END-OVER-END AGITATED THERMAL PROCESSING OF CANNED MODEL FOOD PARTICLES

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ABSTRACT

Heating efficiency at 0 rpm of a Lagarde steam/air simulator evaluated with water filled plastic bowl cans (size 307) revealed no statistically significant differences (p>0.05) in heating rate indices between the 15 different positions, indicating uniform and predictable heat delivery within the retort load. Furthermore, heating rate index ($f_h$), heating lag factor ($j_h$) and accumulated lethality ($F_0$) were also determined in the plastic, retortable bowls during tests at 7 agitation levels (0-30 rpm), and at 3 radial positions ($R_1=0$ mm, $R_2=115$ mm, $R_3=175$ mm). Temperature histories were recorded in the centre of water filled bowls, and in both the liquid and particle portions of bowls filled with water and 6 potato/alginate simulated food particles (18 mm diameter). End-over-end rotation from 0 to 30 rpm significantly influenced all parameters. Response to agitation (rpm) was non-linear, such that higher rpm did not always improve heating rates. Substantial improvements in heating of particle portion were seen at relatively low rpm. Although less important than rpm, even small (115 mm) radial position differences significantly altered $F_0$ in some cases.

The effect of immobilization of simulated food particles on the end of a thermocouple investigated using bacterial spores (Bacillus stearothermophilus ATCC 7953) sealed in DSC pans revealed significantly higher lethality delivered to immobilized, as opposed to freely moving particles. Use of DSC
pans with bioindicators for verification of delivered lethality was shown to be reproducible, and this method may find application not only in the canning industry, but also in testing of aseptic processing systems.
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NOMENCLATURE

a, initial number of viable cells in a population

a_w, water activity

b, number of viable cells in the population after time t

CFU, colony forming unit

D_r, time at 121.1°C to destroy 90% of the cells in a population (min)

f_h, heating rate index (min)

F_0, accumulated lethality (z = 10°C, T_r = 121.1°C), min

j_h, heating lag factor

IS, integrated sterilization value, exposure time at 121.1°C, (min)

MIG, mercury in glass thermometer

MPN, most probable number

O.D., outer diameter

p, probability level for testing statistical significance

P_t, operators' process time (min)

R, radial position (mm)

rpm, agitation level (revolutions/min)

t, time (min)

T_r, reference temperature (°C)

T_R, retort temperature (°C)

z, reciprocal slope of thermal death time curve (°C)
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1. INTRODUCTION

Thermal processing of hermetically sealed food products has as its goal extension of the product shelf-life through inactivation of both pathogenic and spoilage organisms. The basic principles behind this preservation method have not changed for more than a century. However with the advent of new technology, commercial processors today can chose from a variety of static or agitating retort systems utilizing steam, steam/air mixtures or water as the heating media.

Recent consumer trends indicate that market share for ambient, shelf-stable ready meals is expanding. It is expected that in the upcoming years use of lightweight, visually appealing, microwaveable and retortable plastic containers will increase substantially (Tucker, 1990; Rice, 1993). However, thermal processing of such containers, creates some unique problems, including slower internal heat transfer (Berry and Bush, 1988). Furthermore, since most retortable plastic containers have metal lids, the lid orientation during static processing influences the location of the slowest heating point, and also the overall heating rate (Lu et al., 1991).

Because of the costs involved, products currently processed in plastic containers tend to be of the high value, high quality variety, where minimization of product deterioration during the sterilization step is paramount. One way to improve/maintain quality of such products is to use end-over-end (EOE) agitated thermal processing offering rapid heating rates, which in
combination with higher retorting temperatures generally correspond to shorter processing times and higher product quality. Convection heating liquid or semi-liquid particulate products are known to benefit most from such agitated processing.

At present two types of retort designs are available for agitated container processing. The main difference between the types is the position of the container in relation to the axis of rotation, which translates into difference in path travelled by the headspace bubble within the can. Axial rotation retorts (cookers-coolers), have cans positioned horizontally along the rotating outer shell of the retort. The rotation, and thus agitation of the product is obtained by allowing the cans to roll freely across the bottom of the retort shell.

In the second type of retort, cans are positioned vertically, perpendicular to retort axis of rotation. In this arrangement known as the end-over-end rotation (EOE) the headspace bubble travels a longer, more turbulent path resulting in more efficient agitation. Although EOE processing offers significant improvements in heat transfer rates, this topic has not been adequately evaluated and there still are a number of important issues that have to be resolved. Most of the published studies of the effects of EOE rotational speed on the heat transfer have been conducted at very high rpm levels, 120 (Naveh and Kopelman, 1980), 144 (Parchomchuk, 1977) and 200 (Clifcorn et al., 1950). Significantly improved heat transfer was reported
at rotational speeds greater than 40 rpm. These studies provided better understanding of the physics involved in EOE agitation process, however speeds used were outside the normal range of commercial rotary retorts. In case of Lagarde steam/air retorts the maximum rotational speed for the commercial size vessels is 20 rpm, while for the pilot simulator it is 30 rpm. In addition, no information is available on the effects of agitation rate (rpm) and radial position on the difference between particle and liquid portions of canned product in terms of delivered lethality (\( F_0 \)) and heating rate index (\( f_h \)). Such information is of significance for calculation of an appropriate thermal process for a given product. Another subject requiring investigation is the effect of immobilization of a food particle on the end of a thermocouple in relation to freely moving particles within a canned product undergoing thermal processing. Investigation of this question would shed light on the validity of use of thermocouple obtained time/temperature data for process time calculations, especially in particulate products, and also on particle-liquid heat transfer mechanisms.

The main research objectives of this thesis were:

1. Evaluation of heating uniformity of a Lagarde lab-scale steam/air retort under static conditions. No differences in terms of \( F_0 \) values between containers at different locations within the retort are expected, but due to the influence
non-uniform heat delivery would have on subsequent studies, this hypothesis must be verified.

(2) Evaluate the effect of two variables: radial position and rotation level on the extent of the difference in terms of $F_0$ and $f_h$ values between the particle and liquid portions of a canned model food system during EOE processing. Both, radial position and rpm are expected to have significant effect.

(3) Investigate, using encapsulated bacterial spores differences in lethality between thermocouple immobilized and freely moving simulated food particles in a canned model food system undergoing agitated processing.
2. LITERATURE REVIEW

2.1 INTRODUCTION

Evolution of canning from the early half of the 19th century to the present has been extensively reviewed elsewhere, from both the scientific and social perspectives (Anonymous, 1960; Goldblith, 1968; 1971; 1972a,b). Generally three developments are regarded as the most influential on the development of canning, these are: (1) practical developments of Appert, (2) scientific discoveries of Pasteur, and (3) technological developments of Underwood and Prescot (Goldblith, 1971).

Appert, who often is thought of as a father of canning, is famous for preservation of foods in glass bottles. However, more correctly it is Chevallier-Apperts’ French patent of 1852 that is thought to be the first mention in literature of an autoclave use for the specific purpose of food conservation (Goldblith, 1972b). In North America, Shrivers’ 1874 patent for a forerunner of present day vertical retorts is considered to mark the beginning of canning in the U.S. (Ball, 1938; Goldblith, 1971).

The early work of Pasteur laid the foundations of the science of canning, and served as the basis for the transformation of canning from an art to an established branch of applied science (Goldblith, 1968; 1971). Later establishment by Underwood and Prescot of bacteria as the causative agents of can spoilage further advanced canning, and showed the importance of heat penetration in canned food through the use of maximum-
registering thermometers (Goldblith, 1972a).

The twentieth century brought with it numerous additional improvements and innovations to the canning industry including: development and improvement of the thermocouple and its application in studies of heat penetration; discovery of thermophilic bacteria and founding of a new branch of applied science known as thermobacteriology; heat transfer studies, nutritional studies as well as numerous developments and innovations in the area of processing devices.

2.1 THERMAL STERILIZATION SYSTEMS

2.2.1 General Information

Today a variety of thermal processing systems are available for commercial sterilization of foods. They vary in mode of operation (batch-type or continuous, static or agitating), as well as the type of heat transfer medium (steam, steam/air or water) employed. Still retorts were used almost exclusively until about 1950, after which continuous agitating systems became more common. The still retorts can be either horizontal or vertical and are batch operated. These systems generally operate with saturated steam as the heating medium, however they may be modified to operate with steam/air or water when overpressure is required.

Ball and Olson (1957), Fennema (1975) and Lopez (1981) described a number of continuous and/or agitating steam retort systems. Several agitating and non-agitating water processing
systems were also covered by Lopez (1981). Design parameters and operating characteristics of a unique thermal processing design, the direct flame sterilization system developed in France in 1957, were discussed by Beauvais et al., (1961), Casimir (1975) and Leonard et al., (1975).

2.2.2 Axial Rotation Systems

The collective term "static retort" applies to all overpressure vessels used for sterilization. Since no continuous agitation occurs during sterilization or cooling, areas of temperature stratification may exist within the retort. To guarantee sterility, processes have to be based on temperature readings from containers in the coldest zone (the cold spot) of a retort. As a result, the containers in warmer areas are subjected to over-sterilization, and substantial variations in product texture and flavour can occur within the batch. Furthermore, in static retorts, heating medium is not circulated, and entropy release to containers and product is relatively slow.

Early investigations by numerous technologists and scientists indicated that the major quality characteristics of canned food would be better maintained if high temperature-short time thermal processes were used (Ball, 1938; Clifcorn, 1948). Further research by Clifcorn et al.,(1950) showed heating of fluid products was much faster when the containers were agitated during processing, providing adequate headspace was maintained
within the containers. From a practical point of view two agitation methods are possible, end-over-end and axial rotation. They differ, in the relative orientation of the cans relative to the axis of rotation.

Axial rotation has been quite successful in continuous steam overpressure retorts, also known as Steritort cooker-coolers (FMC Corporation, San Jose, CA). Axial movement of cans, and thus the agitation rate, is determined by transfer time of cans through the unit, with agitation achieved in the lower portion of the spiral with cans rolling freely across the bottom of the retort shell. These cooker-cooler systems are designed such that every container experiences the same rotational and temperature history during a process. They are most efficient at processing large volumes of the same commodity. Sterimatic retorts have been commercially employed in the production of high quality peas, corn, asparagus and mushrooms. A substantial number of publications are available evaluating the effect of various processing variables (rotation speed, headspace volume, fill weight etc.) on heating rate index ($f_h$) and accumulated lethality ($F_0$) of various products processed in FMC Sterimatic cookers (Berry and Bradshaw, 1980; Berry et al., 1979; Pflug et al., 1980a; Berry et al., 1985; Berry and Kohnhorst, 1985).

2.2.3 EOE Agitation Systems

There is no question that axial rotation processing of
cans is a great improvement over still processing; however EOE rotation and oscillation are far more effective means of improving heating rates (Hersom, 1967). Early research on agitated processing conducted by Clifcorn during the 1950s, was responsible for the introduction of this new improvement to thermal processing. In Germany at about the same time Stock developed the first full water immersion agitating retort with an overpressure (Eisner, 1988). Further improvements of this type of retort system included addition of a water circulation arrangement, so-called semi-immersion sterilization and the water spray system, which utilized still less water.

Live steam retorting systems have undergone considerable development in France, and today the steam/compressed air sterilizer by Lagarde is available as a static, or rotary sterilizer. This system allows pressurization of the retort shell during sterilization and cooling. In order to eliminate temperature stratification within the retort a high performance fan is used to circulate the compressed air/steam mixture. The air overpressure counteracts expansion of gases inside flexible and semi-rigid containers so that heat transfer may be optimal and predictable, and the likelihood of package rupture may be minimized.

2.2.4 Heat Transfer Mechanism

Heat transfer to canned food product during thermal
processing involves a number of complex mechanisms including not only composition and physical properties of the heating medium, but also the surface heat transfer characteristics between the heating medium and the container, the container and the food, and within the food itself. In addition, during EOE thermal processing, movement of headspace "bubble" along the can's vertical dimension is responsible for improved mixing and turbulence. In the case of axial rotation, the headspace "bubble" tends to follow the can's circumference in a continuous streamline path with substantially less agitation. Headspace bubble motion is known to be periodic, with the frequency determined mainly by rotation speed and position of the can in relation to axis of rotation. Furthermore, the trajectory of the bubble motion is known to depend on the system geometry, while its amplitude depends on headspace volume, liquid properties (viscosity) and system geometry (Naveh and Kopelman, 1980).

Studies with model liquids have shown that with increased rotational speed, amplitude of the bubble motion was dampened until at high rpm the bubble merely vibrated around a localized point, impairing agitation (Naveh and Kopelman, 1980). Viscosity can also exerted strong drag forces on the headspace bubble, and viscous, non-Newtonian liquids have been shown to heat faster closer to the container wall than at the geometric centre (Anantheswaran and Rao, 1985a). With very viscous products, rotation failed to break up the liquid, and the movement of the headspace bubble was confined to the wall of the can.
(Anantheswaran and Rao, 1985b). Particulates present in actual food products can be expected to promote secondary mixing, and thus further improve agitation during the rotation of the can.

Internal conduction, or convection capacity of the product are not the only factors affecting the rate of heat penetration. On a macroscopic scale, heat transfer from the heating medium to the surface of stationary containers depends on circulation of retort heating medium. Cooler film zones form around containers, resulting in a reduced rate of heat transfer from the heating medium. To counteract this effect, static steam/air retorts may be equipped with some form of heating medium circulation, usually involving air make-up or positive steam flow arrangements. On the other hand, agitating retorts are usually designed for constant circulation of the medium around the containers, to minimize temperature stratification. Furthermore, when agitation is employed, additional mixing of the heating media is induced by the motion of the retorted load.

On a microscopic scale, the mechanism of heat release from steam is important. Tung et al.,(1990) reviewed two possible processes of heat release from steam to surfaces of lower temperature. The first type, called drop-wise condensation involves formation of microscopic water droplets on the container surface. This process is reversible, and the liquid droplets are in a state of flux, being constantly evaporated from container surface. As long as the container surface is colder than the surrounding steam, there is a very rapid
transfer of heat by the drop-wise mechanism.

The second type of heat transfer mechanism, film-wise condensation, tends to prevail in thermal processing operations. Upon enthalpy release, steam condenses and builds up a layer of liquid on the container surface. Heat transfer rates are one to two orders of magnitude lower than for drop-wise condensation.

Efficiency of enthalpy release from steam can also be reduced by formation of a layer of non-condensible gas, such as air, adjacent to the solid surface. To prevent this situation, venting of static retorts is necessary to remove air prior to processing. In retorts utilizing steam/air mixtures, forced mixing of the heating medium is very important, especially when a large fraction of air is used to provide an overpressure.

2.2.5 Retortable Plastic Containers

Plastic cans emerged in the late 1980s as a packaging innovation, in response to changes in consumer buying habits. Shelf-stable foods packaged in microwaveable containers offered a convenient hot snack, and delivered three functions instead of the tins' traditional one.

Retortable plastics are new to the food processing community and very little data is available on their behaviour during thermal processing. Due to several inherent characteristics of plastics, including lower thermal conductivity and lower heat transfer coefficients in condensing steam, plastic cans heat more slowly than metal cans (Berry and
Bush, 1988). The increased wall thickness of plastic containers (0.4 to 0.6 mm as compared to 0.2 mm for the metal can) further slows heat transmission. The orientation of the plastic can's metal lid during static processing also affects the heating rate. A downward orientation of the metal lid significantly enhanced natural convection within the can, resulting in faster heating rates, and higher $F_0$ values compared with plastic cans with lids positioned upwards (Lu et al., 1991).

Manufacture of plastic containers is a rather complex process, involving a series of steps (Farrell, 1990). Presently, thermoforming and injection/blow moulding (coextrusion) container making technologies are available. The latter offers greater flexibility in the number of possible layers, and also lower waste proportion. Walls of most plastic containers are composed of at least 5 layers, laminated together. No single plastic can offer both sufficient oxygen and water barrier properties. Lidding systems, and can closures are other critical points in the packaging and merchandising operation. Olfield (1990) discussed currently available materials, including metal ends, preformed plastic lids and flexible lidding materials, with emphasis on problems and limitations affecting the lidding materials' performance.

Plastics can provide a convenient, safe, efficient material for use in packaging but are undoubtedly a visible component of waste. Gallagher (1990) discussed the role of plastics and plastic waste management methods, especially in relation to the
2.3 LETHALITY EVALUATION

2.3.1 Physical Method

In order to establish confidence in the performance of a thermal process, lethality delivered to the product has to be known. Lethality can be measured by biological methods, or by the more common thermocouple method. From the time-temperature histories of the cold spot of the container, factors \( F_0, f_h, j_h \) describing heating behaviour and lethality can be calculated. The canning industry employs copper-constantan thermocouple systems, in which the electromotive force that develops when two dissimilar metals form a circuit is used to indicate the temperature (Lopez, 1981). Errors introduced by conduction of heat along the thermocouple and mounting hardware can be eliminated by use of correction factors published by Ecklund (1956). Alternatively, when heat penetration studies in plastic containers are conducted, plastic (Darlin) thermocouple receptacles may be fitted, which do not usually require correction.

2.3.2 Biological Method

The second method used to evaluate the killing power of a thermal process is the biological method, utilizing bacterial spores. This procedure is simple in concept but quite complex in practice. Biological validation requires accurate, quantitative microbiological procedures. In addition, the experiment must
provide an accurate picture of the actual low acid canning process. Yawger (1978) described two methods of bacteriological process evaluation. The first one, the Inoculated Pack System (NCA, 1968) is basically a system for controlling the initial spore population and counting the number of survivors. The advantage of this method is that by inoculating the cans with a large number of spoilage organisms, the study will approximate the size of a commercial operation. The second one, the Count Reduction System (Yawger, 1967) involves inoculation of a number of cans with a large number of spores, usually thermophilic flat sour organism, followed by processing at various time-temperature combinations. The initial counts are determined from an unprocessed inoculated can and survivors from each processed can. Thermal processing calculations used in the food industry are based on the exponential rate of spore death. Evaluation of process lethality by bacteriological systems is based on a simple equation:

\[ IS = D_r (\log a - \log b) \]

where IS is the Integrated Sterilization value, \( D_r \) time at reference temperature to destroy 90% of the spores in the population, and \( a \) and \( b \) are respectively initial and final spore numbers. Both IS and \( F_0 \) values are expressed as the equivalent number of minutes at 250 °F (121.1 °C). By definition, \( F_0 \) is the equivalent in minutes at 250 °F at the slowest heating point in the container (cold spot). Thus \( F_0 \) represents the minimum lethality in the container, while the remainder of the container
is assumed to be subject to a higher but undefined lethality. By contrast, the IS value represents the average lethality of the entire container contents.

Results of bacteriological evaluation of convection or induced convection heating products are easy explained because product heating is nearly isothermal. Thus convection IS values tend to be close to $F_0$ values since the entire product volume is heated virtually at the same rate. On the other hand, conduction heating products present a very different picture, therefore direct comparison of IS values to $F_0$ values are for most part meaningless.

Dignan et al., (1989) reviewed the three possible approaches for use of microbial methods in evaluation of lethality delivered to food particles carried by fluid in an aseptic processing system and the problems associated with them. All three approaches can be applied in the evaluation of canned particulate products undergoing thermal processing.

2.3.3 Biological Indicator Units

The first approach involves bacterial spores in carriers, BIUs (Biological Indicator Units), in which the spores in the indicator unit are not in direct contact with the food product. BIUs are leak proof, small diameter plastic rods containing spore solutions. There is general agreement between number of researchers, (Jones et al., 1980; Pflug et al., 1980a; Pflug et al., 1980b) in the $F_0$ and IS values from BIUs under varied
processing conditions. A similar principle was used by Hersom and Shore (1981), who employed micro-sized glass bulbs containing a spore suspension for thermal process evaluation. More recently, Ramaswamy and Ghazala (1990) used 75 uL aliquots of ascorbic acid and thiamine solutions sealed in leak-proof, pressure-stable, stainless steel DSC sample pans for evaluation of centre point nutrient degradation in conduction heating canned food which are subjected to thermal processing. The above methods offer the advantages of known spore location, complete spore recovery, elimination of influence of environmental (Ca$^{2+}$, NaCl, pH levels) factors on spore thermal response, and in the case of DSC pans, because of their small size (7.7 mm diameter x 2.9 mm height), possible localization of the indicator in the geometric centre of food particle.

2.3.4 Inoculated Food Particles

The second approach involves direct inoculation of food product particles with a bacterial spore suspension. With this set-up a number of problems and conditions must be considered and solved, including identical spore environment during calibration (TDT tests) and actual testing, possible spore leaching, controllable particle size, and uniform spore distribution within the food particle. In a study by Berry et al.,(1985) IS values were calculated for cheese ravioli inoculated with a suspension of B. stearothermophilus (1503) spores, and processed in brine in 603x700 cans. Because of the
difficulties in post-process spore recovery from food particles and the strong possibility of leaching, this method was found to have limited practical use. More recently, Sastry et al.,(1988) successfully tested *B. stearothermophilus* spores infused and immobilized within individual mushrooms using alginate gels. Particles were made shelf-stable by freeze-drying, and showed negligibly small spore leaching during reconstitution and blanching steps, with no detectable leaching during thermal tests.

### 2.3.5 Simulated Food Particles

The third approach involves use of simulated food particles. Most of the problems or conditions applicable to actual food particles which are directly inoculated with spores must also be considered when using simulated particles. Simulated particles offer the advantage of uniform inoculum throughout the particle and controlled particle size. Sastry et al.,(1988) highlighted most of the important considerations involved in the use of simulated food particles for thermal process verification. Most important, the indicator must contain heat resistant bacterial spores that undergo temperature histories similar to those in the cold spots of real particles. Thus, the bio-indicator should be in the form of a particle possessing the following necessary and/or desirable characteristics: (1) large size (1 inch), containing immobilized bacterial spores throughout the interior and at the slowest
heating zones; (2) geometry, thermal properties similar to real food particles; (3) visually distinguishable from real particles, permitting easy recovery from processed product; (4) retention of spores without leakage through all process steps; (5) physical durability, possessing the ability to withstand process stresses without disintegration.

The initial attempt to use simulated food particles containing bioindicators, employed Bacillus anthracis spores imbedded in "perspex" (polymethylacrylate) beads to monitor processing of particles in continuous flow systems (Hunter, 1972). The embedding procedure employed was complex, and release of surviving spores from the "perspex" after heating required treatment with acetone, which may have been deleterious to the recovery of heat injured spores.

Most procedures for model food particle production use alginate as a binding agent due to alginate's high affinity for water, total lack of toxicity, and ability to form gels very rapidly in the presence of calcium ions under extremely mild conditions. These characteristics have been explored not only for spore immobilization, but also for gel entrapment of other materials, including particulate enzymes (Hussain et al., 1985), plant protoplasts (Draget et al., 1988) and yeast cells (Jain and Ghose, 1984).

Alginates are glycourans extracted from seaweed (Smidsrød, 1970; 1974). Some relevant properties of calcium alginate gels, including mechanical rigidity, swelling and shrinkage
characteristics, depend upon the molecular weight of the alginate (Martinsen et al., 1989). The gelling reaction between sodium alginate and an aqueous solution of calcium ions is a highly controllable event (Littlecott, 1982), influenced by temperature, ion concentration and contact time with the solution (Gilson et al., 1990).

Electron micrographs of gelled sections of calcium alginate beads, revealed very broad distribution in pore diameter (Smidsrød, 1974), with smaller pores on the bead surface than in the core (Klein et al., 1983). This heterogeneity was essentially a result of an irreversible gelling mechanism and relative diffusion rates of the gelling ions (Ca$^{2+}$) and alginate molecules (Skjak-Braek et al., 1989).

In another study Dallyn et al., (1977) reported the use of bacterial spores immobilized in calcium alginate gel, for evaluation of lethal effects of ultra-high temperature (up to 140°C) in a scraped-surface heat exchanger. Small sodium alginate beads (1.6-3.2 mm) contained randomly immobilized heat resistant spores of Bacillus stearothermophilus Th 24, and unlike the "perspex" system subjected the spores to moist rather than dry heat conditions. Recovery of surviving spores involved agitation of alginate particles with potassium citrate to dissolve the alginate gel structure. Citrate worked by chelating calcium ions, and since Ca$^{2+}$ levels have been shown to affect the relative recovery of heat stressed spores, addition of Ca$^{2+}$ ions to the recovery media was required. Reproducibility of spore
counts in beads was demonstrated, and alginate gel at 3% was found to give best mechanical strength.

Brown et al., (1984) demonstrated feasibility of a similar method. Large food/alginate (0.8-2.4 cm) cubes consisting of alginate mixed with pureed potatoes, peas or meat and containing spores of Clostridium sporogenes PA 3679 or Bacillus stearothermophilus NCIB 8910 were used in evaluation of a range of thermal processes. Significant differences however, were observed between experimental and calculated integrated lethalities. The authors attributed these to particle shrinkage, and to limitations of the process calculation procedure.

2.4 BACTERIAL SPORES

2.4.1 Bacterial Spore Heat Resistance

Resistance of bacterial spores to heat has tremendous practical importance in relation to sterilization and pasteurization protocols, and also presents some interesting problems in basic cell biology. Spores are dormant biological systems with no detectable metabolism, yet with full capability of rapid germination on exposure to appropriate stimulants. The above characteristics, and ubiquitous presence make bacterial spores suitable as indicators of thermal process efficiency. Use of spores for monitoring various commercial thermal processes has been reviewed by Brewer et al., (1956), whereas applications in the medical, and pharmaceutical fields have been assessed by
The main factors responsible for bacterial spore heat resistance have been reviewed extensively (Gombas, 1983). Transformation from a dormant spore to a metabolically active cell includes the stages of activation and germination. During the later, the fundamental property of heat resistance is lost (Lefebvre and Antipa, 1982). The actual spore characteristics that are responsible for heat resistance can be divided into three discernable components (Gould, 1984). First one, the inherent component, is related to the growth temperature of sporeforming bacteria, with thermophiles generally producing more resistant spores than mesophiles. The second, cellular component involves peptidoglycan, or murein cortex which plays a major role in heat resistance (Gould, 1984; Warth, 1985), including stabilization of the initial osmotic dehydration of the spore (Marquis et al.,1973). Cortical peptidoglycan is known to have a high level of peptide cross-linking and relatively compact structure (Marquis and Bender, 1990), which provides mechanical strength to support internal spore pressure (Algie and Lindsay, 1983).

Mineralization of internal spore structures is considered to be responsible for yet another component of heat resistance (Ellar, 1978). Most surprising are the findings that mineralization is more important for spore heat resistance at lower killing temperatures than at higher ones (Bender and Marquis, 1985). The exact mechanism is not known, however a
relationship exists between the dipicolinic acid (Dpa) and calcium contents of the spores and heat resistance (Malidis and Scholefield, 1987a). Based on the available information, two theories of Dpa involvement in heat resistance have been proposed. Powell and Strange, (1956) suggested that the protoplast of a resting spore is a highly condensed, water proofed system stabilized by calcium dipicolinate. Grech et al., (1972) proposed that the core is stabilized by a protective cement consisting of chelates of divalent metals with spore ligands. Even though heat killing of spores is usually considered in terms of irreversible heat denaturation of proteins, the recent work of Hanlin and Slepecky (1986) suggests that heat damage to DNA may be more important than previously expected.

Water distribution also plays an important role in spore heat resistance. Most spore water is associated with the cortex, while the core, or the protoplast is relatively dehydrated (Koshikawa et al., 1984; Nakashio and Gerhardt, 1985). Furthermore, both the level of protoplast dehydration, and the amount of cortex have been correlated with heat resistance, although there appears to be more than a single correlation modulus (Beaman et al., 1984; Mallidis and Scholefield, 1987).

2.4.2 Factors Affecting Heat Resistance

A number of environmental factors are known to affect directly, or indirectly, bacterial spore heat resistance and
should be considered if spores are used as thermal indicators. Most often an increase in spore heat resistance is observed following activation at sublethal temperatures, a phenomenon named "heat-induced resistance" (Etoa and Michiels, 1988). Similar observations were noticed in some non-sporebearing bacteria (Yamamori et al., 1978; Mackey and Derrick, 1987, 1986). Alternatively, with prolonged exposure to sublethal temperatures, a fraction of the spore population may enter heat-induced dormancy instead of germination (Rossignol and Vary 1979; Etoa 1985). From an applied point of view, heat-induced resistance may cause errors when estimating the sterilization effect of a thermal process.

Other environmental factors, including the type of ions and ionic concentration affect the heat destruction rate of Bacillus stearothermophilus spores (Gauthier et al., 1978). Lower heat resistance was observed in phosphate buffer than in water (Cook and Gilbert, 1968a), and increasing concentrations of sodium chloride in the heating medium have been shown to progressively reduce the heat resistance of Bacillus stearothermophilus spores (Cook and Gilbert, 1968c).

The effect of food, or laboratory medium pH on spore heat resistance has received considerable attention (Blocher and Busta, 1983; Brown and Thorpe, 1979; Cameron et al., 1980; Hutton et al., 1991; Stumbo, 1965; Xezones and Hutchings, 1965; Walker, 1964). Generally, spore thermal resistance decreased with decreasing pH, with the type of acid also playing a role. An
acidic environment may cause partial demineralization of the spore, with corresponding changes in conformation and stability of nucleic acids (Vamvakopoulos et al., 1977), translating into increased DNA sensitivity to heat (Bender and Marquis, 1985; Sako et al., 1981).

Thermal resistance to dry heat is very different from resistance to wet heat. At lethal temperatures, availability of water molecules that dictates the relative killing power of the thermal process. Thus, both the water activity ($a_w$), and the osmotic potential of the canned product are important. Heat resistance of several types of bacterial spores (Harnulv and Snygg, 1992; Hsieh et al., 1975), including $B. stearothermophilus$ ATCC 7953 (Harnulv et al., 1977) have been shown to increase at intermediate (0.8) water activity ($a_w$), with maximum resistance at $a_w$ of 0.3-0.4 (Murrell and Scott, 1966).

### 2.4.3 Spore Production Methods

Both solid and liquid media can be used for spore production, with thermophile spores more often produced on agar surfaces incubated from 2 to 14 days (Campbell et al., 1965). There are several disadvantages to the use of solid media for spore production, however, most significant is the possibility of a heat induced dormancy (Finely and Fields, 1962).

The critical step in any spore production sequence is triggering of the sporulation cascade in the vegetative, pre-sporulation population. In complex liquid media, sporulation is
initiated by starvation, usually by limiting one of the nutrients. In synthetic media the presence of excess glucose, ammonia, or phosphate (Freese et al., 1978) is necessary. A number of environmental factors have been shown to affecting sporulation of B. stearothermophilus, including sources of carbon, nitrogen and sulphur (Yildiz and Westhoff, 1984). In addition, manganese has been shown to stimulate sporulation, but not the vegetative cell growth, of several species of the genus Bacillus (Charney et al., 1951), including B. stearothermophilus grown in broth medium (Thompson and Thames, 1967).

2.4.4 Spore Activation Methods

In order to accurately enumerate viable spores in a given population, the dormancy of individual spores has to be broken. Several treatments can be used to initiate spore germination. Application of heat (heat shock) is the simplest and most widely used. Bacillus stearothermophilus spores exhibit a high degree of dormancy and require a severe heat shock in the range of 100-115°C for activation (Finely and Fields, 1962), with both intensity and duration of the treatment being important (Busta and Ordal, 1964). In addition, both low pH (Lewis et al., 1985; El-Mabsout and Stevenson, 1979), and Ca-Dpa treatments (Kirk and Hambleton, 1982) have been shown capable of spore activation under certain conditions.
2.4.5 Spore Recovery Media

Use of bacterial spores for verification of sterilization processes requires maximum reproducible recovery of spores. Problems arise when the definition of viability for heat treated spores has to be specified. The ability to enumerate heated spores can vary not only with the type of recovery medium (Cook and Gilbert, 1968a), but also from lot-to-lot for some commercial preparations (Pflug et al., 1981). Yeast-peptone-tryptone-dextrose medium with added starch or charcoal, was found to be superior to commercial media preparations for recovery of heat treated spores of B. stearothermophilus (Mallidis and Scholefield, 1986). Both starch and charcoal contributed, most likely by binding inhibitors of germination and outgrowth of heat injured spores (Adams, 1979). In addition Zechman and Pflug (1991) reported an interesting correlation between improved recovery of heat-stressed spores of B. stearothermophilus on commercial media with 1/10 of the manufacturer’s recommended concentration levels, and increased heating time and temperature. Dilution of sodium chloride and starch-adsorbed inhibitors were suggested as factors responsible for this observation. From commercial media preparations, trypticase soy agar (TSA) was shown to yield significantly higher recovery of heat treated B. stearothermophilus spores than other plating media (Mikolajcik and Rajkowski, 1980).
2.4.6 HGM Filtration Method

The Hydrophobic Grid Membrane (HGM) filtration method is based on a membrane with hydrophobic boundaries dividing the membrane into 1600 growth compartments or grids. The most probable number (MPN) counting range of HGM is about 3 log cycles, much larger than that obtainable with traditional pour, or spread plate techniques (Sharpe and Michaud, 1975). The ISO-grid system for quantitative microbiological analysis of foods combines the features of reliability, flexibility and speed with increased counting precision, and more rapid results (Brodsky et al., 1982). Furthermore, the membrane filtration technique allows removal of water soluble materials that could interfere with the outgrowth of heat stressed bacterial spores.

Use of HGM filtration method for enumeration of heated bacterial spore has not been previously reported. However, Brodsky et al., (1982) reported use of the method for aerobic plate counts in foods. Entis and Boleszczuk (1986) evaluated the HGM filtration method for aerobic plate count using tryptic soy agar (TSA) supplemented with fast green FCF dye against conventional pour plate method. The HGM filtration method not only yielded counts equivalent to, or significantly higher than the pour plate method for 24 of the 25 product categories, it was also superior with respect to sensitivity and reproducibility of results. A recent publication demonstrated an excellent correlation between the ISO-grid aerobic count and conventional pour-plate counts (Chain and Fung, 1991).
3. MATERIALS AND METHODS

3.1 Retort System

Studies were carried out using a Lagarde (Lagarde, Montelinar, France) pilot-scale, batch-type, rotating, steam/air overpressure simulator located at T.J. Lipton processing plant, in Richmond British Columbia. Retort consisted of a horizontal shell (520 mm O.D.), with a powerful, high speed circulation fan mounted in the retort door, to ensure uniform distribution of the heating mixture (96% steam, 4% air). Simulator accommodated containers in a single wire basket (W 300 mm/ L 600 mm/ H 300 mm) held in place during agitation by an overhead, variable pressure clamp. In order to generate the greatest thermal load during the come-up portion of the process, and also to provide a ballast during rotary processes, the retort basket was filled with a total of 105 water filled 307 bowl type cans positioned in 5 individual layers, separated by plastic mats with 3/4 inch holes. The retort could be operated in either manual or fully automatic mode. During all experimental runs, process variables (temperature, pressure, time) were automatically controlled using on-off valves through a pre-programmed sequence (Table 1). Venting time of 10 min, and P_v of 15 min at T_r of 118.0°C were used in all initial experiments. Both temperature and venting time were chosen to represent commercial process conditions. Cooling was achieved by a combination of water shower and partial water immersion of the load. Cooling water from the
Table 1. Programming sequence employed in operation of steam/air simulator.

<table>
<thead>
<tr>
<th>Program segment</th>
<th>Time (min)</th>
<th>Temperature (°C)</th>
<th>Pressure (bar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start</td>
<td>0.00</td>
<td>0.0</td>
<td>0.00</td>
</tr>
<tr>
<td>Come-up period</td>
<td>6.00</td>
<td>100.0</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>4.00</td>
<td>118.0</td>
<td>1.80</td>
</tr>
<tr>
<td>Processing</td>
<td>15.00</td>
<td>118.0</td>
<td>1.80</td>
</tr>
<tr>
<td>Cooling period</td>
<td>4.00</td>
<td>69.0</td>
<td>1.50</td>
</tr>
<tr>
<td></td>
<td>6.00</td>
<td>42.0</td>
<td>1.50</td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>42.0</td>
<td>1.00</td>
</tr>
<tr>
<td>End</td>
<td>1.00</td>
<td>42.0</td>
<td>0.00</td>
</tr>
</tbody>
</table>
bottom of the retort shell was recirculated through a water/water heat exchanger before being added back to the cooling sequence. Prior to any experimental runs, one warm-up run was conducted to heat the retort components to operating temperature.

3.2 Simulated Food Particles

Model food particles were employed in the experiments to eliminate non-uniformity associated with the use of actual food ingredients. Spherical, 18 mm diameter particles were chosen to represent average sized food particles, and also to allow uniform heat transfer to the particle centre from all directions. Particles were produced using a modification of the method of Brown et al.,(1984). In the first step of particle production, locally obtained potatoes were hand peeled, packaged in water in a 2000 mL beaker and autoclaved at 121°C for 45 min, to reduce microbial load, and soften the potato sufficiently for further processing. After overnight cooling (4°C), potato batches (300 g) were added to 400 mL of sterile distilled water and pureed (Osterizer blender) for 5 min. Slurry was then transferred to a 1000 mL beaker, and sodium alginate (Sobalg FD 170. Sobalg, S.A., Landerneau, France) added at 2.5% (w/v). Ingredients were dispersed for 5 min using an overhead mixer (Fasco, 5VA, Hamden, Conn.) fitted with a four-blade propeller (30 mm diameter).

Simulated food particles were cast using a rubber (Dow
Corning, 3120 RTV Silicone Rubber) 2 piece, 10 particle mould. Silicone rubber was chosen for the mould material due to its low cost, good thermal stability (autoclavable), physical flexibility and relative ease of use. Each mould consisted of two mirror image halves produced from a wooden master model.

Particle production sequence involved clamping the two mould halves together, and pumping the potato/alginate mixture (60cc, disposable syringe fitted with 16 gauge needle) into the spherical chambers through circular openings in one of the mould halves. Throughout the pumping step, care was taken to prevent air entrapment in the chambers. Filled moulds were placed in a −35°C freezer for 1 h to solidify the potato/alginate mixture. Upon removal from the mould, frozen particles (10) were placed for 2 h in a 1000 mL beaker containing 500 mL of 4% (w/w) calcium chloride (Fisher Scientific) solution, to induce alginate gelation. The freezing step was essential for production of uniformly spherical particles. It was followed by curing of the particles in sterile deionized water for at least 24 h at 4°C. Prior to use, particles were screened for visible air pockets, and trimmed of excessive alginate/potato mix. Prepared particles could be stored in deionized water at 4°C for up to 2 weeks without any noticeable changes.

3.3 Temperature Histories

Plastic, 307 size (87.3 mm diameter, 46.0 mm height) bowl-type cans (Ball Packaging, Inc.) with easy-open aluminum lids
were used in all the experiments. New cans were used for each experimental run to eliminate influences of can deformation. Cans were filled to a constant weight of 190 ± 1 g corresponding to 13.6% headspace (w/w) based on a maximum fill weight of 220 g, and sealed under vacuum with a commercial seamer. In order to normalize the initial can temperatures all cans were held in an ice water bath for 30 min prior to processing. Time-temperature data were collected using 1.6 mm diameter, copper-constantan thermocouples (Type CNS, Ecklund-Harrison Technologies, Cape Coral, FL) mounted in cans through plastic (Darlin) receptacles, and positioned to record centre point temperature. A Calwest model 32 datalogger (Calwest Technologies, Canyon County, CA) connected to a Toshiba 1000 lap-top computer was employed for data acquisition.

Prior to use, all thermocouples were checked for their accuracy in a constant temperature oil bath (Blue M, Magic Whirl) at 105 and 120°C against a certified MIG thermometer. The difference in temperature readings between thermocouples did not exceed 0.1°C, thus eliminating need for correction factors. During all experimental runs heating medium temperature within the retort was monitored by two wire thermocouple junctions located at the front and the back of the retort basket.

Time/temperature data during all experimental processes were recorded at 1 min intervals, and analyzed using Cal-soft commercial thermal processing software (Technical Inc., Metairie, CA). From centre point temperature histories of each
can, useful parameters describing the heating portion of the thermal process ($j_h$ and $f_h$) were calculated. In addition, accumulated lethalities ($F_0$) associated with temperature histories were calculated using the General Method (Patashnik, 1953).

3.4 Retort Heating Efficiency

Heating uniformity of a fully loaded retort was studied in static mode (0 RPM) at 15 different positions within the retort basket which were chosen to represent possible areas of temperature extremes (Figure 1). Water filled cans with centrally located thermocouples were positioned in the appropriate locations in the lid-down orientation. Thermal histories for each position came from 3 separate runs. An ANOVA of the mean heating rate indices among the positions was conducted.

3.5 Radial Position, RPM Effect

Due to the relatively small retort basket dimensions, only three radial positions (Figure 2) were evaluated at 7 different agitation rates (0-30 rpm). Two types of model food products were used in the evaluations. Cans filled with water were used to model convection heating Newtonian product. Cans filled with water and six simulated food particles modeled behaviour of a convection heated particulate commodity. For cans containing simulated food particles, both liquid and particle centre
Figure 1. Schematic diagram of positions within steam/air simulator evaluated during heating efficiency tests. (not all positions shown, not to scale).
LEGEND: $R_1 = 0 \pm 18 \text{ mm}$
$R_2 = 115 \pm 18 \text{ mm}$
$R_3 = 175 \pm 10 \text{ mm}$

Figure 2. Schematic diagram of location within steam/air simulator of evaluated radial positions.
thermal histories were recorded. Since placement of two thermocouples within a single, relatively small container would have been too disruptive to internal flow, collection of thermal histories of liquid and particle portions was divided into two sets of experiments. In one set of experimental runs, only liquid temperature histories were recorded in cans containing 6 simulated food particles. In a second set of runs, the centre point temperature of one of the 6 particles was recorded.

In order to record simulated food particles' centre point temperature, a unique method of particle mounting was devised (Figure 3). The procedure employed dental floss and small diameter flexible nylon tubing. Initially, a length of dental tubing was threaded using a needle through the outer segment of the potato/alginate particle. Subsequently, both ends of the dental floss section protruding from the particle were then passed through the short segment of nylon tubing, thus forming a loop. The next step involved pressing the nylon tubing onto the thermocouple to the desired position. Finally, the food particle was positioned (pressed) onto the exposed section of the thermocouple end, and the dental floss ends exposed on the other side of the nylon tubing were pulled to immobilize the particle. This simple arrangement held the particles in position at the thermocouple end even at highest agitation rates (30 rpm).

Thermal indices from the experimental runs were analyzed using a two level (rpm x radial position), completely randomized
Figure 3. Schematic cross-section diagram of a plastic 307 bowl can with thermocouple mounted simulated food particle.
factorial design. An analysis of variance (SAS Institute, Inc., 1985) was conducted, and significant interactions found among means of main effects at each agitation level were separated with T-tests (LSD) with a predetermined level of significance set at 5% (p<0.05) for all conditions.

3.6 Spore Production

Spores of Bacillus stearothermophilus ATCC 7953 were produced using a modified method of Kim and Naylor (1966). Pre-sporulation inoculum was grown in Tryptone Yeast Glucose (TYG) Broth with following composition: tryptone (Difco), 1%; yeast extract (Difco), 0.5%; glucose (Fisher), 0.5%; K$_2$HPO$_4$ (Fisher), 0.2%. The sporulation medium, Tryptone Yeast Agar (TYG agar) contained: nutrient broth (Difco), 0.8%; yeast extract (Difco), 0.4%; MnCl$_2$·4H$_2$O (Fisher), 10ppm; agar (Difco), 3.0%. Prior to sterilization, the media pH were adjusted to 7.2 ± 0.1. Cooled sporulation media was deposited in disposable petri dishes (Fisher), allowed to solidify over a period of 45 min in a laminar flow cabinet and stored prior to use at 4°C. Through initial experiments it was found that use of 3% agar in the sporulation medium, rather than the 2% recommended, improved moisture retention at incubation temperature (55°C), and facilitated washing the spores off the agar surface.

Preparation of pre-sporulation spore inoculum was divided into two parts. Initially, freeze dried spores were reconstituted (ATCC, 1976) by adding TYG broth (0.3-0.4 mL) to
the vial, and allowing a few minutes for spore rehydration. The entire vial content was then transferred to a test tube containing 5 mL of TYG broth and mixed well. Approximately 5 mL of such reconstituted (rehydrated) spore culture was then transferred into a 125 mL Erlenmyer flask containing 15 mL of TYG broth, and incubated in an agitated water bath (Blue M) at 55°C for 12-14 h. This step served to increase the microbial numbers and rejuvenate the culture. At this point, a number of TYG agar slants were inoculated with the culture and incubated at 55°C for 48 h. Those slants, subsequently stored at 4°C, served as back-up cultures.

In the second part of the pre-sporulation inoculum production, 10 mL of culture from the first part was transferred into a 2000 mL Erlenmyer flask containing 150 mL of fresh TYG broth, and incubated with agitation at 55°C for additional 16-18 h. The final step involved inoculation of sporulation agar (TYG) plates with approximately 1 mL of pre-sporulation suspension, followed by incubation at 55°C (Blue M, Gravity Convection Bacteriological Incubator) for 8-10 h in the upright position to allow for inoculum absorption by the agar. To reduce further dehydration, plates were inverted and incubated for an additional 36-38 h.

At the end of the incubation period (>48 h), the presence of sporulation was checked by microscopic evaluation of glass slides with stained (Schaeffer-Fulton method) spore preparations. Surface growth from petri plates was then washed
off with ice cold, 0.1 M phosphate buffer (pH 6.5) into sterilized 250 mL Nalgene bottles. Spores were concentrated by centrifugation (Sorvall, RC2-B centrifuge) at 5000 x g for 15 min at 4°C. Supernatant liquid was discarded, the spore sediment was re-suspended in an equal amount of buffer, and then re-centrifuged. The washed spore pellets were transferred to a 500 mL Erlenmyer flask by re-suspending them in 200 mL of 0.1M phosphate buffer containing lysozyme (Sigma, 100 ug/mL), and were incubated with agitation at 37°C for 1 h (Feeherry et al.,1987). Lysozyme treatment served to rupture remaining vegetative cells allowing spore release. The spore stock solution was then, as previously described, centrifuged and washed 5 more times with equal volumes of ice cold buffer. In the final step, spore pellets were pooled and re-suspended in 50 mL of buffer. The spore stock solution was then sub-divided into 10 mL aliquots and stored in screw-top test tubes at 4°C until needed. Initial enumeration indicated that the stock spore suspension contained 1.0 x 10^8 CFU/mL.

3.7 Spore Enumeration

Throughout the TDT studies and thermal process validation experiments, the viable spores were enumerated using the Hydrophobic Grid Membrane (HGM) filtration method (QA Life Sciences, Inc., San Diego, USA). Aliquots (1 mL) of appropriate spore dilutions were filtered through ISO-grid membranes, with the filters subsequently incubated for 48±4 h at 55°C on
tripticase soy (TSA) agar (BBL) in the lids down position. Colonies of *Bacillus stearothermophilus* ATCC 7953 grown on ISO-grid membranes on TSA medium were lightly transparent in appearance and thus hard to enumerate. To improve colony visibility, Fast Green FCF (Fisher) dye was incorporated into TSA media at a level of 0.25 g/L (Entis and Boleszczuk, 1986). For all dilutions, sterile 0.1% peptone (Difco) water was employed. Initial (zero time) counts of spore suspensions, were obtained from appropriate dilutions of 1 mL spore aliquots which were heat shocked (10 min @ 100°C) in 5 mL screw-top test tubes.

### 3.8 Spore Thermal Resistance

The capillary tube method (Stern and Proctor, 1954) was employed to determine the thermal resistance of a *Bacillus stearothermophilus* ATCC 7953 spore population suspended in 0.1M phosphate buffer (pH 6.5). Glass capillary tubes (Fisher) were dry heat sterilized for 180 min at 121°C prior to use. Disposable 1.0 mL syringes fitted with 25 gauge needles were used to fill each tube (100 mm length, 1.9 mm O.D.) with 0.1 mL of mixed spore suspension. Filled capillary tubes were sealed in an oxygen flame, and stored at 4°C prior to use.

Tubes were heated in a constant temperature oil bath (Blue M, Magic Whirl), with temperature monitored by a MIG thermometer. Eight different temperatures spanning the range encountered during sterilization processes were evaluated. For each time-temperature point, 3 individual capillary tubes were
evaluated. Heating times were corrected with a come-up time of 15 sec reported for the capillary tubes (Brown et al., 1984). At the end of each heating time, capillary tubes were removed from the oil bath and placed in an ice water bath to arrest further thermal inactivation. Prior to survivor enumeration, capillary tubes were individually cleaned from the remaining heating oil by washing with detergent and 70% alcohol. Spore aliquots were removed from capillary tubes by sniping one end of the tube, placing it into a dilution test tube, and then breaking off the other end to allow the contents to flow out. To ensure complete spore removal from the capillaries, each tube was flushed with 1.0 mL of diluent (0.1% peptone) introduced by a 1.0 mL disposable syringe fitted with 25 gauge needle. Spore counts were obtained using HGM filtration method, as previously described. From the surviving spore counts, \( D_{121.1} \) and \( z \) values for the spore crop were determined.

3.9 Particle Immobilization

To evaluate the effect of thermocouple immobilization of a food particle on heat transfer, the modified method of Ramaswamy and Ghazala (1990) was employed. This involved the evaluation of changes in viability of a thermal indicator (bacterial spores) which were encapsulated in DSC pans and placed in the centre of simulated food particles. Leak proof, two piece, aluminum (DuPont), Differential Scanning Calorimetry (DSC) pans were used as carrier containers for the spores. Pans
were filled with 0.02 mL of *B. stearothermophilus* spore suspension, crimp-sealed, and stored at 4°C prior to use. Use of this system eliminated the problem of spore leaching, as well as the effects of environmental factors (Ca\(^{2+}\) level, pH) on spore thermal resistance. Furthermore, DSC pans offered relatively small dimensions (2.4 mm height, 7.5 mm diameter) and thus could be positioned in the centre of larger, simulated food particles. IS values so obtained could then be compared to centre point lethality values calculated from thermocouple data.

The following are the main steps for the preparation of simulated food particle preparation. The bottom half of each mould was filled with potato/alginate mixture, and spore containing DSC pans were positioned in the centre. The mould was then placed in a -35°C freezer for 10 min to freeze the mixture and ensure that DSC pans were centrally located. The top of the mould was then positioned and the particle cavities filled with more of the potato/alginate mixture. The rest of the simulated food particle production was conducted as previously described.

Experimental runs were conducted at three different agitation rates (0, 15, 30 rpm). Cans were positioned at the most extreme radial position (R\(_{3}\)=175 mm). It was hoped that differences between freely moving and immobilized particles (if present) would be more pronounced at this radial position. Process times (P\(_{t}\)) to achieve particle centre point lethality of F\(_{0}\) = 5.0 min were calculated (General Method) for each agitation rate (rpm) from previously collected heat penetration data.
Process times had to be normalized to achieve approximately 3 log reductions in spore numbers, which in turn allowed the use of one stock spore solution for all three agitation levels tested. For each run, 6 cans were used. Each can contained 6 simulated potato/alginate food particles with centrally located DSC pans. In each can, 5 particles had unrestricted motion, while one was mounted on a mock thermocouple constructed from 42 mm long (3 mm O.D.), rigid nylon tube attached onto 5 mm long conventional needle thermocouple. Nylon tubing was chosen to eliminate significant heat transfer to the particle along the thermocouple that occurs with needle thermocouples. Simulated food particles were mounted on mock nylon thermocouples using dental floss as previously described (Figure 3). Furthermore, all cans were filled to a constant weight of 190 g with phosphate buffer (pH 6.5) containing *B. stearothermophilus* spore suspension in order to evaluate spore destruction in the liquid portion. The initial concentrations of spore suspensions used were $1.5 \times 10^9$ CFU/mL in the DSC pans, and $1.7 \times 10^7$ CFU/mL in the liquid portion. For each can, initial and final (processed) spore numbers were determined for both the liquid and particle (DSC pans) portions. To recover the spores from DSC pans, alcohol flamed long nose pliers were used to crack the pans along the crimp seal. The pans were subsequently dropped into dilution tubes and vortexed vigorously to re-suspend spores. The spore count reduction technique (Yawger, 1978) was used to calculate the IS values obtained by the freely moving and
immobilized particles, as well as by the liquid portion in each can.

In addition, for each experimental run, 6 cans with 6 simulated food particles and conventional needle thermocouples were placed in same retort position (R₃). Three of the cans had thermocouples positioned to record liquid histories, and the other 3 had thermocouple immobilized food particles. From the temperature histories obtained, accumulated lethality (F) values were calculated (General Method) and compared to the IS values from spore destruction data.
4. RESULTS AND DISCUSSION

4.1 Mapping of Retort Heating Efficiency

Temperature uniformity within a retort is a factor of major significance in thermal processing, affecting delivery of the desired lethality to all packages in the load. To evaluate retort heating efficiency, the heating rate indices ($f_h$) derived from the centre-point temperature histories of the test materials with constant thermal properties may be taken as an indication of the heat transfer conditions during processing (Tung et al., 1990). Another basis for evaluating retort performance is the comparison of accumulated lethalities ($F_o$) recorded at different locations in a retort. $F_o$ values reflect a standardized lethal effect on microbial spores due to the recorded temperature histories in the centre of the containers, and account for variability in heating rate lag times, as well as differences in the cooling conditions.

Reported studies of retort heating efficiency have employed silicone rubber bricks (Morello, 1987), or teflon (polytetraethylene) slabs (Britt, 1993). In this investigation, cans filled with water were employed to simulate actual processing conditions. The locations used in the evaluation of retort heating uniformity were chosen to represent the most extreme positions within the retort shell. Retort process operation was fully automated, thus eliminating operator error. Temperature histories from each location were used to plot semi-
logarithmic heat penetration curves (Figure 4). The sample curve shown is typical of convection-heating products, showing a short lag period followed by a steep rise until retort operating temperature (118.0°C) is approached. To describe each heat penetration curve, two thermal process parameters, the heating rate index $f_h$ and the lag factor ($j_h$) were computed. The heating rate index is the time required for the straight line portion of the Jackson plot to traverse one log cycle of temperature difference, while the lag factor is a measure of responsiveness of the product in establishing a uniform heating rate. In addition, overall accumulated lethalities ($F_0$) associated with the centre point temperature histories recorded for each can were calculated by the Improved General Method.

Analysis of the retort heating efficiency data were carried out by computing individual and overall mean and standard deviation values for the heating indices at all the locations (Table 2). An overall mean heating rate index of 6.63 min ($\pm 0.23$), with a range of 6.50 to 6.80 min was calculated. The overall mean accumulated lethality of 5.44 min ($\pm 0.24$) was obtained with a range of 5.31 to 5.58 min. Heating lag values ranged from 0.55 to 0.62, with an overall mean of 0.59 ($\pm 0.05$). Coefficients of variation for the means of $f_h$ and $F_0$ values (3.46 and 4.41% respectively) were relatively small. More variation, as indicated by larger coefficient of variation (8.58%) was observed among means of the heating lag factor. The heating lag factor is a function of the position of the measured point, the
Figure 4. Typical heat penetration curve observed during retort heating efficiency tests at 0 rpm.
Table 2. Individual and overall mean values and standard deviations of the process indices from heating efficiency evaluation experiments in steam/air simulator at 0 rpm.

<table>
<thead>
<tr>
<th>Retort location</th>
<th>Process Indices¹</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fₜ (min)</td>
<td>F₀ (min)</td>
<td>jₜ</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6.67 (0.27)</td>
<td>5.31 (0.25)</td>
<td>0.62 (0.05)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6.62 (0.11)</td>
<td>5.33 (0.27)</td>
<td>0.60 (0.06)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6.75 (0.22)</td>
<td>5.49 (0.31)</td>
<td>0.59 (0.05)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6.54 (0.34)</td>
<td>5.50 (0.19)</td>
<td>0.61 (0.05)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6.56 (0.10)</td>
<td>5.57 (0.22)</td>
<td>0.57 (0.04)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6.60 (0.23)</td>
<td>5.36 (0.24)</td>
<td>0.59 (0.03)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>6.61 (0.18)</td>
<td>5.41 (0.27)</td>
<td>0.59 (0.05)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>6.67 (0.29)</td>
<td>5.43 (0.12)</td>
<td>0.60 (0.05)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>6.67 (0.23)</td>
<td>5.31 (0.18)</td>
<td>0.62 (0.05)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>6.80 (0.23)</td>
<td>5.53 (0.22)</td>
<td>0.59 (0.07)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>6.51 (0.23)</td>
<td>5.57 (0.25)</td>
<td>0.56 (0.04)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>6.73 (0.38)</td>
<td>5.40 (0.32)</td>
<td>0.55 (0.05)</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>6.65 (0.11)</td>
<td>5.33 (0.22)</td>
<td>0.56 (0.04)</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>6.50 (0.36)</td>
<td>5.44 (0.15)</td>
<td>0.59 (0.04)</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>6.57 (0.15)</td>
<td>5.58 (0.32)</td>
<td>0.58 (0.09)</td>
<td></td>
</tr>
</tbody>
</table>

| Overall C.V.² | 6.63 (0.23) | 5.44 (0.24) | 0.59 (0.05) |
|---------------| 3.46        | 4.41        | 8.58        |

1 - Mean and standard deviation values (n = 3).
2 - Coefficient of variation (%).
shape of the object being heated, and the initial temperature distribution within the object (Ball and Olson, 1957). It is possible that variations, albeit relatively small (8.4 - 10.7 °C) in the initial water temperature of the cans might have played a more significant role. However, an ANOVA of the means of the heating rate index ($f_h$), the achieved lethality ($F_o$) and the heating lag factor ($j_h$) values among different retort positions during mapping runs indicated no statistically significant differences ($p>0.05$) among the positions (Table 3). The results indicate uniform heating within the simulator. This can be attributed to the relatively small retort size combined with forced circulation of heating media employed in Lagarde steam/air retorts.

In a similar study, Morello (1987) evaluated heating efficiency in a lab-scale (FMC Model 500W) water immersion retort. In this system, steam heated water travels through flow channels between stacked, aluminum trays holding retort pouches. Heating rate indices ($f_h$) and accumulated lethality ($F_o$) values of teflon transducers from different tray positions exhibited significant ($p<0.05$) variability. Similarly, a gradient in $j_h$ values was observed. Those results are not unusual since water employed as a heating media is not as easily circulated within the retort as a steam/air mixture.

In another study (Britt, 1993), heating efficiency of a commercial, 4-basket water immersion retort (Stock Rotomat RCS) and a single-basket forced convection steam/air sterilizer
Table 3. ANOVA of means of process indices from 15 locations within steam/air simulator at 0 rpm.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Mean Square</th>
<th>F-Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heating Rate Index ( (f_h) ) (min)</td>
<td>44</td>
<td>0.0840</td>
<td>0.3940ns</td>
</tr>
<tr>
<td>Heating Lag Factor ( (j_h) )</td>
<td>44</td>
<td>0.0030</td>
<td>0.5720ns</td>
</tr>
<tr>
<td>Accumulated Lethality ( (F_o) ) (min)</td>
<td>44</td>
<td>0.0790</td>
<td>0.4360ns</td>
</tr>
</tbody>
</table>

ns - Not Significant at p>0.05
(Lagarde) were conducted with brick-shaped Teflon slabs. Comparisons of $f_h$ data revealed significant ($p<0.05$) positional differences in both steam/air and water immersion retorts. Similarly, in both retort types, positional differences in $F_0$ values were found, with differences in the steam/air retort smaller in magnitude. In the water retort, the water circulation arrangement affected the heating uniformity. In the steam/air retort, the size of the retort was most likely responsible for the differences.

### 4.2 Radial Position, RPM Effect

In this section, effects of both radial position in relation to the axis of rotation, and agitation rates (rpm) in the range employed in the industry on $f_h$ values of a canned model food product were evaluated. Clifcorn et al.,(1950) reported variation in heating rates with radius of rotation, container orientation, rotation speed and product viscosity. Increasing rotational speeds for containers with a headspace bubble improved mixing until gravitational and centrifugal forces became equal, after which the centrifugal forces dominate, and push the more dense fluid to the outside of the arc of rotation. At these high rotational speeds, the headspace "bubble" remains stationary with respect to the container and no longer affects mixing of the fluid.

Naveh and Kopelman (1980) studied the effects of headspace volume and rotational speed on overall heat transfer
coefficients for a viscous glucose syrup (84°Bx, 70 DE). Heat transfer coefficients increased with rotational speeds from 20 to 120 rpm. The above experiments were conducted in a pilot scale vessel with presumably small radius of rotation (not reported). It is likely that the gravitational forces were dominant, which could explain the continued increase in the rate of heating with increased rotation (up to 120 rpm). In other studies, Anantheswaran and Rao studied EOE rotation at pasteurizing temperatures using both Newtonian (1985a) and non-Newtonian fluids (1985b). The heat transfer coefficient was found to be dependent on product viscosity and rotational speed (0-38.6), but independent of the radius of rotation (149 mm) of the vessel used.

Examples of typical time-temperature profiles for the particle ($C_{\text{particle}}$), liquid ($C_{\text{liquid}}$) portion of a particle/liquid system, and for water ($C_{\text{water}}$) only, in plastic 307 bowls are presented in Figure 5. Model food particles exhibited slower heating and cooling responses due to the conductive heat transfer. In the present study, agitation level (rpm) was found to have a significant effect (Table 4) on $f_h$, $j_h$ and $F_0$ values of all three experimental set-ups evaluated; in the centre of water only filled bowls ($C_{\text{water}}$), and in the liquid portion ($C_{\text{liquid}}$) or in the centre of the thermocouple mounted particle ($C_{\text{particle}}$) in cans containing 6 simulated food particles. Figures 6, 7 and 8 show the influence of rpm on the heating rate index ($f_h$) for the three experimental conditions. An increase in the agitation rate (rpm)
Figure 5. Typical time-temperature profile curves observed for particle, liquid and water portions in 307 bowl cans. (R_i=175 mm, 15 rpm).
Table 4. ANOVA of means of process indices obtained from different rpm and radial position settings.

<table>
<thead>
<tr>
<th>Test Conditions</th>
<th>Parameter</th>
<th>F values</th>
<th>Mean square of Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>rpm</td>
<td>Radius</td>
</tr>
<tr>
<td>Water only (C_{water})</td>
<td>f_h</td>
<td>130.6***</td>
<td>5.3**</td>
</tr>
<tr>
<td></td>
<td>j_h</td>
<td>27.8***</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>F_o</td>
<td>200.0***</td>
<td>3.5**</td>
</tr>
<tr>
<td>Particle portion (C_{particle})</td>
<td>f_h</td>
<td>181.6***</td>
<td>15.9***</td>
</tr>
<tr>
<td></td>
<td>j_h</td>
<td>18.1***</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>F_o</td>
<td>117.3***</td>
<td>0.5</td>
</tr>
<tr>
<td>Liquid portion (C_{liquid})</td>
<td>f_h</td>
<td>97.4***</td>
<td>19.6***</td>
</tr>
<tr>
<td></td>
<td>j_h</td>
<td>3.6**</td>
<td>4.3*</td>
</tr>
<tr>
<td></td>
<td>F_o</td>
<td>81.9***</td>
<td>13.5***</td>
</tr>
</tbody>
</table>

*** p ≤ 0.005  
** p ≤ 0.01  
* p ≤ 0.05  

1 - rpm of Retort Basket (0-30).  
2 - Radial Position of Containers (R_1=0 mm, R_2=115 mm, R_3=175 mm) from Axis of Rotation.
Figure 6. Radial position/rpm effect on $f_h$ values of the centre of water ($C_{water}$) only containing cans (error bars indicate standard deviation).
Figure 7. Radial position/rpm effect on $f_h$ values of the centre of simulated food particles ($C_{particle}$) in cans containing 6 particles (error bars indicate standard deviation).
Figure 8. Radial position/rpm effect on $f_h$ values of the liquid portion ($C_{\text{liquid}}$) of cans containing 6 particles (error bars indicate standard deviation).
resulted in a gradual decrease in the magnitude of $f_h$ values at all conditions. In both $C_{\text{water}}$ and $C_{\text{particle}}$ (Figure 6, 7) cases, the most substantial decrease in $f_h$ values occurred at agitation rates between 0 and 5 rpm. Further increases in agitation rate (10–30 rpm) resulted only in a small improvement. The $f_h$ values of the $C_{\text{liquid}}$ (Figure 8) decreased more gradually between 0 and 15 rpm, and showed even lower decreases between 15 to 30 rpm. Results indicated a steady improvement in the mixing efficiency of the head space bubble, and thus improved heat transfer with increased agitation. However the extremely high agitation rates (20–30 rpm) did not translate into correspondingly lower heating rate index ($f_h$) values.

Interestingly, at all agitation rates tested (0–30 rpm), mean $f_h$ values obtained for the $C_{\text{liquid}}$ case were smaller than the corresponding values for the $C_{\text{water}}$ case (Table 5). At 0 rpm (still cook) this discrepancy may be explained by the smaller volume (160 ml vs. 190 ml) of liquid present in cans containing particles ($C_{\text{liquid}}$). Under conditions of agitation (5–30 rpm) the presence of simulated food particles ($C_{\text{liquid}}$ case) may have resulted in additional agitation and mixing, in contrast to containers filled with water only ($C_{\text{water}}$) where headspace bubble was the only means of agitation.

Comparisons of achieved lethalities ($F_0$) under the three experimental set-ups; water only ($C_{\text{water}}$), particle ($C_{\text{particle}}$) and liquid ($C_{\text{liquid}}$) conditions are presented in Figures 9, 10 and 11 respectively. As expected, cans filled with water only (Figure
Table 5. T-test (LSD) of means of process indices at different rpm and radial position settings.

<table>
<thead>
<tr>
<th>Heating Parameters(^1) (C(_{\text{water}}))</th>
<th>Water only (C(_{\text{particle}}))</th>
<th>Particle portion (C(_{\text{particle}}))</th>
<th>Liquid portion (C(_{\text{liquid}}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>rpm (</td>
<td>F_h</td>
<td>(\text{min}))</td>
<td>(R_1)</td>
</tr>
<tr>
<td>0</td>
<td>7.6a</td>
<td>7.4a</td>
<td>7.4a</td>
</tr>
<tr>
<td>5</td>
<td>6.0a</td>
<td>6.1a</td>
<td>5.8a</td>
</tr>
<tr>
<td>10</td>
<td>6.0a</td>
<td>6.1a</td>
<td>5.5a</td>
</tr>
<tr>
<td>15</td>
<td>5.2a</td>
<td>5.6a</td>
<td>5.3a</td>
</tr>
<tr>
<td>20</td>
<td>5.5a</td>
<td>5.3a</td>
<td>5.4a</td>
</tr>
<tr>
<td>25</td>
<td>5.2a</td>
<td>5.0ab</td>
<td>4.8b</td>
</tr>
<tr>
<td>30</td>
<td>4.5a</td>
<td>4.6a</td>
<td>4.3a</td>
</tr>
</tbody>
</table>

\(j_h\) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(F_h) (min)</td>
<td>0</td>
<td>0.5ab</td>
<td>0.6a</td>
</tr>
<tr>
<td>5</td>
<td>0.5a</td>
<td>0.5a</td>
<td>0.5a</td>
</tr>
<tr>
<td>10</td>
<td>0.5a</td>
<td>0.5a</td>
<td>0.6a</td>
</tr>
<tr>
<td>15</td>
<td>0.5a</td>
<td>0.5a</td>
<td>0.5a</td>
</tr>
<tr>
<td>20</td>
<td>0.5a</td>
<td>0.6a</td>
<td>0.5a</td>
</tr>
<tr>
<td>25</td>
<td>0.6a</td>
<td>0.6a</td>
<td>0.6a</td>
</tr>
<tr>
<td>30</td>
<td>0.7a</td>
<td>0.7a</td>
<td>0.7a</td>
</tr>
</tbody>
</table>

\(F_e\) (min) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.0a</td>
<td>5.0ab</td>
<td>5.3b</td>
</tr>
<tr>
<td>5</td>
<td>5.8a</td>
<td>5.8a</td>
<td>5.8a</td>
</tr>
<tr>
<td>10</td>
<td>6.1a</td>
<td>6.2a</td>
<td>6.2a</td>
</tr>
<tr>
<td>15</td>
<td>6.2a</td>
<td>6.0b</td>
<td>6.0ab</td>
</tr>
<tr>
<td>20</td>
<td>6.3a</td>
<td>6.4a</td>
<td>6.4a</td>
</tr>
<tr>
<td>25</td>
<td>6.4a</td>
<td>6.5b</td>
<td>6.6c</td>
</tr>
<tr>
<td>30</td>
<td>6.9a</td>
<td>7.0a</td>
<td>7.1a</td>
</tr>
</tbody>
</table>

1 - Means of heating parameters (n = 3) with different letters within a row at a given rpm are significantly different (p ≤ 0.05).

2 - Distance from axis of rotation (\(R_1\)=0 mm, \(R_2\)=115 mm, \(R_3\)=175 mm).
Figure 9. Radial position/rpm effect on $F_0$ values of the centre of water ($C_{water}$) only containing cans (error bars indicate standard deviation).
Figure 10. Radial position/rpm effect on $F_0$ values of the centre of simulated food particles ($C_{particle}$) in cans containing 6 particles (error bars indicate standard deviation).
Figure 11. Radial position/rpm effect on $F_0$ values of the liquid portion ($C_{\text{liquid}}$) of cans containing 6 particles (error bars indicate standard deviation).
showed a steady, almost linear increase in achieved lethality ($F_o$) with increasing agitation. The $C_{\text{liquid}}$ results (Figure 11) show a linear increase in $F_o$ values between 0 and 10 rpm. Further increases in agitation rate (10-30 rpm) did not result in higher achieved lethality, as evident by the levelling of the values. A similar trend (Figure 10) was observed for the particle portion ($C_{\text{particle}}$), however, unlike the $C_{\text{liquid}}$ case, the particle $F_o$ values increased at the higher agitation rates (25-30 rpm). This observation indicates that an increased surface heat transfer at the particle/liquid interface existed at those higher agitation rates.

The effect of rpm on the heating lag factor ($j_h$) was less regular and not easily explained. Figures 12, 13 and 14 show the effect of agitation rate on $j_h$ values in $C_{\text{water}}$, $C_{\text{particle}}$ and $C_{\text{liquid}}$ cases respectively. Contrary to expectation, for all three experimental set-ups, $j_h$ values increased with increasing agitation. Particle ($C_{\text{particle}}$) $j_h$ values (Table 5) were significantly larger than those of either liquid ($C_{\text{liquid}}$) or water ($C_{\text{water}}$) portions. The larger $j_h$ values of simulated food particles can be explained by the slower thermal response of potato/alginate gel where heating occurred by conduction. Lower $j_h$ values ($j_h < 1$) of the liquid portions ($C_{\text{liquid}}$ and $C_{\text{water}}$ cases) indicate improved mixing. In theory, thermal convection heating gives rise to $j_h = 1$, but in practice, convection in a can results in $j_h < 1$ (Ball and Olson, 1957). This is because the overall coefficient of heat transfer is greater at the beginning of heating within the can.
Figure 12. Radial position/rpm effect on $j_h$ values of the centre of water ($C_{water}$) only containing cans (error bars indicate standard deviation).
Figure 13. Radial position/rpm effect on $j_h$ values of the centre of simulated food particles ($c_{particle}$) in cans containing 6 particles (error bars indicate standard deviation).
Figure 14. Radial position/rpm effect on $j_h$ values of the liquid portion ($C_{\text{liquid}}$) of cans containing 6 particles (error bars indicate standard deviation).
when temperature differential and convection are greatest.

In a study (Abbatemarco and Ramaswamy, 1993) of heating behaviour of 3 and 4% gelatinized starch solutions in 307x409 cans undergoing EOE processing, similar observations with respect to \( j_h \) values were also observed. Lag factors increased with an increase in rotation speed, and authors attributed those observations to the resistance to mixing of the starch solutions at the beginning of the process.

Despite the small size of the retort basket, radial position did influence heating parameters, albeit inconsistently at different rpm levels. In \( C_{\text{water}} \) tests, radius had a significant effect on the \( f_h \) and \( F_0 \) heating parameters (Table 4). Further statistical evaluation of data from the different positions (Table 5) showed that \( f_h \) values were significantly different \( (p<0.05) \) mostly between the extreme positions \( (R_1 \text{ and } R_3) \). Similarly, \( F_0 \) values were significantly different at 0, 15 and 25 rpm, however differences were not limited to the most extreme radial positions.

Radial position had a significant effect on \( f_h \) values of \( C_{\text{particle}} \). LSD multiple range tests indicated possibly significant but minor effects on \( j_h \) and \( F_0 \) values of the particles. Similar trends were observed in the \( C_{\text{liquid}} \) study. Radial position had a strong effect on both \( f_h \) and \( F_0 \) values, whereas \( j_h \) values were affected very little.

Figures 15 and 16 compare the effect of agitation rate and radial position on the achieved lethality \( (F_0) \) and heating rate
Figure 15. Effect of rpm level on the $f_h$ values of particle and liquid portions of canned model food product.
Figure 16. Effect of rpm level on the $F_0$ values of particle and liquid portions of canned model food product.
index \( (f_h) \) values of particle and liquid portions. With all three radial positions evaluated, increased agitation decreased the particle-liquid differences. In the case of heating rate index \( (f_h) \), the most notable improvement was observed at higher agitation rates (25-30 rpm), especially at the two larger \( (R_2 \text{ and } R_3) \) radial positions (Figure 15). The magnitude of the difference in achieved lethality \( (F_0) \) values between particle and liquid portions was much less affected by the agitation rate than the radial positions. Only at the highest agitation (25-30 rpm) and largest radial position \( (R_3) \) was the effect visible (Figure 16).

4.3 Thermal Death Time Studies

Use of the capillary tube method in combination with the HGM filtration method of spore enumeration to evaluate thermal response of *Bacillus stearothermophilus ATCC 7953* spores was found to be relatively simple, albeit time consuming.

Spores of *B. stearothermophilus ATCC 7953* exhibited typical behaviour upon exposure to lethal temperatures, including initial "shoulder" regions, and "tailing" of curves especially at lower temperatures (Figure 17). With the logarithmic order of bacterial spore inactivation, a number of deviations from a straight line destruction can be observed, including shoulder, sigmoid, upward concavity, and a biphasic curve with a tail. Most often the discrepancies are seen early in the exposure interval (Stumbo, 1965), as a consequence of concomitant processes occurring at that time, including heat activation and
Figure 17. Example of spore survivor curves (*Bacillus stearothermophilus ATCC 7953*) in 0.1 M phosphate buffer (pH 6.5).
primary inactivation (early inactivation of less heat-resistant fractions).

In calculations of D-values, only the linear portions of the survivor curves were employed (Figure 18). The fit of the D-value curves was very good, especially at the higher temperatures as indicated by the narrow confidence intervals (Table 6). From the D-values a phantom TDT curve was constructed (Figure 19) from which a z value of 7.80 C° and a $D_{121.1^\circ C}$ of 2.09 min for the spore crop were calculated. Both numbers are within values previously reported in literature.

4.4 Particle Immobilization

The paramount concern over introduction of new thermal preservation technologies such as agitated processing is that the resulting products do not compromise consumer safety. Heat transfer to a food particle during an agitated cook is a complex phenomena involving composition and physical properties of the heating medium, the heating characteristics of the food within a particular container, and the surface heat transfer characteristics between the heating medium and the container, as well as between the container and the food. Current technology does not allow measurement of the temperature profile at the slowest heating zone within a moving particle without disturbing its movement. Therefore lethality calculations based on physical mathematical approaches such as the General Method and formula methods are impossible. As a consequence researchers are forced
Figure 18. Example of linear portions of spore survivor curves (Bacillus stearothermophilus ATCC 7953) in 0.1 M phosphate buffer (pH 6.5) at 118.2, 120.0, 123.0 and 125.0°C.
Table 6. Summary of data from TDT studies of *Bacillus stearothermophilus* spores in 0.1 M phosphate buffer (pH 6.5) at various temperatures.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Number of Observations</th>
<th>D Value (min)(^1)</th>
<th>95% Confidence Interval (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>105.0</td>
<td>24</td>
<td>212.3</td>
<td>189.0 - 242.3</td>
</tr>
<tr>
<td>109.6</td>
<td>12</td>
<td>72.6</td>
<td>61.5 - 88.4</td>
</tr>
<tr>
<td>112.6</td>
<td>12</td>
<td>29.4</td>
<td>27.3 - 31.9</td>
</tr>
<tr>
<td>115.0</td>
<td>18</td>
<td>11.3</td>
<td>10.9 - 11.7</td>
</tr>
<tr>
<td>118.2</td>
<td>18</td>
<td>5.1</td>
<td>4.9 - 5.2</td>
</tr>
<tr>
<td>120.0</td>
<td>21</td>
<td>2.5</td>
<td>2.4 - 2.6</td>
</tr>
<tr>
<td>123.0</td>
<td>15</td>
<td>1.3</td>
<td>1.2 - 1.3</td>
</tr>
<tr>
<td>125.0</td>
<td>15</td>
<td>0.7</td>
<td>0.6 - 0.7</td>
</tr>
</tbody>
</table>

\(^1\) Calculated from the linear portion of the TDT curve.
Figure 19. Phantom thermal death time curve for spores of *Bacillus stearothermophilus* ATCC 7953 in 0.1 M phosphate buffer (pH 6.5).
to use mathematical modelling to simulate the time-temperature profile at the centre of a moving particle, and subsequently verify the lethality with thermal indicators and proper integration methods.

In this part of the study, a combination of methods was used to evaluate the effect of placing a simulated food particle on the end of a rigid thermocouple, on the lethality of such a particle in relation to a freely moving food particle within the same container. In order to accurately evaluate the effect of particle immobilization, bacterial spores sealed in DSC pans were used as thermal indicators. Thermal indicators not only allowed unhindered motion of canned model food particles, but also allowed central localization of the indicator within the simulated food particle.

Some rather surprising results were obtained when integrated sterilization (IS) values between freely moving and thermocouple mounted model food particles were compared. As expected, the thermocouple data showed higher accumulated lethality ($F_{07.80}$) values in the liquid portion than in the particles (Table 7). The $F_{07.80}$ values were calculated with a z value obtained for *B. stearothermophilus* spore crop ($z = 7.80 \degree C$ vs. $z = 10.0 \degree C$) so that more meaningful comparison with integrated sterilization (IS) values could be made. Analysis of variance (Table 8) of the IS values from the liquid portion and from both freely moving, and thermocouple immobilized food particles, showed that significantly higher IS values were
Table 7. Overall mean $F_0$ values of particle and liquid portions of canned model food product processed at different agitation rates.

<table>
<thead>
<tr>
<th>Agitation Rate (rpm)</th>
<th>Process Time $P_t$ (min)$^1$</th>
<th>Accumulated Lethality$^2$ $F_0$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Particle</td>
</tr>
<tr>
<td>0</td>
<td>15.70</td>
<td>5.30 (0.11)</td>
</tr>
<tr>
<td>15</td>
<td>11.50</td>
<td>4.50 (0.23)</td>
</tr>
<tr>
<td>30</td>
<td>10.40</td>
<td>4.28 (0.03)</td>
</tr>
</tbody>
</table>

1 - Mean process times ($P_t$) to $F_0 = 5$ min calculated using General Method from previously acquired thermal history Data ($n = 3$)

2 - Mean accumulated lethality $F_0^{7.80}$ values and standard deviations calculated using General Method ($z = 7.80 \, ^\circ C$, $n = 3$)
Table 8. IS values obtained by particle and liquid portions of model food product processed at different rpm levels.

<table>
<thead>
<tr>
<th>Agitation Rate (rpm)</th>
<th>Model Food Particles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liquid²</td>
</tr>
<tr>
<td>0</td>
<td>5.253b(0.056)</td>
</tr>
<tr>
<td>15</td>
<td>5.750b(0.084)</td>
</tr>
<tr>
<td>30</td>
<td>5.763a(0.118)</td>
</tr>
</tbody>
</table>

1 - Mean and standard deviations of IS values. Mean values with different letters within a row are significantly different at p>0.05.

2 - Sample size, n = 6.

3 - Sample size, n = 30.
Particle-fluid heat transfer depends mainly on the relative velocity of fluid and particle. The worst case situation occurs when no relative motion between particle and fluid exists. The predominant force governing the motion of a solid particle in a liquid is the drag force, which is responsible for pulling the particle with the liquid. Both liquid viscosity and particle density can interact, affecting the relative drag force between liquid and particle, and thus the heat transfer rate. In the case of this experiment, lower IS values obtained by freely moving model food particles were a result of similar particle and liquid densities which diminished the effect of drag forces and resulted in minimal particle rotation and translation in relation to the liquid. This decreased the efficiency of heat transfer relative to the immobilized particles.
5. CONCLUSION

Retort heating efficiency tests of a Lagarde steam/air simulator at 0 rpm conducted with water filled 307 bowl cans revealed no significant differences (p>0.05) among 15 positions within the retort load in terms of heating rate index ($f_h$), accumulated lethality ($F_0$) and heating lag index ($j_h$). This indicates uniform heating conditions.

The effect of radial positions (0-175 mm) from the axis of rotation at 7 rpm levels (0-30) in 307 bowl cans filled with water only, or with water and 6 simulated potato/alginate food particles (18 mm diameter) were investigated. Both rpm, and radial position were found to significantly influence the heating rate indices. However, the influence of agitation rate was found to be non-linear, and effects of radial position were greatest at the largest radial position tested (R3).

To evaluate the effect of thermocouple immobilization of a food particle in the centre of a can with convective liquid, spores of *Bacillus stearothermophilus ATCC 7953* ($D_{121.1}=2.04$ min, $z=7.80 \, ^\circ C$) were encapsulated in DSC pans and located in the centre of simulated food particles. Integrated sterilization (IS) values obtained from the spore count reduction showed that thermocouple immobilized particles obtained significantly larger lethality than freely moving particles. These results indicate a need for further studies of EOE agitated processing of particulate products, and especially of the effects of thermocouple mounting of food particles.
6. REFERENCES


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