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SUMMARY

Time-temperature integrators (TTI) from a-amylases were used to estimate pasteurisation values in a variety of complex food processes where temperature probe systems were difficult or impossible to use. The amylase TTIs were from either a *Bacillus amyloliquefaciens* (BAA) or *Bacillus licheniformis* (BLA) source. Amylase activity measured before and after processing was converted to pasteurisation values from which log-reductions in target microorganisms were estimated. BAA had an equivalent measuring range from 3 to 16 minutes at 85 "C and BLA from 3 to 16 minutes at 93.3 "C, with z-values of 9.4 and 9.1 C° respectively. These ranges covered several commercial pasteurisation processes, for example, heat resistant spore-forming yeasts and moulds (5 minutes at 85 "C, z = 10 C°), butyric anaerobes (5-10 minutes at 93.3 "C, z = 8.3 C°) and psychrotrophic strains of botulinum (10 minutes at 90 °C, z = 9-10 C°). Several TTI case studies were conducted and reported, covering a wide range of food products, processing systems and package formats. TTI data allowed the pasteurisation processes to be defined more accurately and in some instances the thermal processes optimised to increase production throughput by as much as 25%. This innovative analysis method has many applications in food processing because of its benefits of being simple to use, relatively inexpensive, non-toxic and accurate.

CONTENTS

1. INTRODUCTION AND BACKGROUND

This report covers work on a DEFRA LINK funded project to develop and apply time-temperature integrators to measure the safety of thermal processes. Validation of thermal processes is an essential step in order to prove that the numbers of micro-organisms within a product have been reduced to a level that is deemed safe from a public health perspective. The requirement is for companies that manufacture heat preserved foods to document the severity of their thermal processes in terms of the achieved reductions in the target micro-organisms; for example *Salmonella* spp., Listeria monocytogenes, and Clostridium botulinum. Various methods can be used to validate a thermal process.

1.1 Process Validation Methods

The most common process validation method involves converting the measured time-temperature profile experienced by a food into the resulting destruction of target micro-organisms. This relies on accurate methods of measuring temperatures in foods that do not interfere with the temperature response of the food to its environment.

As the number and variety of manufactured food products increases, food companies are faced with the challenge of proving that these products are safely pasteurised or sterilised. This can sometimes be difficult if conventional temperature probe systems cannot be used and other more complex approaches need to be adopted. The main product categories that introduce these complexities include products cooked in continuous ovens or fryers (e.g. poultry joints, chicken nuggets, burgers, bread) and products with discrete pieces cooked in steam-jacketed agitated vessels (e.g. ready meals, soups, cook-in-sauces, fruit preparations) or in heat exchangers (e.g. cook-in-sauces, preserves, dressings). If an alternative to temperature probes is needed, the following approaches to validating microbiological process safety are the options available to prove the microbiological process safety:

a) Microbiological methods can be used whereby cells or spores of a non-pathogenic organism, with similar temperature-induced death kinetics to the target pathogen, are embedded into an alginate bead (Brown et al. 1984). The beads are made to mimic the food pieces in their thermal and physical behaviour and so pass through the process with the food. Enumeration of the surviving organisms allows the log reduction and process value to be calculated.

b) Simulated trials are carried out in a laboratory where the heat transfer conditions of the process are replicated.

c) No validation is attempted, with the process safety being inferred from temperature probing of the bulk product or the environment. Substantial over-processing is allowed, in order that the thermal process delivered to the product thermal centre is sufficient. End product testing for microbiological activity is usual.

d) Process models can be developed that predict, for example, the temperature-time history of the critical food particles as they travel through the heating, holding and cooling zones of the process (McKenna and Tucker, 1991).

e) Time-temperature integrators (TTIs) can be applied to gather similar process data to that from microorganisms: this is an emerging method and is the focus of this report.

1.2 Process Modelling Issues and Optimisation

Having indirectly measured the impact of the thermal process on the microbial population, and thus proven that the process delivers a consistently safe product, it is important that product quality is considered. This is where process optimisation studies are employed. The basis for an optimisation study is the application of a mathematical model of the process, and in order to develop such a model it is imperative that the process measurement system (from which the microbiological impact is deduced) is accurate and repeatable. These models are not often accepted without proof that the process can deliver the correct level of microbiological reduction. For example, the FDA has recently licensed a method of characterising aseptic particulate processes which involves the sampling of a large number of particles treated with spores in conjunction with finite difference modelling (Palanappian and Sizer, 1997). The single and multiple TTI particles that were developed in the project offered this type of proof.

Process modelling issues were dealt with almost exclusively within this project by the University of Birmingham, and so will not be discussed at length in this report. Further details of the scope of the modelling studies can be found in Cox and Fryer (2001) and Cox et al. (2002).

1.3 Potential Time-Temperature Integrators

This project utilised biochemical time and temperature integrators to obtain data for validating the process efficacy and for process modelling purposes. These TTIs were enzymes, such as amylase or peroxidase, that denatured (an unwinding of the structure) in a repeatable way as they were heated. The reaction kinetics of their temperature-induced denaturing matched those of the microbial death kinetics and so it was possible to use such TTIs as non-microbiological markers of a process. Since most published heat resistance data for micro-organisms followed first order reaction kinetics, good mimics of their kinetic pathways were obtained with the TTIs.

One of the early aims of this project was to identify a range of TTIs that could be used in the temperature range associated with both pasteurisation (60-105 "C) and sterilisation (105-14.0"C) treatments, and which could be used either singly or in combination. Most of the interest from the food industry was to define the levels of microbiological destruction in thermal processes, which required a TTI with a z-value close to 10 C° (this was the 'safety TTI'). For sterilisation, the target was to reduce the numbers of *Clostridium botulinum* spores to an acceptable level: at least a 12 log reduction. For pasteurisation, the target

2. MATERIALS AND METHODS

2.1 Pasteurisation TTIs

2.1.1 Preparation of amylase solutions

To prepare the arnylase solutions, 200 mg of α-amylase from a *Bacillus amyloliquefaciens* or *Bacillus licheniformis* source (EC 3.2.1.1 Type 11-A, Sigma A-6380) was dissolved in 20 mL of 0.1 M Tris buffer (pH 8.6 at 25 °C). This was stored at 4 °C for no more than 10 days before use (Adams, 1996). The enzyme activity was determined by first diluting 10 μL of the α-amylase solution with 290 pl of Tris buffer, then adding 20 μl of this to 1 mL of Sigma amylase reagent at 30 °C (Sigma Diagnostics, catalogue number 577-20). The rate of increase of absorbance at 405 nm was measured for the unheated (control) samples between 1.5 and 2.0 minutes after insertion into a spectrophotometer (ThermoSpectronic PU8755). For the heat-treated samples, this rate was usually determined between 3.0 and 4.5 minutes, although the peak rate could occur over a different period.

2.1.2 Calculation of pasteurisation values

The calculation of a pasteurisation, or P-value, eniployed a similar first order equation as for the destruction of microorganisms, except that the calculation used the initial and final enzyme activities instead of numbers of organisms (Equation 1).

$$P = D_T \cdot \log\left(\frac{A_{initial}}{A_{final}}\right) \quad \text{..........(1)}$$

where, A_{∞} is the final activity after a specific time-temperature history

$A_{initial}$ is the initial activity

D_T is the decimal reduction time at a fixed temperature (T) to reduce the enzyme activity by a factor of ten (minutes).

P-values can also be calculated by integrating the killing power of a thermal process over the time-temperature history experienced by the product, providing that this can be measured. A P-value calculated using Equation 1 will be the same as that calculated from the time-temperature integration, provided that first order kinetics have been followed for the enzyme destruction throughout the heat process (see Equation 2).

$$P = \int_0^t 10^{\frac{T(t)-T_{ref}}{z}} \cdot dt = D_T \cdot \log\left(\frac{A_{initial}}{A_{final}}\right) \quad \text{..........(2)}$$

where, T(t) is the product temperature, which is a function of time, "C

T_{ref} is the reference temperature for the D_T value, °C

t is the process time, minutes

z, the kinetic factor, is the temperature change required to effect a ten-fold change in the D_T value (C°)

2.1.3 Preparation of the silicone particles

Previous methods for TTI particle construction (Tucker, 1999a) used air bubbles in silicone to encapsulate a TTI solution and prevent its contact with the surrounding food. Creating the air bubbles was time-consuming and technically difficult, and they could not be created to a consistent size and shape. The encapsulation method was improved by using leiigtlis of silicone tubing, of 2.0mm bore, 0.5mm wall, or of 2.5mm bore, 0.5mm wall. To prepare the tubes, 10 mm lengths of tubing were cut, one end sealed off by dipping it into uncured Sylgard 184 elastomer (Dow Corning Ltd), allowing capillary action to create a 2-3mm plug, then heating the tube at 70 °C for a short time to cure. 15µL aliquots of amylase solution were injected into each plugged tube using a hypodermic syringe. To create a seal, the tubes were gently squeezed to force the amylase solution just proud of the end, and the end was dipped into liquid Sylgard so that when the pressure was released, the silicone was drawn into the tube to form another 2-3 mm plug. Intimate contact between the amylase solution and the Sylgard was important. The tubes were immersed in 4.0 "C water and cured in an oven set at 40 °C for about 2 hours. This avoided any possibilities of drying. The finished TTI tubes were cut down to 7-8 mm in length.

The finished TTI tubes were stronger and more uniform than the filled air bubbles, and could be inserted more easily into food products by making a small hole. However, many of the TTI trials required the tubes to be made into a simulated food particle because of their greater strength and ease of finding them afterwards. The material chosen for this was again Sylgard 184 because it was transparent, robust, chemically inert and could be moulded into particles. To match the thermal characteristics of the silicone particles precisely to those of the target fruit pieces, the size and shape of the silicone particles were calculated using Equation 3 for a spherical particle and Equation 4 for a cubic particle (Ball and Olsen, 1957). The thermal diffusivity value, a measure of how fast the centre temperature of a solid body responds to a change in ambient temperature, was $1.0 \times 10^{-7} m^2.s^{-1}$ for Sylgard 184 (Dow Coming, 1986) compared with $1.4 \times 10^{-7} m^2.s^{-1}$ for a typical high water content food (McKenna and Tucker, 1991). These equations used the calculated logarithmic heating rate (f_h) as the factor to equate heating rates of solid bodies of varying shape, size and dimensions.

$$\alpha = \frac{0.233a^2}{f_h} \qquad\qquad ....(3)$$

$$\alpha = \frac{0.933}{f_h(1/a^2 + 1/b^2 + 1/c^2)} \qquad\qquad ....(4)$$

where, a is thermal diffusivity $(m^2.s^{-1})$

f_h is the logarithmic heating rate, or the time required to effect a ten fold increase in the centre temperature once the temperature rise is logarithmic (s);

a, b, c are half dimensions (m)

As a first approximation, a silicone TTI particle would be made slightly smaller than the target food particulate. For example, 9-10 mm silicone cubes would heat and cool at almost the same rate as 12-14 mm cubes of most high water content foods. In fact, for tests in flowing situations it was important that the heat transfer conditions experienced by the silicone and food particulates were similar, otherwise the fluid to particle heat transfer coefficients may have differed. For particulates below 10-15mm this can become critical (McKenna and Tucker, 1991) and may in fact be just as important as calculating the correct dimensions.

2.1.4 Industrial trials

A number of different pasteurisation processes were investigated using the two amylase TTIs in solution. Much of the detail of this work remains confidential to the companies involved in each study. However, generic information from this work can be published. Table 2 presents the type of products and processes that were evaluated, together with some detail on how the TTIs were constructed.

Table 2: **Type of products and processes evaluated with pasteurisation TTIs.**
(BAA is B. *amyloliquefaciens* **amylase and** *BLA* **is B.** *licheniformis* **amylase)**

Product Type	Process Description	Amylase Type (BAA or BLA)	TTI Particle method
Fruit preparations with particulates	Ohmic heating	BAA	Silicone particulates moulded to represent h i t pieces
Cook-in-sauces	Sprayed water pasteurisation-cooling tunnel	BAA and BLA	Tubes suspended within sauce
Poultry pieces	Continuous oven cooking-cooling	BAA	Tubes inserted directly into product
Fruit preparations with particulates	Tubular heat exchanger	BAA	Silicone particulates moulded to represent fruit pieces
Fruit products in liqueurs	In-jar pasteurisation	BAA	Tubes inserted directly into larger products (e.g. peaches). Silicone particulates moulded to represent smaller fruit pieces (e.g. blackcurrants)
Ready meals	Sous-vide processing in water baths	BLA	Tubes inserted directly into the meat components
Hot-fill sauces	Sprayed water top-up pasteurisation of internal jar surfaces	BLA and BAA	Tubes attached onto jar surfaces

Examples of how some of these TTI trials were conducted are given in the results section.

2.2   Sterilisation TTI

Enhancement of the amylase heat stability was achieved by drying. Kinetic data for D_T and z-values of a dried a-amylase fiom *Bacillus subtilis* were reported to have a $D_{...}$ of 25.3 ± 0.9 minutes and a z value of $9.4 \pm 0.5\ C°$ (Van Loey et al., 1997b). These values were close to those required to represent the death kinetics of *Clostridium botulinum* over the commercial F, range. However, in the final stages of the reported work, the supplier of the *Bacillus subtilis* aniylase changed their preparation procedures, which resulted in a less pure form on the market. An alternative amylase source was required, which was fiom *Bacillus licheniformis*, another bacteria that was reported to produce high heat stability enzymes (Vielle and Zeikus, 2001).

Drying over phosphorus pentoxide at 4 °C for 10 days removed almost all of the fiee water fiom the amylase. The next stage was to equilibrate the dried amylase above a saturated salt solution in order that a specific moisture content could be achieved. Saturated sodium chloride solution at 4 °C for 6 days was used by Van Loey et al. (1997b) with *Bacillus subtilis* amylase: this has an Equilibrium Relative Humidity (ERH) of 76. Moisture content has been reported to have a significant effect on both D_T and z-values and therefore some manipulation of values was possible. Various salt solutions were evaluated for their suitability at providing different ERH conditions. It was found that amylase fiom *Bacillus licheniformis* gave the most suitable kinetics when equilibrated over saturated ammonium sulphate solution at an ERH of 82.

Kinetic paranieters (D_T and z) were estimated initially using standard isothermal methods. However, non-isothermal methods were chosen because they represented more closely the conditions experienced by the food products during a sterilisation process. The analysis method measured enthalpy peaks with a Perkin-Elmer Differential Scanning Calorimeter (DSC). Arnylase is a protein that gave a characteristic peak in enthalpy (see figure 1) over a consistent temperature range; the area under the peak was estimated within the DSC software. The difference between initial unheated enthalpy and final heated enthalpy allowed the F-values to be calculated (see equation 2). The DSC allowed a series of temperature-time profiles to be programmed into a macro, which automated the entire procedure of conducting non-isothermal kinetic parameter estimation. Fifteen series of ramps, hold times and hold temperatures were used to generate fifteen different sterilisation values.

Figure 1: **Example of the enthalpy peak for an amylase sample heated in the DSC**
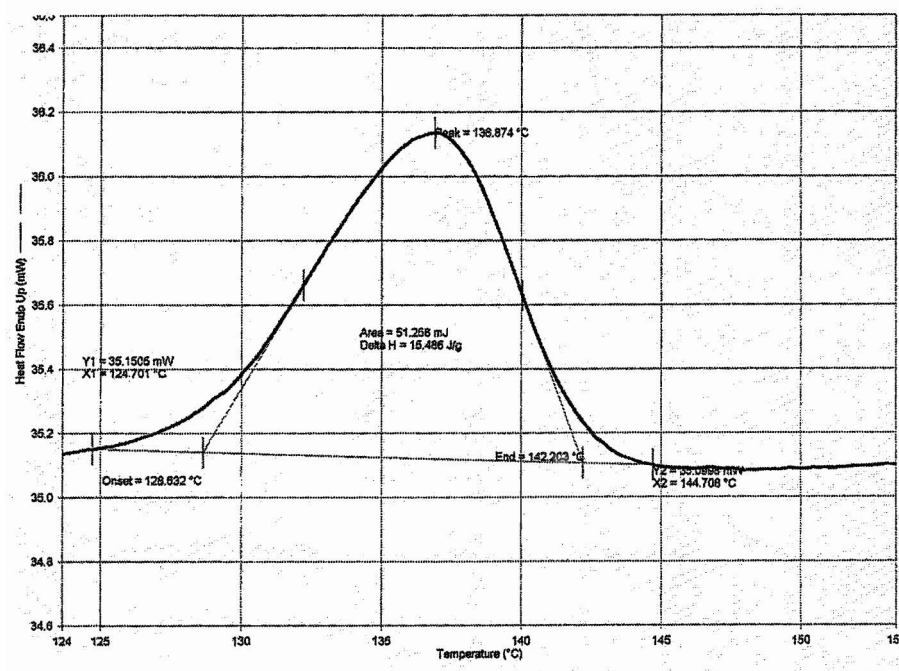
Having encapsulated the dried and equilibrated amylase inside a steel DSC capsule, it was ready for inserting into a canned food product. **An** advantage of the DSC method was that the capsule did not have to be opened **during** the analysis. However, several disadvantages were identified during the research, which are discussed in the results section.

3. RESULTS FROM INDUSTRY TTI TRIALS

A feature of this project was the application of TTIs to industrial processes as a means to measure the levels of pasteurisation and then to optimise processes based on the data taken. Examples were selected from industrial trials to illustrate the type of studies conducted.

3.1 Ohmic heating of fruit preparations

Several TTI trials have been conducted within this project to evaluate the thermal process achieved in a commercial 75kW ohmic heater. The example presented here is for the pasteurisation of blackcurrant and pineapple fruit preparations, using the *Bacillus licheniformis* amylase. The objective was to challenge the microbiological process safety by setting higher product flowrates than in normal production.

In order to produce TTI particles that were of similar dimensions to blackcurrants, the TTI tubes were placed into the centre of 8mm sphere moulds that were filled with the uncured silicone compound. Thermal characteristics of the silicone were such that an 8mm silicone sphere heated and cooled at the same rate as a 10mm blackcurrant. A similar method was used to produce 10mm silicone cubes that represented the 12mm pineapple pieces. Forty-eight x 8mm spheres and forty-seven x 10mm cubes were taken to the factory. Four of each were kept as controls from which the initial amylase activity was measured; the remaining TTI particles were incorporated randomly into the feed tank. *Bacillus licheniformis* amylase was the TTI.

The electrical insulation properties of the silicone were such that the silicone TTI particles would not heat up by resistance heating (Tucker et al. 2002). P-values measured using these TTI particles represented thermal conduction to the particle centres from the hot surrounding liquid, giving conservative P-values. It is also worth emphasising that the P-values included any contributions during cooling.

For *Bacillus licheniformis* amylase, the kinetics of destruction by heat were represented by decimal reduction times (D_T value) of 8.8 minutes at 93 "C or 66.6 minutes at 85 "C, with a kinetic factor (z-value) of 9.1 C°. This enzyme had suitable kinetics that would allow it to measure the equivalent target process for the blackcurrant and pineapple pieces, against a P-value target of at least 5 minutes during the holding tube. The ohmic process delivered a significant proportion of the microbiological kill during the tubular cooling stages where the heat transfer was less favourable than during electrical heating. Hence, although the holding tube target was 5 minutes at 85 "C, it was expected that the overall P-value would exceed this by a substantial margin.

The fruit products were pumped through the 75kW ohmic heater, which led to 16.4m long holding tubes, before being cooled in tubular coolers and packaged in aseptic 800 kg tanks (see Figure 2). The ohmic heater was running at an average power of 60kW, giving an outlet temperature of 100 "C. The flowrate was increased from the normal 750kg/h to investigate whether the process time could be shortened safely. After processing, a starch solution was pumped through the system in order to pig out the product and minimise wastage. Some of

this solution containing the product/starch interface was collected in buckets and the remaining amount fed down the drain.

Figure 2: **Schematic diagram of the ohmic heater used to process fruit preparations**
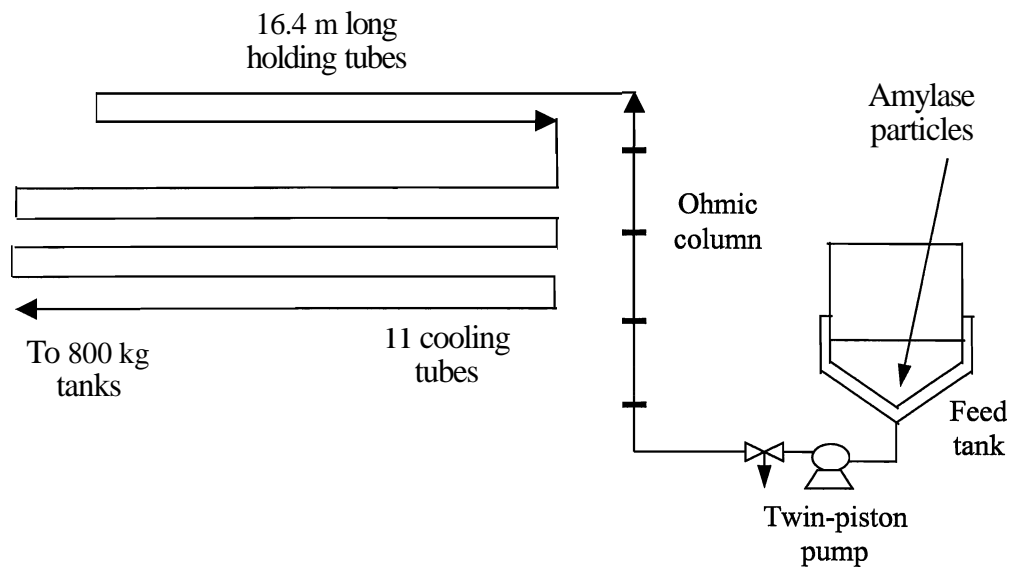
For the blackcurrant trial, forty-four TTI particles were put into a 700kg batch and processed at a flowrate of 850kg/h. The TTI particles were recovered by rinsing the blackcurrant mix with water through a sieve that removed the liquid, making the silicone particles more visible. Figure 3 presents the frequency distribution for P-values at 85 ℃, showing a flat distribution of values, all in excess of the 5 minutes minimum. For the pineapple, forty-three TTI particles were put into a 650kg batch and processed at a flowrate of 790kg/h. The TTI particles were marked with a blue cross on opposite faces, making them easily visible in the light yellow fruit mixture. Figure 4 presents the frequency distribution for P-values at 85 °C again showing a flat distribution of values in excess of 5 minutes. Further detail on this work can be found in Tucker et al. (2002).

Figure 3: **Frequency distribution of P-values for the blackcurrant fruit preparation processed in a 75kW ohmic heater at 850 kg/h**

Blackcurrant Fruit Preparation
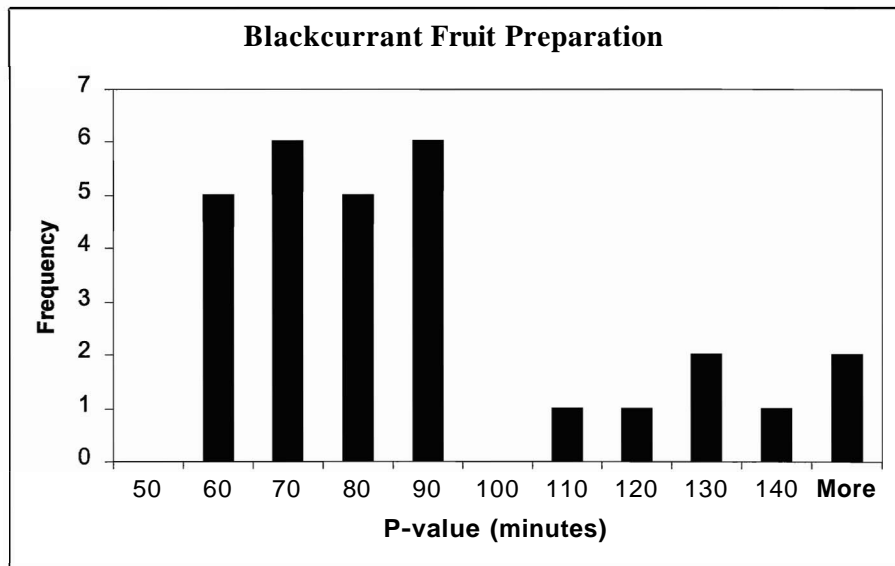
Figure 4: **Frequency distribution of P-values for the pineapple fruit preparation processed in a 75kW ohmic heater at 790 kg/h**

Pineapple Fruit Preparation
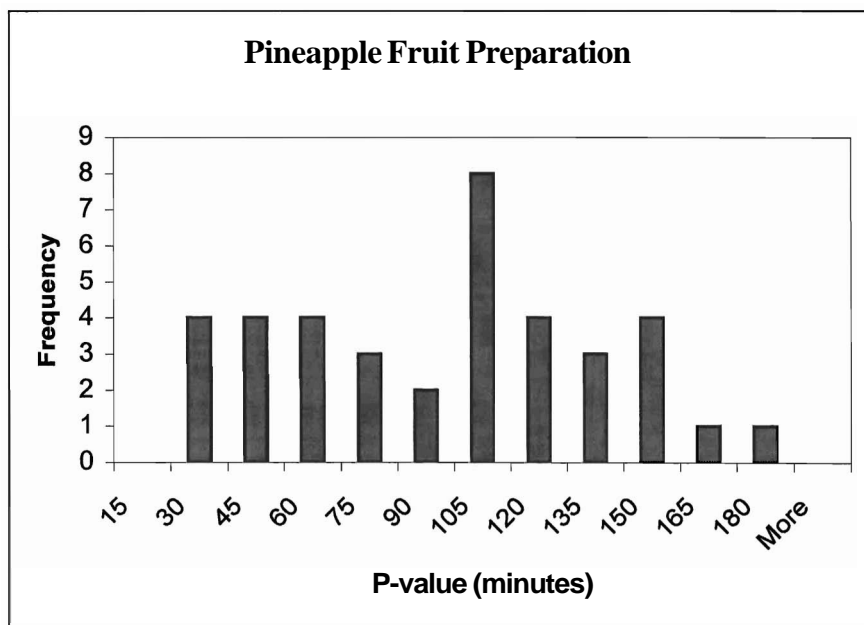
3.2 Sprayed water pasteurisation-coolingtunnel for cook-in-sauces

The aim of this trial was to map the pasteurisation achieved at various locations within a jar of tomato cook-in-sauce during processing in a sprayed water pasteuriser. Data from the trials carried out at the industrial site were confidential, and so to illustrate the type of data that was obtained, a repeat trial was done at CCFRA using off-the-shelf product. Amylase TTIs were used to provide P-values from 16 positions in the jar and comparisons were made with those measured by probes, where possible. The probes were thin-wire type K thermocouples with soldered junctions, which allowed several temperature measuring points within the single jar.

TTI tubes were 2.0mm bore, 0.5mm wall thickness and 10.0mm long. 15μL of *Bacillus licheniformis* amylase was encapsulated inside and sealed with Sylgard plugs at either end. The amylase kinetics of destruction by heat were represented by decimal reduction times (D, value) at 93.3 °C of 8.2 minutes and a kinetic factor (z-value) of 9.1 C°. These kinetics were suitable for measuring the butyricum process for these sauces, which was equivalent to 5 minutes at 93.3 °C with a z value of 8.3 C°.

Figure 5 shows the location of paired TTIs and probes in the jar, with estimated P-values against each position. Silicone sealant (RS Non Corrosive Silicone Rubber 494-118) was used to attach the TTIs to the jar sides and base. This sealant did not produce acetic acid during its cure and so previous issues with diffusion of acetic acid through the thin silicone tube walls was eliminated (Lambourne and Tucker, 2001).

A TTI calibration was introduced into this type of process evaluation trial to ensure that minor changes in amylase D_n values did not affect the accuracy of the results. In previous industrial trials, D-value changes up to 10% had been noted. Such D-value variation can arise because of impurities in the amylase and when fresh amylase solution was made up for each trial, the D-value was checked. A calibration test involved heating three to four TTI tubes in water at a temperature close to the reference temperature (93.3 "C) to achieve approximately 1-log reduction in activity. A cross-check against the expected probe P-value allowed the accuracy of the TTI P-value to be assessed.

At all locations other than the headspace, the TTI and thermocouple P-values agreed within the experimental limits. The major and undefined source of error was in the precise location of TTIs and thermocouple junctions, partly as a result of the physical TTI size, but also due to the movement of TTIs or thermocouple junctions during the processing. Hence, the P-value results provide a detailed map of the thermal treatment received throughout the jar.

The discrepancy in headspace P-values between TTIs and thermocouples was caused by the TTIs just penetrating the top surface. Evidence for this was the uptake of red/orange coloration from the sauce. The thermocouple junction was not in contact with the sauce and as such would have measured a P-value in the headspace above the sauce.

TTI results given in this test showed that a wide range of pasteurisation values occurred within a single jar of sauce, ranging from 2.6 minutes at the centre to 20.9 minutes towards

the sides. The jar centre P-values measured with TTIs and probes showed lower values than the target minimum of 5 minutes at **93.3** °C. However, the initial sauce temperature, the **pasteuriser** zone temperatures and residence times were set at deliberately conservative values.

Figure 5: **P-values measured with thermocouples (left jar) and TTIs (right jar) at various locations within a jar of tomato cook-in-sauce.**
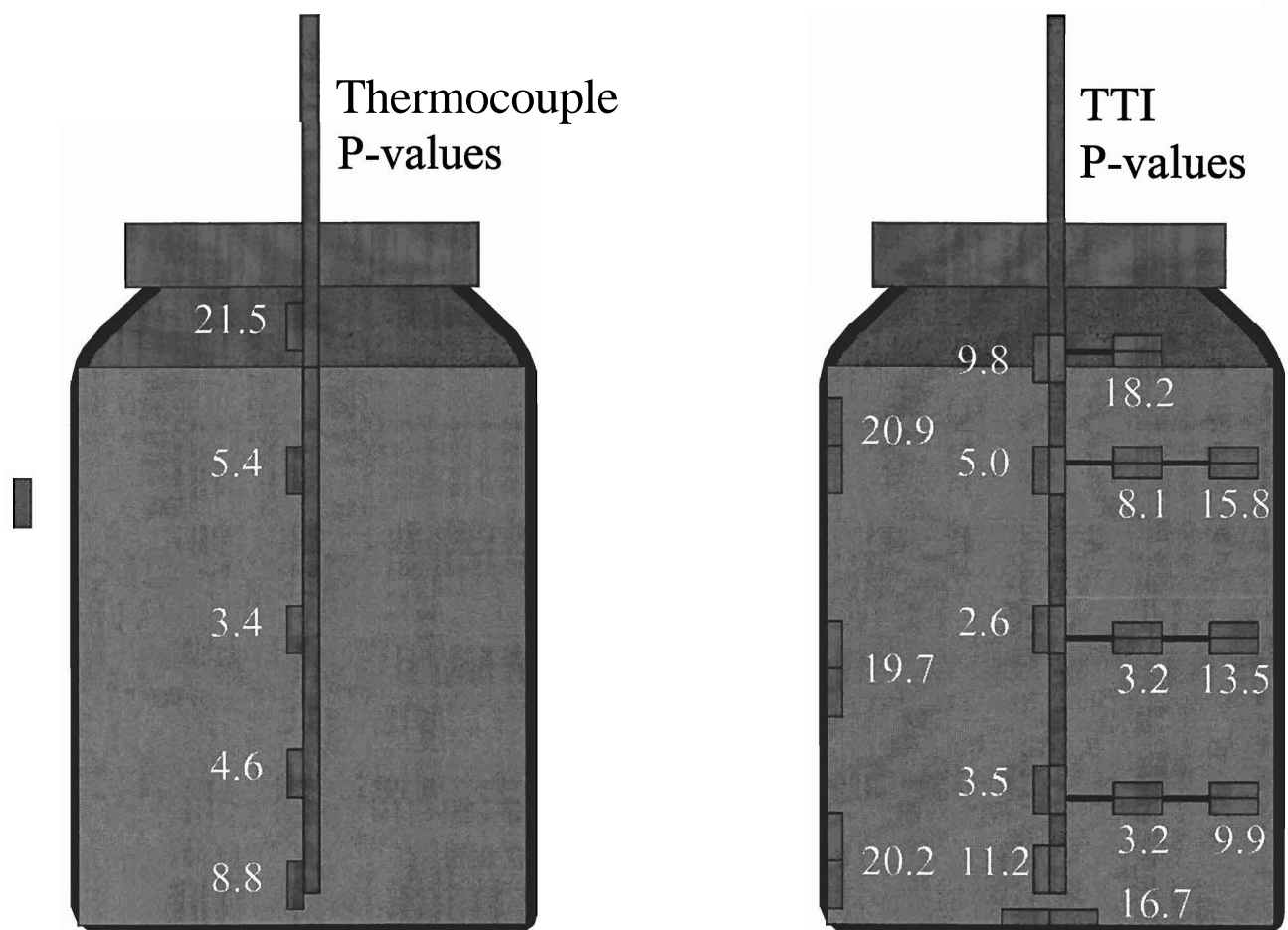
3.3 Continuous oven cooking-cooling of poultry pieces

Reformed meat and poultry products are known to present difficulties for inserting and maintaining the position of temperature sensors used with conventional process establishment. The aim was to use TTIs to measure the processes achieved in chicken fillets cooked in a continuous oven and to assess the effects of taking 2 minutes off the cook time.

TTI particles were silicone tubes of diameter 2.5mm and length 7-10mm, containing approximately 15μL of Bacillus *amyloliquefaciens* amylase. Kinetics of destruction by heat were represented by a decimal reduction time (D_T value) of 6.8 minutes at 85 "C or 268 minutes at 70 "C, with a z-value of 9.4 C°. To ensure the process achieved at least a 6-log reduction in 'aerobic pathogens' such as Listeria, Salmonella and E. coli, the target P-value was 2 minutes at 70 "C (CCFRA Pasteurisation Treatments, Guideline No.27). Although this was a low process to measure with the amylase when compared with the D. value, there was a need to operate with a substantial margin and data from previous trials had resulted in measurable activity loss.

Wherever possible, duplication or triplication of TTI tubes in a fillet was used. Tubes were inserted by slicing the fillets to expose the centre and placing the tubes 'end-to-end' in a row along the centreline. This created a good seal when the cut half was folded back. Fillets with TTIs were placed onto a wire mesh tray in order to identify them from the production fillets. They were retrieved immediately after the oven, and taken to the chiller for a few minutes to remove sufficient heat to enable the TTIs to be handled. The normal production route was for the products to pass to a gas-fired searer before entering the chiller. This provided an additional 45 seconds residence tinie between oven and searer, and 30 seconds in the searer, both of which maintained the fillet centre temperature above 85 "C. Thus, the TTI trials deliberately removed this extra pasteurisation and so estimated P-values lower than expected in production.

The first products tested were 25-40g chicken fillets marinaded in lime and coriander, and the second were 120-130g chicken fillets. For each product, fifteen TTIs were used to assess the level of pasteurisation at the normal production speed and fifteen at a faster throughput. Conventional temperature probing was not used because of the difficulties in positioning probe tips at the fillet centres and holding them in place for the duration of the process. Temperature probing of the fillets after the oven followed standard company practices to ensure that the fillet centres had reached a threshold of 85 $\pm$ 3 "C. Tables **3 and** 4 give the operational settings of the oven for the run under normal production conditions and for a shortened cook time.

Table 3: **Oven settings for the 25-40g fillets marinaded in lime and coriander, using hot air**

25-40g **Chicken Fillets**	Run 1: Normal cook time	Run 2: Reduced cook time
Cook time (mins)	10.0	8.4
Infeed temperature (°C)	162	162
Outfeed temperature (C)	192	192
Fillet temperatures ex. oven (°C)	87-88	84-85
Number of TTIs used	15	15

Table 4: **Oven settings for the 25-40g fillets marinaded in lime and coriander, using steam**

120-130g **Chicken Fillets**	Run 1: Normal cook time	Run 2: Reduced cook time
Cook time (mins)	22.1	20.0
Infeed temperature (C)	95	96
Outfeed temperature (C)	95	96
Fillet temperatures ex. oven (C)	88-90	86-90
Number of TTIs used	15	15

P-values at 70°C for the 25-40g and 120-130g fillets showed high levels of pasteurisation, substantially in excess of the target of 2 minutes at 70 °C for achieving 6-log reduction in 'aerobic pathogens'. Typical P-values for production conditions were in the range 250-300 minutes for the 25-40g fillets and 430-500 minutes for the 120-130g fillets. Reducing the cook time by 2 minutes for each product showed reductions in P-value to 35-200 and 400-450 minutes respectively, but the lowest values were still substantially greater than the minimum safety value of 2 minutes. The lowest process measured at P-value 33.6 minutes represents a 100-log reduction in 'aerobic pathogens'.

These poultry processes were shown to operate with a high level of microbiological safety. This is important to allow for variations in processing and product conditions (e.g. fillet size, belt loading, air temperatures etc). For small products such as the 25-40g fillets, where temperature probes cannot be used with accuracy, the TTI approach can give commercially valuable results. A 100-log reduction represented a satisfactory safety margin, particularly when the trials did not take account of the additional pasteurisation between the oven, searer and chiller.

3.4 Tubular heat exchanger processing of fruit preparations

The aim was to use TTIs to measure the thermal process achieved in a 2" tubular heat exchanger for the pasteurisation of a pear and toffee mixture. Amylases from both Bacillus *licheniformis* and Bacillus amyloliquefaciens were used because of the uncertainty about the achieved thermal process. These two TTIs provided a wide measuring range.

In order to produce TTI particles of similar dimensions to 12mm pear cubes, TTI tubes were placed in the centre of 10mm cubic moulds that were filled with the uncured Sylgard elastomeric compound. The thermal characteristics of the silicone were such that a 10mm silicone cube heated and cooled at the same rate as a 12mm pear piece. Forty cubes contained 15μL amylase from Bacillus *licheniformis*, and forty contained 15μL amylase from Bacillus amyloliquefaciens, to cover a wide pasteurisation range. For the Bacillus lichenijiormis amylase, the kinetics of destruction by heat were represented by decimal reduction times (D_T value) of 8.8 minutes at 93 °C or 66.6 minutes at 85 "C, with a kinetic factor (z-value) of 9.1 C°. For the Bacillus *amyloliquefaciens* amylase, the kinetics of destruction by heat were represented by a decimal reduction time (D_T value) of 6.8 minutes at 85 °C, with a kinetic factor (z-value) of 9.4 C°. For each set of TTI particles, five were kept as controls, from which the initial amylase activity was measured. The remaining seventy TTI particles were incorporated randomly into the feed tank.

The tubular process involved seven steam-heated heating tubes, seven insulated holding tubes and eleven chilled water-cooled tubes, each of six metres length (see Figure 6). The target process was to exceed 88 °C for 3 minutes in the insulated holding tubes, using a normal operating temperature of 92 "C to allow for fluctuations. At 88 "C, this process achieves a P,,-value of 6 minutes, compared with the minimum for high-acid fruit products of 5 minutes (CCFRA Technical Manual No.27 on Food Pasteurisation Treatments). The measured temperatures quoted in the setpoints were for the liquid surrounding the pear pieces.

Figure 6: **Schematic diagram of the tubular heat exchanger used to process the pear and toffee product**
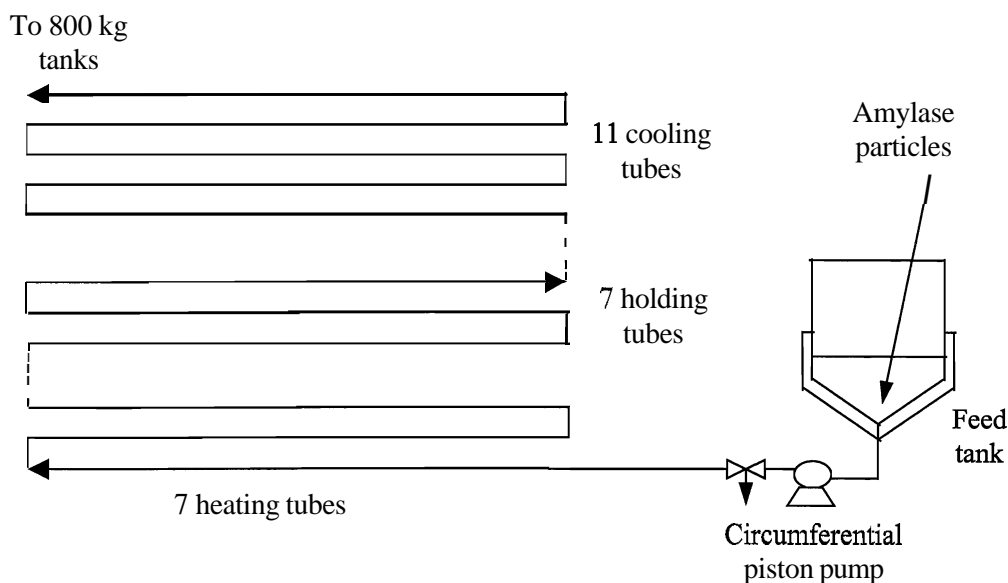
At the end of the batch, a plastic pig operating with 6 bar air pressure was used to push out the product and minimise wastage. Towards the end of pigging, the back pressure from the product decreased as less product remained in the tubes; consequently the flowrate increased steadily from 1,000kg/h. This resulted in product receiving a reduced cool and entering the filled tanks at increasing temperature. All seventy TTI particles were recovered from the two tanks of processed product, although one particle had been cut by one of the valves.

The amylase activities indicated a wide range of P-values, which was expected with the continuous process. For a reference temperature of 85 "C, the highest P-value was 109.3 minutes and the minimum value was 32.9 minutes, compared with the target of 5 minutes. Residual aniylase activity was very low or zero for TTI particles containing the *Bacillus amyloliquefaciens* amylase. The frequency distribution in Figure 7 shows that the P-values measured with *Bacillus licheniformis* amylase were distributed normally. This was quite different from the distribution measured for ohmic heating of similar fruit products where a flatter distribution was typical. The explanation for the differences was the nature of the heating: ohmic heating is volumetric and so all particulates receive similar quantities of heat as they enter the non-heated parts of the process.

The TTI particle results showed that the 12mm pear pieces had received adequate processes, substantially in excess of the target P,, of 5 minutes. In fact, there was scope for process optimisation.

Figure 7: Frequency distribution of P_{85}-values for the pear and toffee preparation, measured with B. *licheniformis* amylase.

Pasteurisation Values for Pear and Toffee
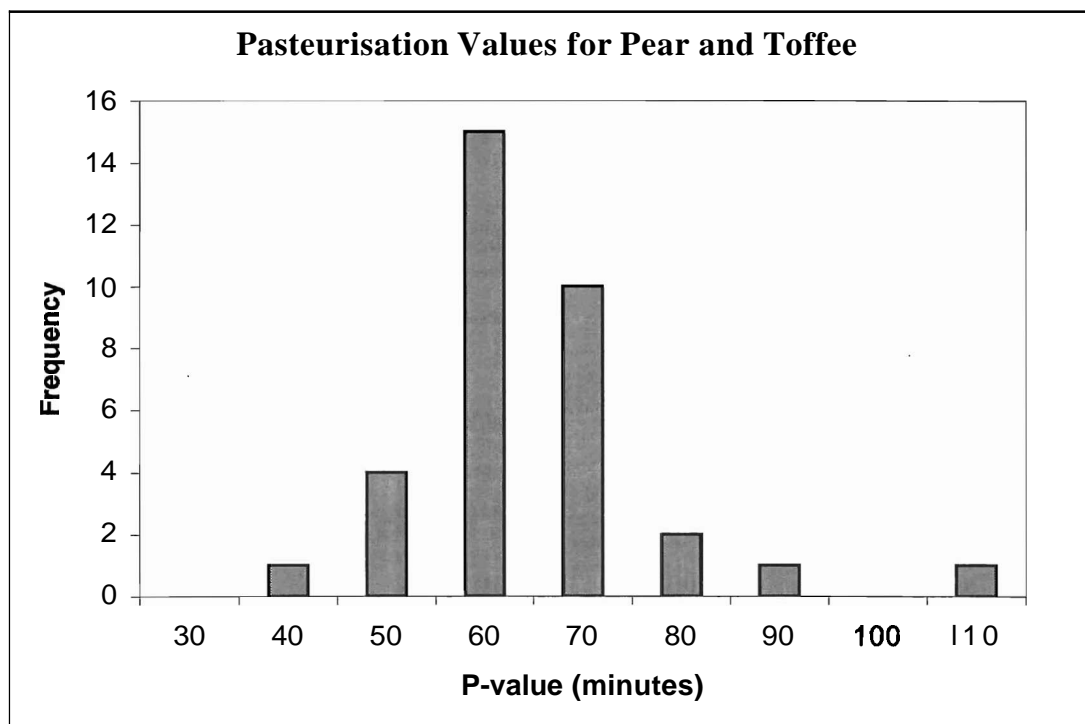
3.5 In-jar processing of fruit products

This was one of the first TTI trials conducted within the project. The aim was to measure baseline process values by inserting TTI particles directly into various fruit products processed in glass jars immersed in water baths. These fruit products are difficult to probe because of the soft fruit that does not retain a probe in position during the process. Further complications arise with the unusual jar shapes and sizes. Peach halves in brandy were the product described here.

TTI particles were silicone tubes of length 8mm and inside diameter 2.5mm containing approximately 15 μL of *Bacillus amyloliquefaciens* amylase. Kinetics of destruction by heat were represented by decimal reduction times (D_T value) at 85 °C of 6.17 minutes and a z-value of 8.6 C°, which compares with the target of 5 minutes equivalent at 85 "C for a z of 10 C°. TTI particles were inserted into the centre of thick peach halves by cutting a hole in the peach with a scalpel. One peach per jar contained a TTI particle, with the peach halves located at the jar centre.

The production process involved several stages of moving batches of jars between water baths. Jars of fruit were first immersed in a water bath set to 50 °C to avoid thermal shock on the glass. Once the glass temperature had equilibrated to 50 "C, the batch was transferred to one at 95 "C for the 80 minutes pasteurisation process. On cooling, the jars moved forwards into a bath at 50 °C to prevent thermal shock and finally into a chilled water bath to take the core temperature down to below 40 "C in the shortest time. A standard 80 minute process at

95 °C was considered too severe for the *Bacillus amyloliquefaciens* amylase, which was the only TTI available at the time, therefore the jars were removed after 35 minutes. This estimate was based on time-temperature data taken previously.

The three TTIs gave P-values of 2.2, 2.5 and 2.8 minutes, with one particle falling out of the peach half and receiving a higher heat process of 10.9 minutes. This trial demonstrated the convenience of using TTI particles to measure a process in a soft, slippery particulate. Further work was completed during the project to better define the scheduled 80 minutes process using *Bacillus licheniformis* amylase, which had a higher degree of heat stability.

3.6   Sous-vide processing of ready meals

Sous-vide processing involves pasteurisation of high quality foods under vacuum packing. This was a feasibility study to demonstrate the ease of using amylase TTIs to measure a process where wire-based probes cannot be used with confidence because of the loss in vacuum that usually occurs. The aim was to evaluate the thermal process achieved in a sous-vide pasteurisation of chicken breast in white wine sauce, using an amylase from a *Bacillus licheniformis.*

Kinetics of destruction by heat of the *Bacillus licheniformis* amylase were represented by decimal reduction times (D_T value) of 8.8 minutes at 93 °C or 18.8 minutes at 90 °C.   Sous-vide processing targets psychrotrophic strains of *Clostridium botulinum.* A 6-log kill can be achieved if the core temperature is at 90 °C for 10 minutes or any equivalent process (CCFRA, 1992a). Psychrotrophic strains of *Clostridium botulinum* are reported to show z-values of 9 C° (CCFRA, 1992b) or 10 C° (CCFRA, 1992a). The z-value for *Bacillus licheniformis* amylase was 9.1 C°.

Amylase TTI tubes were placed into chicken breasts by dissecting the breast with a scalpel and carefully lining up three TTIs along the centre cut. Table 5 shows where the TTIs were placed. The middle TTI was marked with a red dot. The chicken was folded back down to create a good seal. Heated and unheated control TTIs were used to ensure that:

- the environment had not affected the initial amylase activity,
- TTIs vacuum-sealed in the packs and heated in the same water bath gave the expected results,
- TTIs heated in a different water bath confirmed the accuracy of the measurements and provided a calibration factor for the P-values.

Table 5: **Description and location of the amylase TTIs in a chicken in sauce sous-vide product**

	Number of TTIs	TTI Location	TTI P-values (minutes at 90°C)
Pack 1	3	Chicken breast in 80 g sauce	23.4, 34.5, 36.9
Pack 2	3	Chicken breast in 80 g sauce	36.4, 43.4, -
Pack 3	3	Chicken breast in 80 g sauce	41.3, 27.3, 42.3
Pack 4	3	Chicken breast in 80 g sauce	38.8, 38.8, 42.3
Pack 5	3	Chicken breast in 80 g sauce	40.4, 32.6, 41.3
Pack 6	3	Chicken breast in 80 g sauce	33.3, 30.8, 34.5
Pack 7	3	Chicken breast NO SAUCE	40.4, 38.1, 48.1

The thermal process given to the vacuum-packed chicken in white wine sauce was to cook the packs in a water bath set at 85 "C for 80 minutes, followed by immediate immersion in chilled water. The aim was to achieve the 'psychrotrophic botulinum' process of 10 minutes at 90 "C at the centre of the chicken breasts. All of the P-values for TTIs in chicken breasts were considerably higher than the minimum target of 10 minutes equivalent at 90 °C, with most values ranging from 30 to 40 minutes. The pack with no sauce gave P-values towards the higher end of the range. This was thought to be due to a lower pack heat capacity.

Process validation with temperature probes had proved troublesome because of loss in vacuum and so the TTI method was seen as a major step forward. Further confidential trials were conducted during the project to take this work forward by using TTIs to optimise the water bath cooking processes.

3.7 Surface pasteurisation of hot-fill sauces

Surface pasteurisation was an application that was not considered in the project workplans but became possible because of the small TTI tube size. The aim was to evaluate the surface pasteurisation of plastic pots hot-filled with a tomato-based pasta sauce and to determine the effects of adverse conditions on the surface pasteurisation value. Conditions evaluated were low fill temperatures, increased delay times before pasteurisation and reduced pasteuriser residence times. Current operating conditions were challenged to determine the margins for increasing throughput. The tomato-based sauces were pasteurised in agitated vessels prior to hot-filling into 150 g plastic pots, and the pot surfaces were pasteurised by a combination of the heat from the hot sauce and raining hot water in a tunnel pasteuriser. It was the latter that was of concern in these trials.

Bacillus amyloliquefaciens arnylase TTIs were adhered to various positions on the pot surfaces using a silicone sealant (RS Non Corrosive Silicone Rubber 494-118), with each position measured in duplicate. Amylase kinetics of destruction by heat were represented by decimal reduction times (D_T value) at 85 °C of 10.6 minutes and a kinetic factor (z-value) of

9.4 C°. The target group of microorganisms were the spore-forming heat resistant yeasts and moulds, which required at least 5 minutes at 85 "C to effect a 6-log reduction in numbers (CCFRA, 1992a).

There were a total of seven different runs, with each having a different variant, as shown in Table 6. TTIs were placed at the Tracksense probe tips to compare P-values between probes and TTIs. No safety implications were raised with P-values below 5 minutes because the sauce had already been pasteurised prior to filling, and the TTI trials were to evaluate the surface pasteurisation.

Table 6: **Conditions evaluated to determine levels of surface pasteurisation for a 150g pot of tomato-based cook-in-sauce.**

TTI numbers	Holding time between filler and pasteuriser (minutes)	Retention time in pasteuriser (minutes)	Filler temperature (°C)
1 – 8	0.5	6.5	85
9 – 16	4.0	6.5	85
17 – 24	10.0	6.5	85
25 – 32	4.0	6.5	80
33 – 40	4.0	6.5	75
41 – 48	4.0	5.5	85
49 - 56	4.0	4.8	85

Effect of Changing Stoppage Time: holding times of 0.5, 4.0 and 10.0 minutes were used to simulate any possible stoppages on the production line. The current process schedule allowed a holding time of 4.0 minutes before the pots entered the pasteuriser. Pots that had remained between the filler and pasteuriser for greater than 4.0 minutes were discarded. Table 7 presents the measured P-values at 85 °C from the TTIs, showing that the required P-value of 5 minutes was achieved at all surface positions. Thus, further increases in delay time could be accommodated.

Table 7: Surface P-values for 0.4, 4.0 and 10.0 minutes stoppage time between filler and pasteuriser for a 150 g pot of tomato-based cook-in-sauce.

0.5 Minutes				
Position of TTI	**Sample**	**Rate 1**	**Rate 2**	**P-Value**
Probe	1	0.23	0.21	**4.3**
Probe	2	0.183	0.202	**4.7**
Head Space	3	0.01	0.009	**14.0**
Head Space	4	0.015	0.014	**12.6**
Below Top Rim	5	0.019	0.018	**11.9**
Below Top Rim	6	0.026	0.024	**11.0**
Bottom Rim	7	0.006	0.009	**14.7**
Bottom Rim	8	0.006	0.006	**15.4**

4 Minutes				
Position of TTI	**Sample**	**Rate 1**	**Rate 2**	**P-Value**
Probe	9	0.304	0.31	**3.2**
Probe	10	0.355	0.311	**3.0**
Head Space	11	0.013	0.014	**12.9**
Head Space	12	0.016	0.016	**12.3**
Below Top Rim	13	0.018	0.022	**11.7**
Below Top Rim	14	0.026	0.025	**10.9**
Bottom Rim	15	0.016	0.016	**12.3**
Bottom Rim	16	0.013	0.012	**13.1**

10 Minutes				
Position of TTI	**Sample**	**Rate 1**	**Rate 2**	**P-Value**
Probe	17	0.193	0.184	**4.7**
Probe	18	0.229	0.216	**4.2**
Head Space	19	0.035	0.034	**10.0**
Head Space	20	0.032	0.029	**10.4**
Below Top Rim	21	0.061	0.052	**8.5**
Below Top Rim	22	0.041	0.035	**9.7**
Bottom Rim	23	0.039	0.035	**9.8**
Bottom Rim	24	0.051	0.048	**8.9**

Effect of Changing Residence Time: pasteuriser residence times of 6.5, 5.5 and 4.8 minutes were used in the tunnel pasteuriser to assess whether the current scheduled time of 6.5 minutes could be reduced. Water temperature in the sprayed water pasteuriser was set at 96 °C. Table 8 presents the measured P-values from the TTIs, showing that the required P-value of 5 minutes was achieved at all surface positions. Further reduction in pasteuriser residence time could be accommodated.

Table 8: Surface P-values for pasteuriser residence times of 6.5, 5.5 and 4.8 minutes for a 150g pot of tomato-based cook-in-sauce.

6.5 minutes				
Position of TTI	**Sample**	**Rate 1**	**Rate 2**	**P-Value**
Probe	9	0.304	0.31	**3.2**
Probe	10	0.355	0.311	**3.0**
Head Space	11	0.013	0.014	**12.9**
Head Space	12	0.016	0.016	**12.3**
Below Top Rim	13	0.018	0.022	**11.7**
Below Top Rim	14	0.026	0.025	**10.9**
Bottom Rim	15	0.016	0.016	**12.3**
Bottom Rim	16	0.013	0.012	**13.1**
5.5 Minutes				
Position of TTI	**Sample**	**Rate 1**	**Rate 2**	**P-Value**
Probe	41	0.288	0.238	**3.7**
Probe	42	0.261	0.275	**3.7**
Head Space	43	0.023	0.026	**11**
Head Space	44	0.019	0.02	**11.7**
Below Top Rim	45	0.04	0.034	**9.8**
Below Top Rim	46	0.034	0.037	**9.9**
Bottom Rim	47	0.022	0.019	**11.6**
Bottom Rim	48	0.024	0.022	**11.2**
4.8 Minutes				
Position of TTI	**Sample**	**Rate 1**	**Rate 2**	**P-Value**
Probe	49	0.264	0.243	**3.8**
Probe	50	0.238	0.217	**4.2**
Head Space	51	0.035	0.038	**9.8**
Head Space	52	0.023	0.032	**10.7**
Below Top Rim	53	0.069	0.068	**7.9**
Below Top Rim	54	0.064	0.085	**7.6**
Bottom Rim	55	0.033	0.031	**10.2**
Bottom Rim	56	0.035	0.036	**9.9**

Effect of Changing Filling Temperature: the pots were hot filled at temperatures 85, 80 and 75 °C to determine if the current set point of 85°C could be reduced. Table 9 presents the measured P-values at 85 °C from the TTIs, showing that the required P-value of 5 minutes was achieved at all surface positions. Further reduction in filling temperature could be accommodated.

Table 9: **Surface P-values for filling temperatures of 85, 80 and 75 °C for a 150g pot of tomato-based cook-in-sauce.**

85°C				
Position of TTI	Sample	Rate 1	Rate 2	P-Value
Probe	9	0.304	0.31	3.2
Probe	10	0.355	0.311	3.0
Head Space	11	0.013	0.014	12.9
Head Space	12	0.016	0.016	12.3
Below Top Rim	13	0.018	0.022	11.7
Below Top Rim	14	0.026	0.025	10.9
Bottom Rim	15	0.016	0.016	12.3
Bottom Rim	16	0.013	0.012	13.1

80°C				
Position of TTI	Sample	Rate 1	Rate 2	P-Value
Probe	25	0.46	0.394	2.2
Probe	26	0.477	0.406	2.1
Head Space	27	0.017	0.019	12
Head Space	28	0.032	0.029	10.4
Below Top Rim	29	0.056	0.056	8.5
Below Top Rim	30	0.065	0.06	8.1
Bottom Rim	31	0.021	0.025	11.2
Bottom Rim	32	0.033	0.033	10.1

75°C				
Position of TTI	Sample	Rate 1	Rate 2	P-Value
Probe	33	0.454	0.397	2.2
Probe	34	0.601	0.505	1.4
Head Space	35	0.018	0.016	12.2
Head Space	36	0.02	0.022	11.5
Below Top Rim	37	0.025	0.025	11
Below Top Rim	38	0.057	0.058	8.4
Bottom Rim	39	0.025	0.027	10.8
Bottom Rim	40	0.051	0.048	8.9

Surface P-values suggested that factory safety margins were high and that the adverse conditions of increased delay, low fill temperature and reduced pasteuriser residence time had not compromised safety. In fact, scope existed for further process optimisation, which was evaluated later in the project. Lowest surface P-values in each test were found below the top rim, which was used as the position to focus on for further tests.

3.8 In-can sterilisation of products with particulates

Achievements with the sterilisation TTI were limited to experiments under controlled laboratory conditions and to partial success in canning factories. Drying and equilibrating amylase to a specific moisture level proved to be a feasible but difficult method for developing a sterilisation TTI. Few industrial tests were conducted with the sterilisation TTI for a number of reasons:

- Controlling amylase moisture content within a fraction of a percent at levels between 15-20% could not be achieved consistently. This led to greater variation in kinetic parameters (D,,,,, and z) compared with those for the pasteurisation TTIs.

- The DSC pans contained an O-ring gasket to seal the base and lid. Under laboratory conditions, this seal remained intact but it was often compromised when subjected to canning processes where substantial pressure swings occurred in very short times. Major seal damage resulted in ingress of the food materials and was easily identified by a total lack of characteristic enthalpy peak. However, minor damage would result in moisture changes or 'noisy' peaks, which affected the residual enthalpy calculation and gave erroneous F-values.

- The DSC pans were stainless steel and therefore of a high density when compared with most food particulates. Applications of these pans were only appropriate to particulates where the heavy pans did not affect the flow behaviour.

Non-isothermal methods for obtaining kinetic data gave excellent agreement between F-values measured with probes and TTIs. Table 10 presents the DSC heating conditions together with F-value results. Figure 8 illustrates the similarity in F-values over the wide range tested. The key factor in achieving this level of agreement was in controlling the moisture content of the amylase to within a few fractions of a percent.

Table 10: Non-linear temperature-time conditions programmed into the DSC for determination of the kinetic parameters for *Bacillus licheniformis* amylase equilibrated over ammonium sulphate

Trial No.	Start temp	Hold time	Ramp time	Hold temp				Meas H (J/g)	Calc F (t-T)	Calc F (TTI)	Diff	% Diff	Abs Diff	% Abs Diff
1	25	8	8	120				9.279	5.5	5.3	0.15	2.7	0.15	2.7
2	25	3	5	120	z	6.00	°C	15.69	2.2	1.9	0.22	10.0	0.22	10.0
3	25	12	3	120	Tref	121.1	°C	5.140	8.0	9.1	-1.11	-13.8	1.11	13.8
4	25	3	10	123	$D_{121.1}$	14.85	mins	6.533	7.1	7.6	-0.53	-7.5	0.53	7.5
5	25	4	4	123				5.272	8.9	9.0	-0.12	-1.3	0.12	1.3
6	25	3	2	123	H_0	21.22	J/g	7.037	6.7	7.1	-0.38	-5.6	0.38	5.6
7	25	2	3	126		6		1.978	14.8	15.3	-0.49	-3.3	0.49	3.3
8	25	3	2	126				0.597	21.3	23.0	-1.71	-8.0	1.71	8.0
9	25	1	3	126				5.657	8.3	8.5	-0.27	-3.2	0.27	3.2
10	25	10	10	117				15.12	2.2	2.2	-0.02	-1.1	0.02	1.1
11	25	10	10	118				34.57	3.2	2.4	0.75	23.6	0.75	23.6
12	25	10	10	119				91.74	4.7	3.8	0.84	18.0	0.84	18.0
13	25	8	7	121				37.444	8.0	6.8	1.27	15.8	1.27	15.8
14	25	4	5	122				8.698	6.1	5.8	0.31	5.2	0.31	5.2
15	25	5	3	124				2.092	16.0	14.9	1.07	6.7	1.07	6.7
											0.00	2.55	0.61	8.39

Figure 8: F-values calculated from thermocouple temperatures and TTI enthalpies, for reference temperature 121.1°C, z of 6.0C°, decimal reduction time at 121.1°C of 14.85 minutes.

Scatter plot with linear fit: $y = 1.0626x - 0.5123$; x-axis F (t-T), y-axis F (TTI).
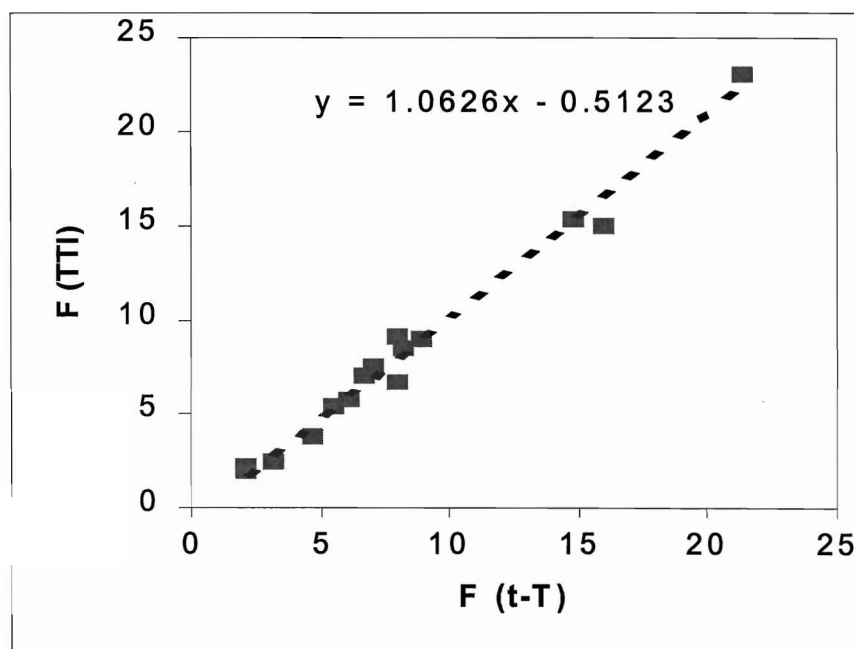
The aim of the canning trials reported here was to measure the F-values in large particulates that were processed under conditions of high axial rotation in a continuous reel and spiral system. Conditions experienced by the process were replicated in the factory simulator, referred to as a bar simulator (FMC FoodTech, Belgium). Twenty DSC capsules were prepared: four to measure the initial amylase enthalpy at CCFRA, one to re-check this after the work was conducted at the factory, and sixteen to insert into the product. The steel DSC capsules were cylinders 8mm in diameter and 3mm in height. A cork borer was used to make a hole to insert them into the frozen sausages and egg balls that were the particulates of concern in the chosen product. Table 11 shows where the TTIs were placed and what F-values were measured from the residual amylase enthalpies. The applied thermal process was less than that given in the factory cookers because of concerns that there would be little amylase activity left.

Table 11: **Location of TTIs, residual amylase enthalpies and calculated F-values for a canned particulate food in a reel & spiral simulator.**

Sample No.	TTI Placement	Enthalpy (J/g)	F-value (min)	Comments
5	Control, no heating	22.60	-	
6	Sausage	0.00	> 12.6	Fell out of sausage
7	Sausage	0.00	> 12.6	Fell out of sausage
8	Egg ball	6.95	2.2	
9	Egg ball	7.81	1.9	
10	Free-moving	0.00	> 12.6	
11	Sausage	0.02	12.6	
12	Sausage	0.00	> 12.6	
13	Egg ball	0.00	> 12.6	Fell out of Scotch egg
14	Egg ball	0.00	> 12.6	Fell out of Scotch egg
15	Free-moving	0.00	> 12.6	
16	Sausage	0.01	13.5	
17	Sausage	0.07	10.5	
18	Egg ball	17.17	0.5	
19	Egg ball	16.62	0.6	
20	Free-moving	0.00	> 12.6	

The TTI F-values indicated that the egg balls were the slowest heating particulates, which was not a surprising finding given their spherical dimensions. Surface area to volume was less favourable than with the sausages. Differences between the measured processes at the centre of the egg balls and the sausages were larger than expected. It is inevitable that measuring processes in particulates will incur a degree of error if temperature sensors are used because of the heat conduction effect along a very short distance. TTIs offer the potential to remove this source of error by being placed entirely inside the particulate.

4.   DISCUSSION OF RESULTS

The examples of process validation and optimisation presented in this report highlight the industrial benefits that were obtained in using TTIs. The common theme with all of the TTI studies was that the processes under investigation were difficult or impossible to validate using temperature probe systems. Table 12 illustrates some of the difficulties that existed with the processes and the benefits obtained from measuring the process values using pasteurisation TTIs.

Table 12:   Difficulties experienced with existing processes and the benefits obtained by using the pasteurisation TTIs to measure process values.

Process Description	Technical difficulty **with** process validation	Method used to validate process safety	Benefit from **TTI** work
Ohmic heating of fruit products	Continuous process with fruit particulates of unknown pathway	Alginate spores with limited products. Micro-tests on end product.	Data on achieved pasteurisation levels. Some processes optimised to increase throughput by up to 20%.
Sprayed water pasteurisation-cooling tunnel for cook-in-sauces	Cold spot in jar not easy to define and depends on many factors.	Temperature loggers that travel with jars, with one measuring point per jar.	Detailed mapping of P-value distribution. Optimisation of filling temperature allowed pre-process temperature to be reduced by 3 C°.
Continuous oven cooking-cooling of poultry pieces	Reformed products too soft to hold probes in place and some too small for precise location. Presence of logger hardware on conveyor belt may interfere with air flow around product.	Micro-tests on end product. Single temperature readings after oven.	Data on achieved pasteurisation levels. Some processes optimised to increase throughput by up to 20%.
Tubular heat exchanger processing of fruits	Continuous process with fruit particulates of unknown pathway	Alginate spores with limited products. Micro-tests on end product.	Data on achieved pasteurisation levels.
In-jar processing of fruit products	Fruit pieces too soft to retain probes. Jars moved between water baths. Jars can be unusual shapes and sizes.	Temperature probes with trailing wires.	Data on achieved pasteurisation levels with greater certainty in TTI location at cold spot.
Sous-vide processing of ready meals	Vacuum-packed in plastic film. Packs moved between water baths.	Alginate spores with limited products. Micro-tests on end product.	Data on achieved pasteurisation levels with greater certainty in TTI location at cold spot. No loss in vacuum when TTIs used.
Surface pasteurisation of hot-fill sauces	Cannot measure surface temperatures with commercial logger systems.	Substantial overprocessing.	Data on achieved pasteurisation levels. Scheduled process parameters challenged.

The breakthrough that enabled these complex processes to be evaluated was in encapsulating the amylase solutions in silicone tubes. When stored in chilled water to minimise the rate of amylase degradation, the TTI tubes could be used for up to 14 days, with reports of one company in the project finding minimal loss in activity after 1 month. Chilling the amylase increases the scope for applying TTIs to factories overseas. To further extend their useable shelf life, filled TTIs can be frozen in large numbers, and TTI tubes removed as and when required. Freezing has little impact on amylase structure or on the rate at which its structure degrades by heat. It is conceivable that several hundred TTIs could be made at one time, frozen individually, and used over a period of months. This would be an economical method for producing TTIs and would ensure that the kinetics for each tube would be similar. Studies are ongoing to confirm the useable shelf life when held at -12°C for greater than 2 months.

Another application that was investigated towards the end of the project was in using TTIs to measure the achieved pasteurisation during microwave reheating of foods. This is an application where existing technology cannot provide a good solution because of two primary hurdles: (1) metallic probes cannot be used in a microwave environment, so expensive and delicate fibre-optic probes are needed, and (2) the turntables with most modern microwave ovens prevent the use of fibre-optic probes. Current best practice is to use static microwave ovens with fibre-optic probes entering through large holes and to assume that these oven types will heat less effectively than those with turntables. There is some evidence to support this theory, but it would be better to have a method that is not restricted to static ovens. Feasibility studies with TTI tubes shielded from the microwave energy with aluminium foil gave P-value agreement with fibre-optic measurements. TTIs gave slightly higher P-values (5-15%) than fibre-optic probes when the TTIs were not shielded. This application for TTIs is potentially immense and should be studied in greater depth.

5. CONCLUSIONS AND FUTURE WORK

Much of the TTI work considered food processing systems where the food packages or particulates flowed from raw material preparation through to final product labelling. Significant advances were made in better defining many processes of interest to the companies that were part of the project. However, there were instances where the TTI methods were not ideal and in some cases they did not work effectively. To be able to use TTIs correctly in flow situations, it will be necessary to enhance our understanding of the flow behaviour of the TTI particles and of food fluids in realistic situations. This will ensure that the values recorded by the TTIs are fully representative of the process and can be used in process design and specification. Particles must be designed so that:

- they can be correctly introduced to the processing system,
- the trajectory of the TTIs closely follows the flow patterns of the process fluid or particulates,
- flowing TTI particles pass through all parts of the flow channel or process vessel,
- the TTI particles can be recovered from the food systems, and,
- the TTI encapsulation method prevents cross-contamination from the food.

These are non-trivial issues that do not have a common solution for all food applications. For example, Sylgard 184 (silicone) encapsulating and moulding compound has a density close to that of water, which is ideal for many food applications. However, work on high sugar solutions requires a density closer to $1,400 kg.m^{-3}$ to prevent the TTI particles from floating and travelling through a continuous processing system at a different rate to the target food particulates.

Although the pasteurisation TTI was developed successfblly, major experimental difficulties were encountered in controlling the sterilisation TTI in a process. The approach was to enhance heat stability by drying the amylase, but the encapsulation method (in a steel DSC pan) did not provide adequate isolation when used in industrial sterilisation processes. One proposed route to find a TTI with greater application is to extract amylase from microorganisms of high heat stability. These are referred to as thermophiles or hyperthermophiles (e.g. Pyrococcus woesei, *Pyrococcus* furiosus) and represent a new commercial area for microbiological research. Amylases from these microorganisms must be heat stable in order to hydrolyse starches in their favoured environmental conditions (Leuschner and Antranikian, 1995; Vielle and Zeikus, 2001). This route may allow an amylase solution to be developed for sterilisation work with suitable kinetics to mimic destruction of the target microbial species: *Clostridium botulinum* spores. Methods for culturing the organisms at 100 °C and extracting the amylase were given in Koch et al. (1990) and Koch et al. (1991), together with some data that suggested that the amylase heat stability was very close to that required.

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