and Reynolds Metal Company. It was called as "Retortable Pouches" or Flexican or Flexipack. It is a 3-ply laminate of polyester, aluminium foil and cast polypropylene. It eliminated most of the disadvantages of metal cans. It is important to retain the physical, textural and sensory properties of thermally processed pouched products. Information is not available on the thermal processing of buffalo meat products in retortable pouches. Based on the above facts, this study was taken with the objectives of determination of process time, optimization of product quality and evaluation of shelf life of retort pouch processed meat blocks at ambient temperature of $30\pm 1^{\circ}\text{C}$.

1. DETERMINATION OF PROCESS TIME FOR BUFFALO MEAT BLOCKS IN RETORT POUCHES

The 'D' values of *C. sporogenes* PA 3679 in phosphate buffer saline (PBS - pH 7.0) as reference medium were determined as 12.90, 5.16, 2.66 and 1.60 min at temperatures of 110°, 115°, 118° and 121°C respectively. The 'Z' value was determined as 12.30°C. The 'D' values and 'Z' values calculated for *C. sporogenes* PA 3679 confirmed that the spore suspension was best suited for conducting thermal resistance studies. The 'D' values of *C. sporogenes* PA 3679 in buffalo meat emulsion were determined as 12.25, 4.41, 2.45 and 1.35 min at temperatures of 110°, 115°, 118° and 121°C respectively and the Z value was determined as °C which is more than the classical 'Z' value of 10°C. The heat resistance of *C. sporogenes* PA 3679 in buffalo meat emulsion (pH 6.28) was lesser than the heat resistance in neutral PBS, but higher when compared to resistance in other low acid foods.

The F_0 value in buffalo meat emulsion was determined as 6.75 min (5D of *C. sporogenes* PA 3679) which is lesser than the F_0 value determined in neutral PBS calculated in this study (8.0 min). For heat penetration studies, the coldest point was determined as the geometric centre of the pouch. For shelf life studies, the products were processed to a targeted F_0 value of 12.13 min which is equivalent to a 9D of *C. sporogenes* PA 3679. The heat penetration studies indicated that a come up time (CUT) of 13 min, a heating time of 20 min (HT), and a cooling time (CT) of 8 min to achieve the targeted F_0 value of 12.13 min with a total process time of 41 min in a Stock-Rotomat Sterilizer, Germany. For indirect confirmation of microbial safety of the products, inoculated pack studies were conducted. The buffalo meat emulsion filled in pouches, inoculated with *C. sporogenes* PA 3679 and processed at F_0 12.13 min had no growth of microorganisms. The pouches inoculated without processing (control) had showed positive growth. This study, thus, indicated that the process determined was microbiologically safe upto a period of 135 days.

2. OPTIMIZATION OF PRODUCT QUALITY

The functional performance of 4 binders was evaluated in order to improve the quality of buffalo meat blocks processed in retort pouches. Incorporation of corn starch had contributed better emulsion stability than other binders namely, refined wheat flour, tapioca starch and wheat samolina. Product yield, drip loss and pH did not vary significantly between wheat flour, corn and tapioca starch. Shear force value was significantly higher for product with corn starch followed by refined wheat flour and other binders. Type of binders used had no significant effect on frying loss, moisture and protein content of the product. However, fat content was higher in products with corn starch when compared to products with other binders. Texture profiles indicated that corn starch and refined wheat flour had contributed firmer texture to the product whereas tapioca starch and wheat samolina with soft texture. Corn starch contributed greater chewiness to the products, resulting in higher sensory scores for texture and overall palatability. Products containing corn starch showed higher sensory scores in all attributes in comparison to products with other binders. Panelists preferred products containing different binders in the order of corn starch > refined wheat flour > tapioca starch > wheat semolina. Histological studies indicated that products with corn starch showed dense protein matrix, uniform fat globules and less number of vacuoles, when compared to products made with other binders.

3. SHELF LIFE STUDIES

The keeping quality of the buffalo meat blocks processed in retortable pouches at F_0 12.13 in Stock sterilizer stored in an oven $30 \pm 1^\circ\text{C}$ was evaluated at 15 days interval for changes in physico-chemical, microbiological and sensory attributes. The pH of the product was 6.28 at 0 day and a gradual decline was noticed during storage upto 135 days. Texture of the product, as indicated by shear force values, had decreased slowly. The residual nitrite content significantly declined from 82.67 ppm at 0 day to 30 ppm on 135th day of storage. The TBARS values were 0.24 and 0.98 mg malonaldehyde/kg respectively at 0 day and 135 days of storage. The values were well within the acceptable limit of 1 mg

malonaldehyde/kg. Tyrosine value significantly increased from 0.37 mg/100 g at 0 day to 0.74 mg/100 g at 135 day of storage. Free amino acid content increased gradually from an initial level of 124.32 to 257.5 mg/kg at 135 days of storage. The SDS-PAGE hydrolysis pattern showed barely discernible 205 KDa protein and presence lot of subfragments in the molecular range of 63 KDa to 29 KDa protein. The sensory studies indicated that the products were well acceptable upto a period of 90 days. After 90 days of storage, a rapid decline in the sensory scores for all attributes were noticed. The products were found to be not acceptable at 135 day of storage. As the storage period increased, pH, residual nitrite, sensory attributes declined significantly and TBARS value, tyrosine value and free amino acid content significantly increased. Mesophilic aerobes and anaerobes were found to be absent. The shelf life study indicated that the products were acceptable upto a period of 90 days based on the assessment of physical, chemical and sensory attributes.

4. CONCLUSIONS

1. Heat resistance of test organism *C. sporogenes* PA 3679 in PBS (pH 7.0) showed a $D_{115\text{ }^{\circ}\text{C}}$ value of 5.16 min which is more suitable for heat resistant studies.
2. The D values of *C. sporogenes* PA 3679 in buffalo meat emulsion (pH 6.28) were $D_{110\text{ }^{\circ}\text{C}} = 1.35$ min and showed a Z value of 11.40°C which is higher than the classical 'Z' value of 10°C. The F_0 value was determined as 6.75 min.
3. The coldest point for heat penetration studies was found to be the geometric centre of the pouch.
4. The heat penetration studies for the targeted $F_0 = 12.13$ min (9D) showed a CUT = 13 min, HT = 20 min, CT= 8 min and a total process time of 41 min.
5. The inoculated pack studies showed no microbial growth in the pouches heated for a $F_0 = 12.13$ confirming microbiological safety of the process.
6. The product optimization experiment showed corn starch as a better binder than other binders viz., refined wheat flour, tapioca starch and wheat semolina based on physical, textural and sensory properties of the products.
7. The shelf life studies at room temperature (30±1°C) indicated good acceptability of the products upto 90 days based on physico-chemical, textural, microbiological and sensory studies.

MINI ABSTRACT

Buffalo meat contributes 36% of the total red meat produced in our country. Abundant quantity of available low cost buffalo meat needs to be processed into value added convenience meat products of better acceptability for higher returns. Comminuted meat products like sausages, patties, blocks, nuggets etc have limited shelf life even during refrigerated storage. Lack of cold chains and frequent power failures are major constraints in the distribution and marketing of highly perishable meat products. Thermal processing paved the way for developing shelf stable food products at ambient temperature. Hence the objective of the study was to develop buffalo meat blocks in retort pouches, stable at room temperature for a considerable period of time.

The thermal death time was determined based on the heat resistance of *C. sporogenes* PA 3679 in PBS and buffalo meat emulsion and the Z-value was determined as 12.30°C and 11.40°C respectively. The F_0 value was determined based on the heat resistance of *C. sporogenes* PA. 3679 as 6.75 min. Buffalo meat emulsion filled in 3 ply retortable pouches were vacuum sealed and were processed using stock sterilizer, for a targeted F_0 value of 12.13 min (9 D of PA. 36.79) to provide built in safety to the product. The process time was calculated as 41 min with 8 min come up time, 20 min heating time and 13 min cooling time. The inoculated pack study revealed that the thermal process used was microbiologically safe upto a period of 135 days.

Quality of the buffalo meat blocks were optimized using four different binders namely cornstarch, wheat flour, tapioca starch, and wheat semolina. The blocks processed using corn starch as binder showed higher emulsion stability, product yield, lower drip loss and frying loss, optimum texture profiles as indicated by instron readings and also significantly greater sensory attributes compared to other binders. The micro structure of buffalo meat blocks containing corn starch also showed dense protein matrix, with uniform size fat globules and less number of vacuoles.

The shelf life of buffalo meat blocks using corn starch, as binder processed to a target $F_0 = 12.13$ min was evaluated at controlled temperature ($30 \pm 1^\circ\text{C}$) at 15 days interval for 135 days. The pH, shear force, value, and residual nitrite content decreased and TBARS number, tyrosine value and free amino acids content increased simultaneously as storage period increased. The SDS-PAGE hydrolysis pattern showed barely discernible 205 KDa protein and presence of lot of subfragments in the molecular range of 63 KDa to 29 KDa. The sensory scores have indicated the well acceptability of product upto a period of 90 days (3 months). This study indicated that buffalo meat blocks processed to a F_0 value of 12.13 min are found to be well acceptable and upto 90 days of storage at ($30 \pm 1^\circ\text{C}$) based on the evaluation of physical, chemical, microbiological, textural and sensory characteristics.

लघु सारांश

भारत में कुल मांस के उत्पादन का 36 प्रतिशत से भी अधिक भैंस से प्राप्त होता है। भैंस का मांस जो कि बहुतायत मात्रा में एवं सस्ता होता है, से सुविधाजनक उत्पाद जैसे-सासेज, पैटीज, ब्लाक्स इत्यादि तैयार करके अधिक लाभ अर्जित किया जा सकता है। लेकिन प्रशीतन श्रंखला एवं बार-बार विद्युत कटौती के कारण इन उत्पादों को अधिक समय तक खाने योग्य दशा में नहीं रखा जा सकता है। तापीय प्रसंस्करण द्वारा हम सामान्य ताप पर भी खराब न होने वाले उत्पाद तैयार कर सकते हैं। इस शोध कार्य में भैंस के मांस से बने ब्लाक्स को रिपोर्ट थैली में सामान्य तापक्रम पर सुरक्षित रखने पर कार्य किया गया।

क्लास्ट्रीडियम स्पोरोजीन्स पी.ए. 3679 को आधार मानकर " जैड वैल्यू " एवं " 12.13 मिनट एफ. वैल्यू " का लक्ष्य निर्धारित करके स्टरलाइजर में प्रसंस्कृत किया गया। प्रसंस्करण का समय 41 मिनट निश्चित किया गया। जिसमें 8 मिनट निश्चित ताप आने के लिए, 20 मिनट गर्म करने के लिए एवं 13 मिनट ठंडा करने के लिए उपयोग किये गये।

ब्लाक्स की गुणवत्ता सुनिश्चित करने के लिए मक्के की मांड, गेहूँ का आटा, टैपिओका पाउडर एवं सिवईयों का बन्धक के रूप में प्रयोग करके परीक्षण किये गये। विभिन्न गुणों जैसे - इमलशन स्टेबिलिटी, पकाते समय हानि, संरचना इत्यादि से यह निष्कर्ष निकाला गया कि बन्धक के रूप में मक्का की मांड का प्रयोग करके उत्तम गुणवत्ता वाले ब्लाक्स बनाये जा सकते हैं।

मांस के ब्लाक्स में मक्के की मांड का उपयोग करके उसको " 12.13 मिनट एफ. वैल्यू " का लक्ष्य मानकर प्रसंस्कृत किया गया एवं सामान्य तापक्रम ($30 \pm 1^\circ\text{C}$) पर 135 दिनों तक उसकी निधानी आयु परखी गई। विभिन्न गुणों जैसे- पी.एच., काटने के लिए बल, शेष नाइट्राइट, अमीनों एसिड, टायरोसिन, एस. डी. एस. पेज, सेन्सरी गुण इत्यादि परखने पर यह निष्कर्ष निकाला गया कि इस तरह प्रसंस्कृत किये गये, भैंस के मांस के ब्लाक्स को 90 दिनों तक सामान्य तापक्रम ($30 \pm 1^\circ\text{C}$) पर आसानी से रखा जा सकता है।

FURTHER RESEARCH NEEDS

1. This present study utilized F_{121}^{10} for processing buffalo meat blocks in retort pouches. In the same manner F_{110} , F_{115} , F_{118} or any other F value can be chosen and their acceptability can be assessed by means of physico-chemical, textural, microbiological and sensory attributes in order to develop excellent quality products with longer shelf life.
2. Different varieties of meat products viz., sausages, patties, balls, restructured meat products and soups can be developed in retort pouches for wide distribution and marketing.
3. Nutritional retention of the products can be studied based on the kinetics of thermolabile vitamin "thiamine", so as to perfect the process for minimum loss of valuable nutrients.
4. Products can be developed by utilizing different types of laminates (2 ply laminates to 4 ply laminates) for economical reasons.
5. Bulk quantities (500 g and 1000 g) of products for use in institutional catering can be developed with optimum process time schedules.

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