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Time-temperature integrators for validation of thermal processes

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TIME TEMPERATURE INTEGRATORS FOR VALIDATION OF THERMAL PROCESSES

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Summary

A process validation method has been developed using enzymes as time temperature integrators (TTIs): α-amylases from *Bacillus amyloliquefaciens* and *Bacillus licheniformis*. The kinetics of these TTIs were tested and confirmed as suitable for use in monitoring pasteurisation processes ($D_{85} = 6.8$ minutes, $z = 9.4$ C° and $D_{93} = 8.8$ minutes, $z = 9.1$ C° for the two enzymes respectively).

Extensive validation tests were carried out in order to assess the accuracy of calculated pasteurisation values (P-values) measured with TTIs compared with those from thermocouples. The results showed that the accuracy of measurement increased with minor alterations in the method of encapsulation of the TTIs. Heating trials in a stirred pan of water resulted in mean B-values for the two methods within 6.8%. This confirmed that the TTIs were a suitable means of validating thermal processes with sufficient accuracy.

Industrial processing trials using the TTIs in a number of different applications highlighted substantial safety margins in several of the processes tested. The TTIs can be applied to any process for foods that contain liquids and/or solids and, with a large number of replicates that can be quickly analysed, say 50-100, provide data on the distribution of process values achieved.

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1. Introduction

With the increasing demand for new and higher quality food products, industries must focus on optimising their processes further in order to maximise the throughput of products. One area investigated is the thermal process, where the aim is to apply the minimum amount of heat treatment required to destroy or at least inhibit the growth of pathogenic and spoilage micro-organisms - leaving a product that is safe and preserved to give a longer shelf-life. To ensure that this objective is achieved, the thermal process must be fully monitored. Conventional validation methods used to collect data are not always appropriate for the form in which the product is being produced. This often leads to over processing in order to compensate for any inaccuracies, and will ensure that the product is always safe.

The work reported here was based on that previously published by Hendrickx et al (1992-1997) and Van Loey et al (1992-1997), where a number of detailed studies were done on the theoretical development, applications and limitations of TTI systems. The TTIs studied were Bacillus spp. amylases, peroxidases and nitrophenol glucoside (NPG). Their investigations reported on the thermal inactivation of these enzymes in different model food systems such as pasteurisation of packs in a water cascading retort and processes employing end-over-end rotational mixing of packs. The experimental procedures were carried out using both large (25 mm) silicone particles and immobilising the enzymes on miniature (2 mm) glass beads and in many cases included both isothermal and non-isothermal inactivation experiments. This report focuses on the practical adaptation and application of this work.

The development of the time temperature integrator (TTI) particle offers an alternative means to thermocouples or microbiological methods of tracking the time-temperature history during thermal processing. The TTI particle also has the advantages of being safe, relatively low priced, easy to prepare and analyse, and can be manufactured in a form that gives a good representation of the food particle itself.

The objective of this work was to develop the TTI particle to enable it to be used in a wider range of processes and products including solids and liquids. This involved experimenting with new methods of encapsulating the enzyme and investigations to calculate the exact destruction kinetics of the enzymes. The TTI particles were used to mimic the slowest heating region in the products tested and allowed the pasteurisation or sterilisation values to

be calculated. This information was then used to improve and optimise the processes, where appropriate.

1.1 TTI systems

A TTI is defined as 'a small measuring device that shows a time-temperature-dependent, easily, accurately and precisely measurable in-eversible change that mimics the changes of a target attribute undergoing the same variable exposure' (Taoukis & Labuza, 1989).

The TTIs studied were of a biological nature and based on the thermal inactivation of enzymes. As the enzyme is heated, the bonds holding the structure of the molecule begin to break and the protein macromolecule unfolds. This level of structure change is dependent upon the level of heat treatment it receives. Reacting the enzyme with a reagent after processing gives an indication of the amount of activity the enzyme has lost, which can then be related to a pasteurisation value.

The use of a TTI can eliminate a number of problems that are encountered when using conventional equipment such as rigid temperature/thermocouple probes to collect processing data. Probes can sometimes be unsuitable for monitoring temperatures inside sealed containers holding products such as sauces that contain small particulates such as rice suspended in them. Although the rice grain is only *2-5* mm in size, there will be a thermal lag at the grain core, which will result in a. lower integrated lethality than that of the surrounding liquid. It is essential that the thermal process is established at the grain core and therefore an alternative method to probes is needed. The trailing wires associated with probes can also cause problems when agitated vessels or heat exchangers are employed for processing particulate materials. A method that does not suffer from these problems is the alginate spore technique, in which the spores of a non-pathogenic organism are dispersed in an alginate gel to mimic food particles and then put through the process. However, this method can be expensive and involves deliberately introducing bacterial spores into the processing environment, which could be perceived as a safety issue and causes concern with some food processors. The TTI particle, however, is inexpensive and does not contain materials that create a safety issue.

1.2 Heat Treatments

The purpose of the pasteurisation and sterilisation treatments is to destroy or reduce the numbers of the likely pathogenic and spoilage microorganisms to an acceptable level (DH, 1994). Table 1 shows some examples of the types of processes and the target treatments that should be achieved in order to prevent spoilage and to produce a final 'safe' product.

Process Type	pH	Time/temperature Equivalent	T_{ref} (°C)	z-value (C°)
Cook–chill	>4.5	2 minutes at 70 "C	70.0	10.0
Sous Vide	>4.5	40 minutes at 70 "C	70.0	10.0
Psychrotrophic bot.	>4.5	10 minutes at 90 °C	90.0	10.0
Acid Fruits	3.7-4.2	5 minutes at 85 °C	85.0	10.0
Tomato Products	4.0-4.3	5 minutes at 93.3 °C	93.3	8.3
Tomato Products	>4.3	10 minutes at 93.3 °C	93.3	8.3
Sterilisation		3 minutes at 121.2 °C	121.1	10.0

Table 1. Selected treatments recommended for thermally processed foods (CCFRA, 1992)

In industry, these time/temperatures are used as a minimum guideline and are usually exceeded by 2-3 times the stated integrated process value. Therefore, the enzyme inside the TTI must have a sufficient decimal reduction time to allow it to undergo up to 2 log reductions with enough residual activity to give accurate results when the enzyme is assayed.

The only other criterion that the TTI has to meet in order to be successful is that the temperature sensitivity of the rate constant of the TTI and the targeted microbial species need to be similar. This is referred to as the z-value. Therefore, the enzymes chosen to represent the TTIs were amylase enzymes from Bacillus *amyloliquefaciens* and Bacillus *licheniformis*, with z-values of 9.4 and 9.1 C° respectively. A number of studies by Hendrickx et al. (1992-1997) and Van Loey et al. (1992-1997) have shown these enzymes to be successful in monitoring pasteurisation processes. Another advantage was that their reaction rates were

found to be minimal at ambient temperatures and almost non-existent in chilled conditions. This meant that no special transportation arrangements between the laboratory and the processing environments were required.

1.3 Mathematics of the Kinetics of Heat Destruction

The mathematical model that relates the thermal destruction rate to time and temperature can be described by the first order kinetics thermal inactivation equation:

$$N = N_o \, e^{-k.t} \qquad [1]$$

Where, N_o is the initial level of a quality attribute,
N is the actual level of the quality attribute,
k is the reaction rate constant at the constant lethal temperature (T),
t is the processing time.

From this expression, the decimal reduction time (D_T) can be derived. D_T is defined as the time required at a constant temperature (T) to reduce a microbial population or a chemical reaction rate by a factor of ten. The relationship between the reaction rate constant and D_T is written as:

$$D_T = \ln (10) / k \qquad [2]$$

Combining these two equations gives an expression to access the effect of the heat treatment on a quality attribute.

$$P = D_T \log (N_o / N) \qquad [3]$$

The pasteurisation value (P-value) is the time required at a constant lethal temperature to reduce the level of the attribute in question from an initial level N_o to a final level N. The N values for the amylase TTIs can be expressed as a change of absorbance per unit time that refers to the enzyme activity when measured by a spectrophotometer.

An alternative method to find the P-value, which will give the same results providing that the z-value of the TTI matches exactly that of the microbial species, can be calculated from the time temperature integration.

$$P = \int_0^t 10^{\frac{T-T_{ref}}{z}} \cdot dt = D_T \cdot \log\left(\frac{A_{initial}}{A_{final}}\right) \qquad [4]$$

Where, A is the change of absorbance per unit time,

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

2. Methods

2.1 Enzyme Preparation and Encapsulation

Sigma Chemicals (EC 3.2.1.1 Type II-A, Sigma A-6380) supplied both the *Bacillus licheniformis* and *Bacillus amyloliquefaciens* amylase enzymes in a dried form for extended shelf life. For use in all of the applications, the enzyme was made up at 10 mg/ml using a Tris buffer solution (pH 8.5 at 25°C). This buffer stabilised and maintained the pH of the enzymes whilst they were being used as a TTI.

The silicone cube particles were prepared according to the procedure of Tucker (1999). Particles of sizes 5 mm, 10 mm and 14 mm were used for the trials. Previous work had shown that the physical/thermal properties of the Sylgard 184 silicone were of a similar order in magnitude to those of food particles, and so could be used for good representation during the thermal processing.

Another form of encapsulation was also employed using PTFE tubes. To enclose the required amount of amylase (0.015 ml), two tube sizes were used: 1.0mm diameter by 24.0mm length and 3.0 mm diameter by 8.0 mm length. One end of the tube was sealed off by dipping it into uncured Sylgard 184 elastomer (Dow Coming), and allowing capillary action to create a 2-3 mm plug, then heating the tube at 70°C for a short time to cure. Amylase was injected into the tubes using a hypodermic needle, and the opposite end of the tube sealed off using a larger needle to inject the Sylgard to form another 2-3 mm plug. The tubes were then cured in an oven set at 40 °C.

After some problems with leakage, this encapsulation method was improved by using silicone tubing in place of the PTFE tubing (see section 2.3.3). This tubing was a high strength silicone with dimensions of: 2 mm bore, 0.5 mm wall and 2.5 mm bore, 0.5 mm wall. The finished TTI tube was 7-8 mm in length.

To measure the activity of the enzymes after they have been exposed to a heat treatment, 0.01 ml of the enzyme was diluted with 0.29 ml of Tris buffer. 0.02 ml of this solution was added to 1 ml of Sigma Chemicals amylase reagent pre-equilibrated at 30°C, and mixed by inversion. The change of absorbance was measured over a short period of time in a Unicam

spectrophotometer set at 405 nm to give the enzyme activity. The P-values were then calculated from the initial activity values taken from an unheated amylase sample and the final activity values from the processed samples, using equation [4].

2.2 Isothermal Inactivation Kinetics of *Bacillus amyloliquefaciens*

A series of heating trials were performed to determine the kinetics of the *Bacillus amyloliquefaciens* α-amylase. To do this, the amylase was sealed inside glass tubes (approx. length 10 mm, internal diameter 2 mm). The glass had a minimal effect on the heat transfer to the enzyme due to the thin walls. The tubes were immersed in an oil bath set at the exact temperature required to measure the decimal reduction time. At chosen time intervals, two tubes were removed for analysis. The amylase was then extracted and tested for activity using the spectrophotometer. The log of the activity rate was plotted against heating time to give a gradient of $1/D_T$, as shown in Figures 1 to 4.

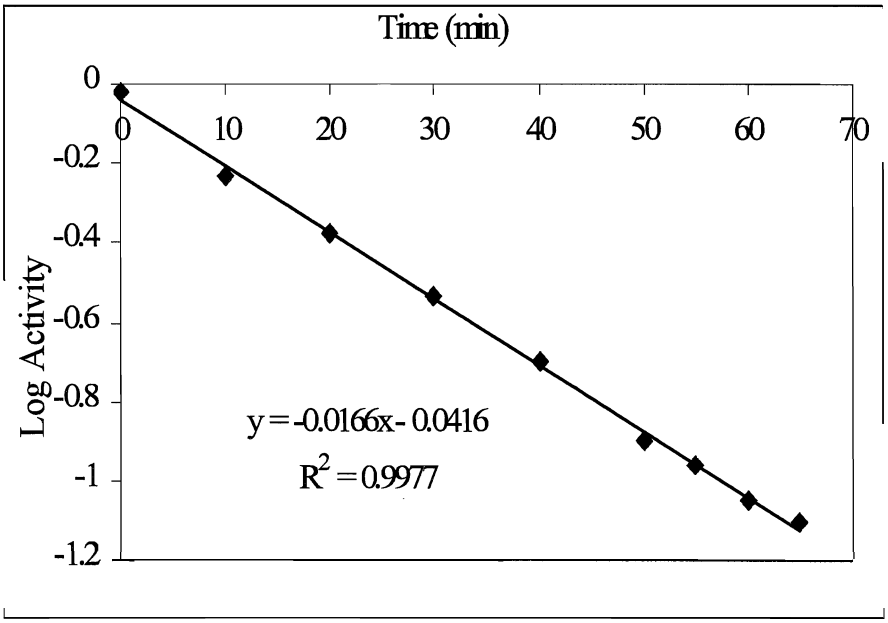

Figure 1. Effect of heating *B. amyloliquefaciens* amylase at 76 °C on the enzyme activity.

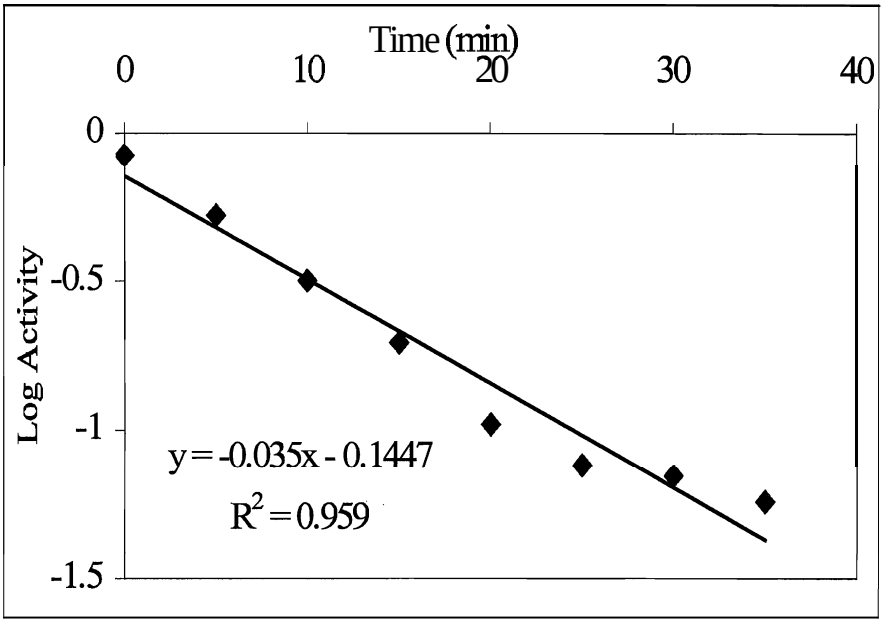

Figure 2. Effect of heating B. *amyloliquefaciens* amylase at *79* °C on the enzyme activity.

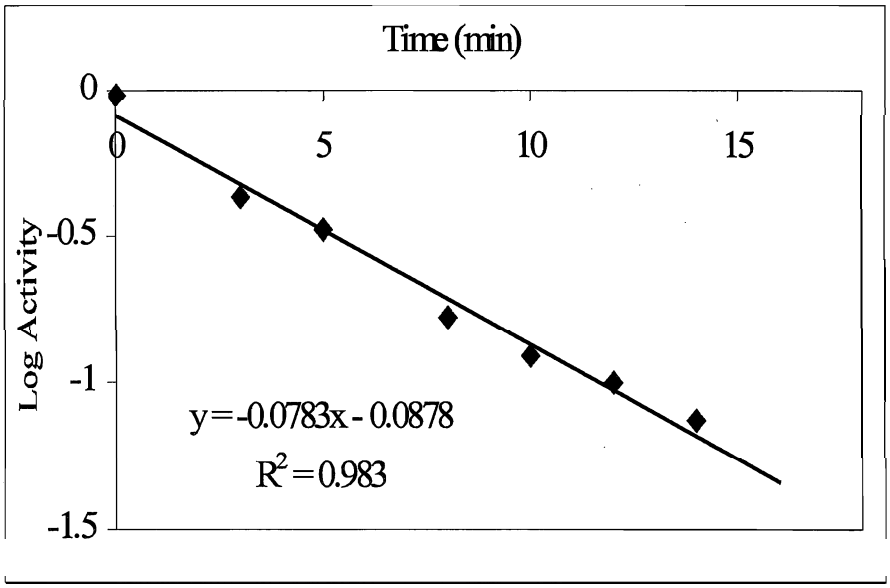

Figure 3. Effect of heating B. *amyloliquefaciens* amylase at *82* °C on the enzyme activity.

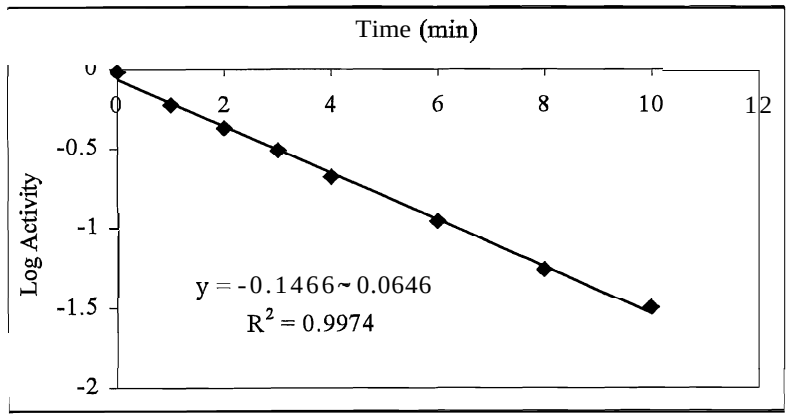

Figure 4. Effect of heating B. *amyloliquefaciens* amylase at *85* °C on the enzyme activity.

Reference Temperature (°C)	Decimal Reduction Time (minutes)
76	60.2
79	28.6
82	12.8
85	6.8

Table 2. Summary of the decimal reduction times for B. *amyloliquefaciens* amylase.

The log of these D_T values was plotted against their respective temperatures to give a z-value of 9.4 C°. (z = -1 / gradient)

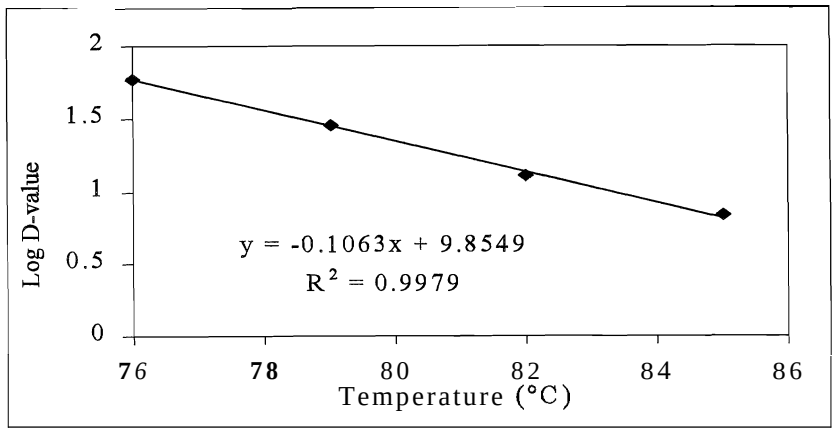

Figure 5. Effect of heating temperature on the D-value of B. *amyloliquefaciens* amylase.

Similar work was carried out on the *Bacillus licheniformis* amylase to give a Decimal reduction time of 8.8 minutes at a reference temperature of 93 "C (Figure 6) and a z-value of 9.1 C° (Figure 7).

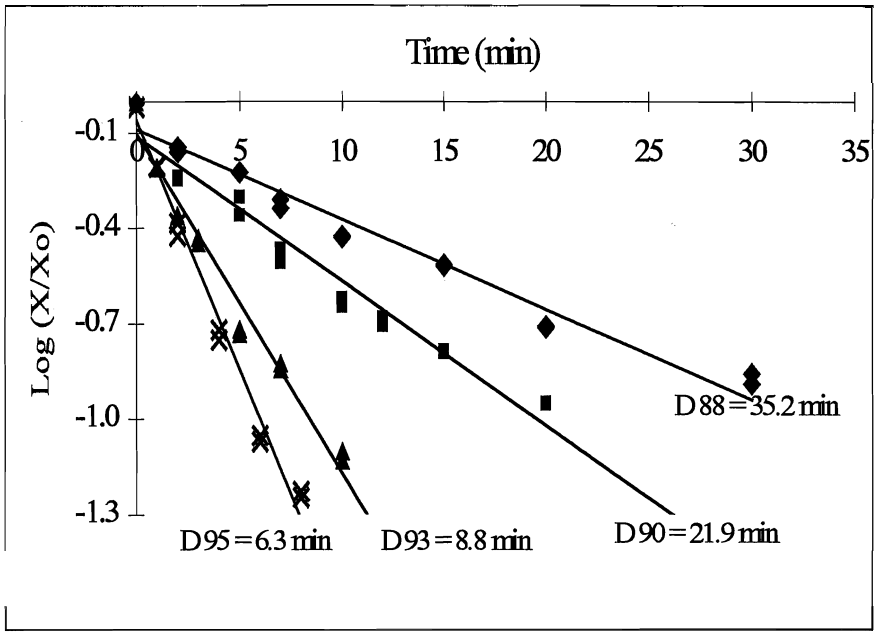

Figure 6. Effect of heating B. *amyloliquefaciens* amylase in the temperature range 88 – 95°C on the enzyme activity.

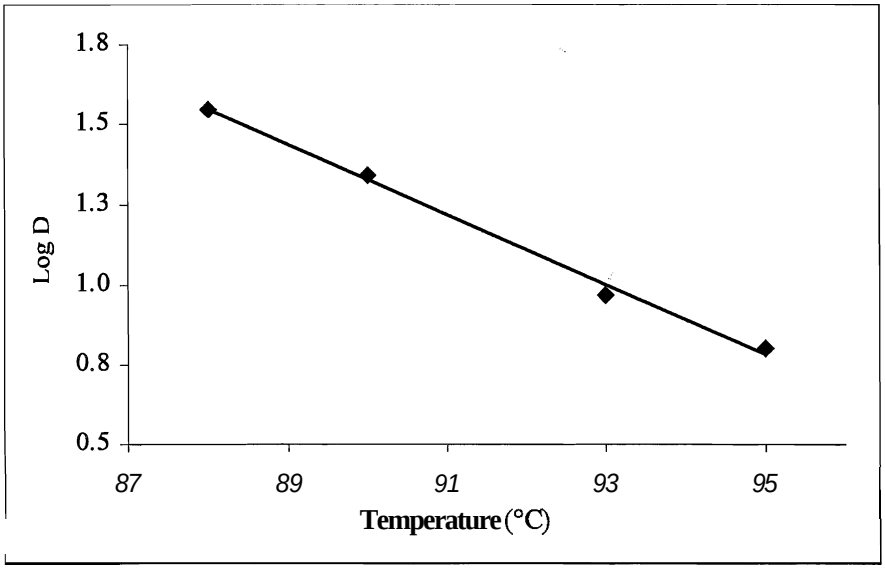

Figure 7. Effect of heating temperature on the D-value for inactivation of B. licheniformis amylase.

2.3 Verification of the TTIs against Thermocouples and Models

In order to assess the accuracy of the pasteurisation values determined using the TTIs when compared with values calculated from measured or predicted temperature data, a number of trials were conducted.

2.3.1 Feasibility Study using TTIs and Thermocouples in a Stirred Pan

The first set of experimental trials used 0.20 mm diameter copper-constantan cable (type T) thermocouples against P-values measured by 14 mm cubed encapsulated TTIs. The uncertainty with the cables was in the effects of heat conduction at stages where the temperature driving force was high, such as the early stages of heating and cooling. The latter stages were of more concern in terms of the integrated lethality. The results were compared with a predictive model fiom CCFRA's PCTemp program (McKenna and Tucker, 1991) for thermal conduction within a food particle that assumed a heat transfer coefficient on the cube surfaces of greater than 5,000 $W.m^{2}.K^{-1}$, i.e. effectively no resistance to heat transfer. Predictions of lethality fiom PCTemp would be accurate provided the thermal properties of the silicone were measured with sufficient accuracy.

Silicone particles sized 14 mm cubed were prepared with a bubble sphere of a minimum volume 25 µl in the centres, according to a previously published procedure (Tucker 1999). Thermocouples (connected to a Grant Squirrel data-logger) were inserted into 5 of these particles with the centre of their junction situated in the midpoint of the particle. The remaining particles were filled with the enzyme *Bacillus amyloliquefaciens* α-amylase. All of these were placed into a pan of water set at a specified temperature and held for a period of time. During this time the water was continuously stirred and controlled at the set temperature which was monitored by a further 2 thermocouples that were not encapsulated. At the end of heating, all of the particles were removed fiom the water at the same time and immediately placed into a cold water bath to prevent further denaturing of the enzyme. A number of runs of this procedure were completed with the teniperatures and holding times given in Table 3. After each run, the enzyme was extracted and analysed following the standard procedure.

Run 1	85 "C, 5 minutes heating and holding time.
Run 2	80 "C, 13 minutes heating and holding time.
Run 3	80 "C, 25 minutes heating and holding time.
Run 4	80 "C, 25 minutes heating and holding time.

Table 3. Details of experimental conditions to test agreement between thermocouples and TTIs.

2.3.2 Feasibility Study using TTIs and Ellab Thermocouples in a Steam Retort

Industrial trials were done to test the validation of TTIs using the 8 mm length PTFE tubes. The TTIs and probes were processed in cans filled with water. Some cans having the particles secured to the probes at the coldest point using a silicone sealant and others with the TTI particles unrestrained and free to move about as the cans rotated. Three runs were completed with the TTI positioning and processing temperatures as shown in Table 4. For the first two runs, the process target was an end of cooling P-value of 7 minutes; for the third run the target was a P-value at end of heating of 10 minutes with a total of 12 minutes.

Run 1	Rotational Speed 5 rpm, temperature 95 °C, P of 7 min
A1	3 TTIs unrestrained with probe in centre of can
B1	3 TTIs fixed to probe in centre of can
Run 2	Rotational Speed 5 rpm, temperature 85 °C, P of 7 min
A2	3 TTIs unrestrained with probe in centre of can
B2	3 TTIs fixed to probe in centre of can
Run 3	Rotational Speed 5 rpm, temperature 95 °C, P of 12 min
A3	3 TTIs unrestrained with probe in centre of can

Table 4. Details of experimental conditions to evaluate P-values measured by thermocouples and TTIs in can of water processed in a steam retort.

2.3.3 Feasibility Study using TTIs and Ellab Thermocouples in Retorts with End-Over-End Rotation

An improved form of encapsulation was undertaken following the work performed using the retort. A series of heating and rapid cooling trials in water containing a dye had identified that the PTFE tubes were leaking. This was likely to have been caused by the pressures experienced inside the tubes and a difference in thermal expansivity values between the PTFE and the Sylgard plugs at either end. In order to eliminate this problem, high strength silicone tubing was purchased and used to encapsulate the enzyme in place of the PTFE. The new tubing had dimensions: 2 mm bore, 0.5 mm wall and 2.5 mm bore, 0.5 mm wall and had a thermal expansivity value close to that of the Sylgard.

Using this new tubing, a trial similar to the previous one described in 2.3.2 was conipleted using three UT sized cans (73 mm diameter, 115 mm height) of water in one run with the retort operating at 95 °C and a rotation of 5 rpm. The cans were prepared with Ellab probes with TTIs secured to them with a small amount of silicone sealant approximately 5 mm from the tip. Although this meant that the TTI was not in the exact centre of the can, it prevented the silicone from insulating the measuring point on the probe which may have affected the results in the previous trial. Four TTIs were also placed in each can and left unrestrained. Details of the cans are given in Table 5.

Can A	2 Probes, 1 TTI fixed to each probe, 4 unrestrained TTIs
Can B	1 Probe, 2 TTIs fixed to the probe, 4 unrestrained TTIs
Can C	1 Probe, **2** TTIs fixed to the probe, 4 unrestrained TTIs

Table 5. Details of can preparation with TTIs processed with end-over-end rotation.

2.3.4 Feasibility Study using TTIs and Ellab Thermocouples in Jars of Water Heated in a Water Bath

Having improved on the method of encapsulation, further validation tests were carried out using silicone tubes prepared with 15 µl of B. *amyloliquefaciens* α-amylase. Two tubes were attached approximately 5 mm from the tip of Ellab probes using a silicone sealant. The Ellab probes were positioned through the lids to the centre of 3 glass jars containing 450 g of water.

The jars were processed in a static water bath set at 85 "C. When an P-value of 6 had been achieved (indicated by the data logging equipment), the jars were removed from the water and cooled first in water at 45 "C, to prevent the glass from breaking, and then in ambient temperature tap water. Once cooled, the TTIs were removed and assayed following the standard procedure.

A repeat of this work was done using 4 jars of water, with all other operating conditions remaining the same.

2.4 Contamination by Chemicals that Affect the Amylase Heat Degradation Rate

As a result of the validation trials described in 2.3.4, work was carried out to determine the reasons for differences in P-values measured with TTIs and thermocouples. The difference was evident when silicone sealant had been used to secure the TTIs on to the tips of the probes. By covering a number of TTI tubes with two different types of silicone sealants and heating the tubes in a water bath, the possibility of the silicone having an effect on the enzyme was tested and compared to the control TTI tubes.

2.5 Distribution of P-values in Stirred Vessels

One of the potential applications for TTIs is to measure P-values in large agitated vessels, used for heating and cooling viscous particulate foods. Conventional temperature probing of these vessels is not straightforward due to the presence of the agitator, the thermal boundary layers that develop adjacent to the heated surfaces, and significant temperature distributions within the viscous food materials. To evaluate the magnitude of these temperature distributions, trials were carried out at CCFRA in a 250 litre stirred vessel (Winkworth), using 50 silicone particles (size 10 mm cubes) filled with 25 ml of a *Bacillus licheniformis* amylase solution. The particles were placed in a 200 litre batch of 5 wt% Colflo 67 starch solution at 90 "C and held at this temperature for 15 minutes before being removed from the starch by rapidly draining the batch through a sieve. The recovered particles were immediately placed into cold water to prevent any further denaturation of the amylase. The amylase was then extracted and tested for its remaining activity.

Recovery of the particles took approximately 5 minutes, with those captured last spending this time immersed in the hot stirred starch solution and those in the sieve being washed with the same solution. The difference in heat transfer between the immersed and captured particles should have been insignificant, because of favourable surface heat transfer conditions in both cases. Thus, the distribution of P-values represented a heated batch with a hold time at a constant temperature.

3. Results

3.1 Feasibility Study Using TTIs and Thermocouples in a Stirred Pan

Four trials were completed with various heating times and temperatures, the pan of water being continuously stirred throughout the heating procedure. Runs 1 and 2 showed that there was close agreement between thermocouple and TTI measured P-values, but run 3 showed mean P-values for TTIs that were 50% greater than those measured by the thermocouples. This result was unexpected and so the run was repeated using identical processing conditions which gave comparable results, as shown in Figure 8.

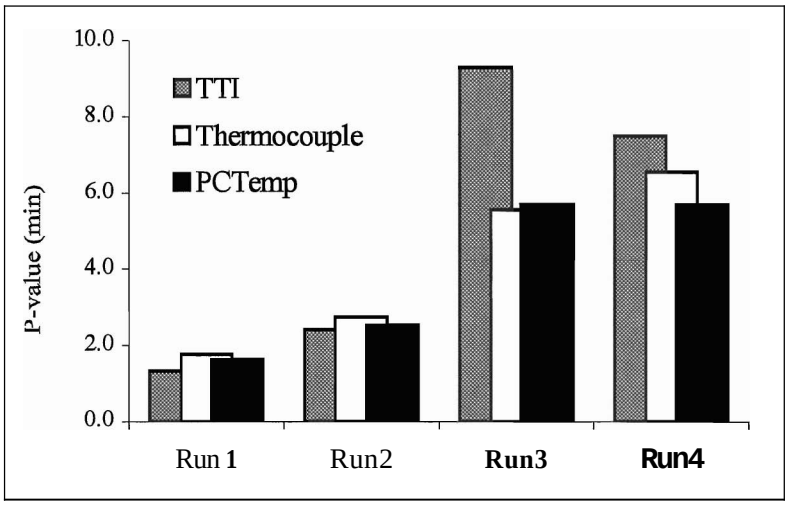

Figure 8. Comparison of P-values calculated from thermocouples and TTIs from trials in a stirred water bath.

The modelled data created by CCFRA's PCTemp program (based on the parameters given in Table 6) for 14 mm silicone cubes gave results in good agreement with those obtained experimentally (McKenna and Tucker, 1991). The PCTemp program was used to provide further evidence that the P-values measured by TTIs and thermocouples were correct, and to explain the slightly lower TTI P-values, thought to have been caused by thermal conduction along the thermocouple wires when the temperature driving force was high.

Initial temperature	22	°C
Mass flow rate	20	$kg.min^{-1}$
Slip velocity	2	$m.s^{-1}$
Thermal conductivity (TTI)	0.134	$W.m^{-1}K^{-1}$
Density (TTI)	1050	$kg.m^{-3}$
Heat capacity (TTI)	1160	$kJ.kg^{-1}K^{-1}$
Thermal conductivity (fluid)	0.6	$W.m^{-1}K^{-1}$
Density (fluid)	1000	$kg.m^{-3}$
Heat capacity (fluid)	4200	$kJ.kg^{-1}K^{-1}$
Consistency coefficient k	2	$Pa.s^{n}$
Flow behaviour index n	0.35	

Table 6. Thermal and physical properties used to model P-vales using PCTemp.

3.2 Feasibility Study using TTIs and Ellab Thermocouples in a Steam Retort

As shown by the results in Table 7, all of the P-values calculated from the TTIs were higher than those measured by the thermocouples, and the range in each run was unacceptable. It was later found that one of the possible reasons for this was the particles leaking and taking in water. This caused the enzyme to become more dilute and reduced its activity, which in turn resulted in higher P-values. Tube leakage was due to differences in the thermal expansivity of the Sylgard plugs and the PTFE tubing. Also poor adhesion of the Sylgard plugs onto the PTFE, which is known for its non-stick properties. The effects were enhanced by the changes in pressure that the tubes undergo during the heating and cooling cycles in the retort. Improvements were made on all further experimental trials by replacing the PTFE with a high strength silicone tubing, compatible with the Sylgard plug.

Run 1	**P-values (minutes)**	**Average**
Fixed	6.6, 10.5, 17.1, 17.1, 18.0, 17.1	14.4
Unrestrained	18.0, 17.1, 18.6, 16.7, 14.4 16.0, 17.1, 18.6, 19.3, 19.3	17.5
Thermocouple probes	7.1, 7.2, 8.6, 8.7, 8.5, 8.6	8.1

Run 2	P-values (minutes)	Average
Fixed	12.5, 10.9, 15.7, 14.4, 14.6, 14.4	13.8
Unrestrained	16.0, 15.7, 12.0, 14.6, 16.0, 7.4 8.5, 15.1, 13.0, 16.3	13.5
Thermocouple probes	5.8, 5.7, 5.8, 5.8, 5.8	5.8

Run 3	P-values (minutes)	Average
Fixed	16.7, 18.0, 19.3, 20.2, 20.2	18.9
Unrestrained	21.3, 21.3, 18.6, 21.3, 20.2, 22.8 20.2, 22.8, 22.8, 21.3	21.3
Thermocouple probes	12.3, 12.3, 12.3, 12.5, 12.5	12.4

Table 7. P-values measured by TTIs and thermocouples after processing in a steam retort at 95°C rotating at 5 rpm.

3.3 Feasibility Study using TTIs and Ellab Thermocouples in Retorts with End-Over-End Rotation

In each can, two TTIs were fixed to the probe tip and four TTIs were left unrestrained, with three replicate cans in one run. The Ellab thermocouples gave P-value readings of 6.4, 6.7 and 6.8 minutes, all in close agreement. The TTIs that were free to move about the can gave results that were in close agreement with these (Figure 9), but the TTIs secured to the probes gave higher P-values. In one case, the TTI gave a result that was more than 3 times larger than the probe P-value to which the TTI was secured. This suggested that the silicone was having an effect on the enzyme, causing the activity to be reduced by an effect other than heating (see section 3.5).

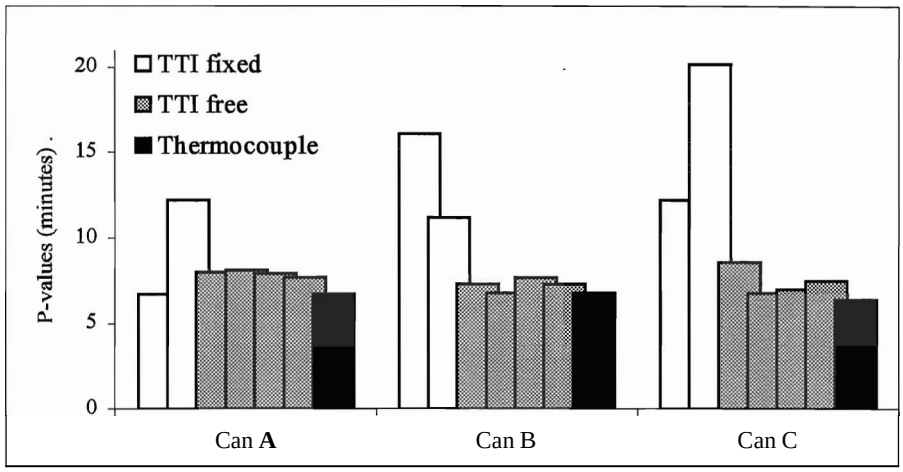

Figure 9. P-values measured by thermocouples and TTIs in cans of water processed with end-over-end rotation.

3.4 Feasibility Study using TTIs and Ellab Thermocouples in Jars of Water Heated in a Water Bath

When the TTIs were analysed from the initial run of this trial, little activity remained, indicating that the P-values were high. When calculated, the P-values were found to range between 5 and 14 minutes and the Ellab thermocouple probes measured 7.5 to 8.5 minutes, as shown in Figure 10. Because of this inconsistency in results in the TTIs, the trial was repeated using 4 jars of water heated under the same conditions to reduce experimental errors from these results.

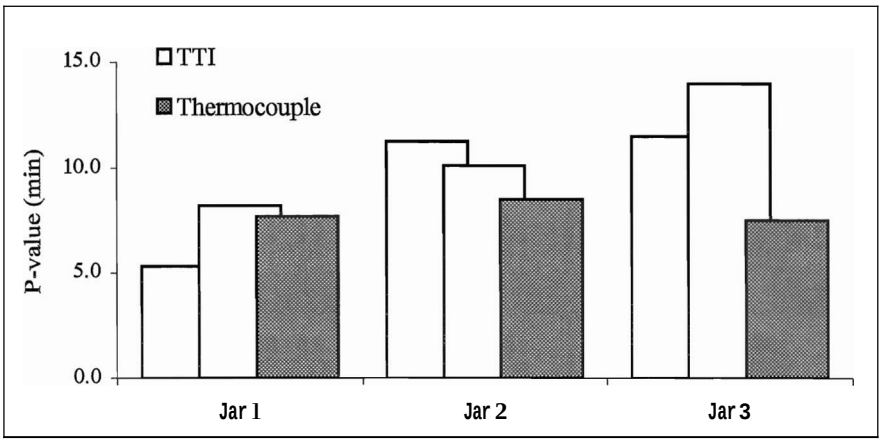

Figure 10. Run 1 results from validation tests carried out in jars of water with TTIs and thermocouples measuring the process lethality.

For the second run (Figure 11), the end of heating was when the probes had reached a P-value of *6* minutes. The final P-value measured by the probes was *6.2* to *6.6* minutes. The corresponding TTIs were again generally much higher and in Jar 2 the TTI gave a P-value that was more than 50% higher. These results indicated that something was affecting the performance of the enzyme and causing a decrease in its activity.

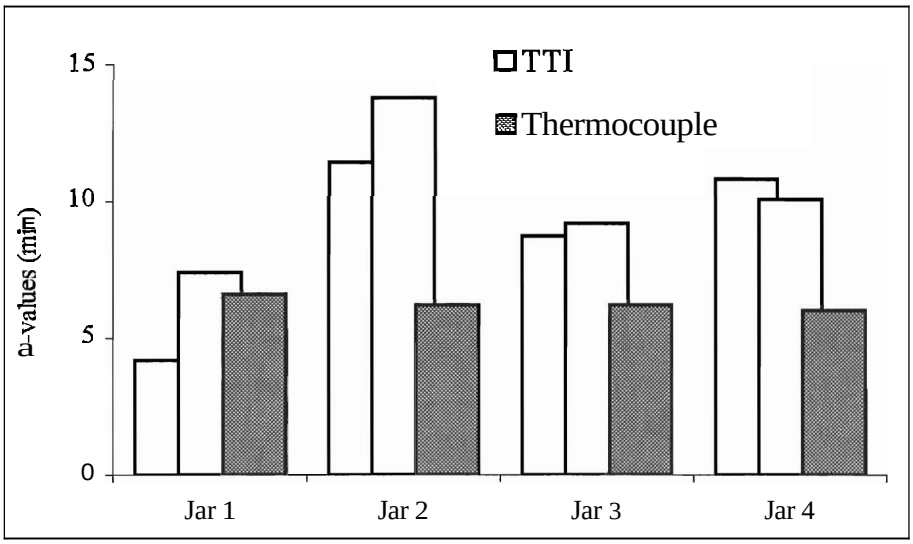

Figure 11. Run 2 results from validation tests between TTIs and thermocouples processed in jars of water in a water bath.

3.5 Contamination by Chemicals that Affect the Amylase Heat Degradation Rate

From the previous sets of trials, it was observed that when the tubes had been secured to the Ellab probes using silicone sealant, the calculated P-values were too high. This was most obvious in the trial with the cans of water processed with end-over-end rotation in the steam retort. TTIs that had been free to move about in the can gave P-values close to those measured by the thermocouples. By coating TTIs with different sealants and comparing the P-values to those from control TTIs, the following results were obtained (Table **8).**

P-values (minutes) measured by different methods			
THERMO-COUPLES	CONTROL TTIs (FREE TO MOVE)	TTIs COATED WITH R.S. SILICONE	TTIs COATED WITH SILICONE SEALANT
	6.1	15.5	15.0
6.8	6.7	18.5	17.8
8.1	6.8	22.0	23.1
	7.1	19.4	19.4

Table 8. P-values measured and compared between thermocouples and TTIs coated with different silicone sealants.

The TTIs that were left untreated and used as controls gave P-values that were consistent and in close agreement with those measured by the thermocouples. The tubes that had been coated with the two types of sealant gave P-values that were up to nearly three times higher. This was thought to be because of the release of acetic acid from the silicone in high concentration as the sealant cured. The acetic acid must have diffused through the thin (0.5 mm) wall tubing to alter the pH of the amylase solution which increased the rate of enzyme degradation and hence lowered the activity. For all further work where TTIs needed to be secured into position it was recommended that a superglue be used, or a sealant that did not release acetic acid in its cure.

High P-values were a common occurrence whenever the silicone sealant had been used, with the TTIs that had been secured by other means or left unrestrained giving P-values consistent with those measured by thermocouples. Industrial trials to measure P-values in acidic food products have not shown this activity reduction, presumably because the acid concentration was not sufficient to diffuse through the 0.5 mm silicone tubing in the timescale of the experiments. The results of these trials are reported in section 4.3.

3.6 Distribution of P-values in Stirred Vessels

Within the 200 L stirred vessel, a range of P-values were obtained, as shown in Figure 12. This was thought to be due to the temperature distribution within the vessel. The highest P-value achieved was 11.3 minutes and the lowest 1.2 minutes with an average of 3.9 minutes.

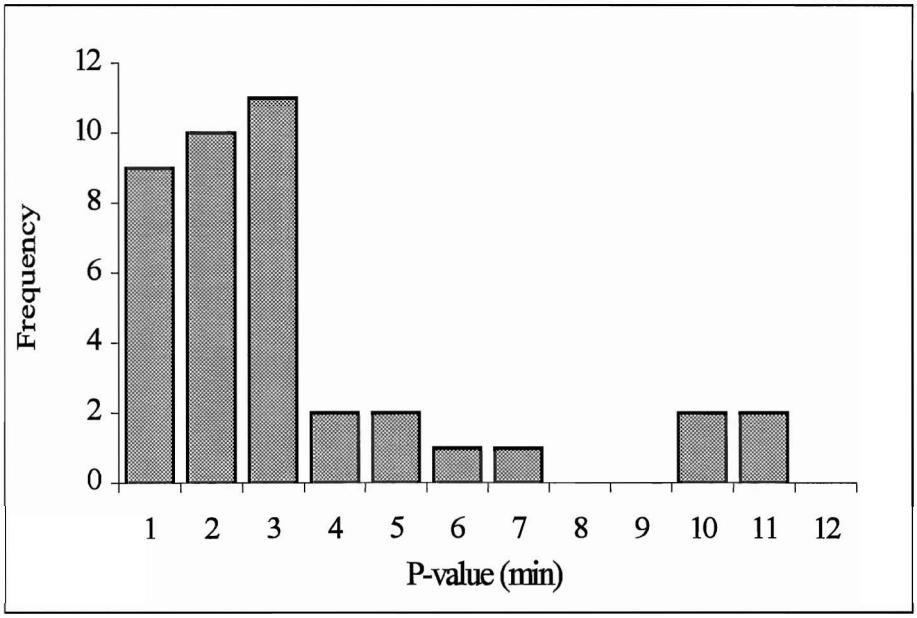

Figure 12. Chart showing distribution of P-values for 42 TTI particles processed in 200 L of 5 wt% Colflo 67 starch solution.

To prove that a temperature gradient existed throughout the stirred vessel, a temperature distribution was taken from 200 L of 5% Colflo 67 starch. The batch was heated to 90°C and the steam supply and mixer turned off temporarily. A rapid response thermocouple (thin wire type T) was attached to a paddle and moved in a random motion/pattern throughout the tank. The mixer was stopped for safety reasons in order to perform this operation. Temperature readings were taken every second using a Squirrel data logger and the data obtained is shown in Figure 13.

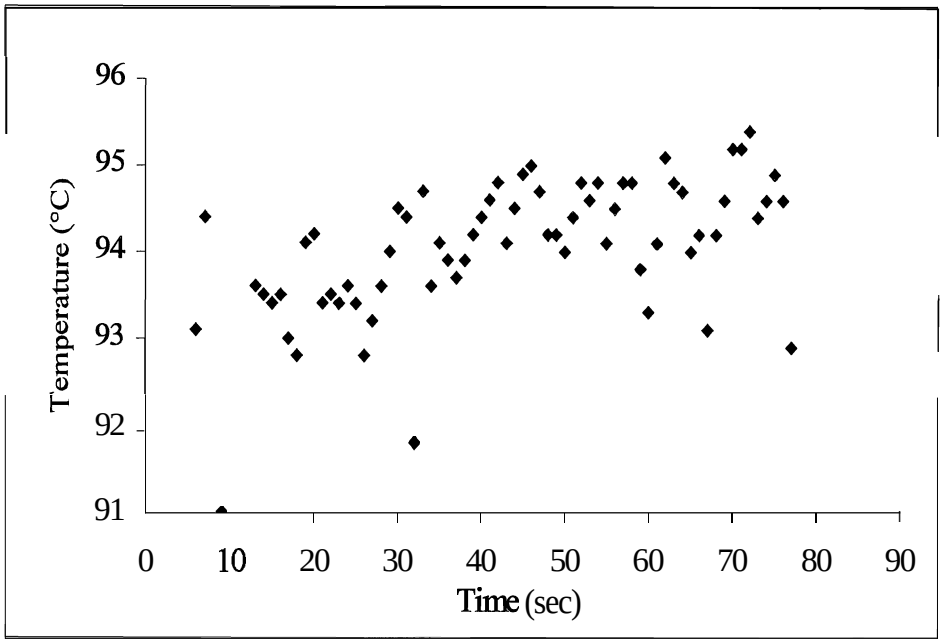

Figure 13. Temperature distribution in a stirred vessel containing 200 L of 5 wt % Colflo 67 starch solution controlled at 94 °C.

4. Industrial applications

This section introduces some of the industrial applications for TTIs, fiom work undertaken with the companies supporting this work. Certain aspects of the work remain confidential to those companies, for example details of formulation and process specifications. However, the aim of this section is to illustrate the potential for using TTIs to measure P-values within a range of food products and process types.

4.1 Batch Cooking of Sweet & Sour Sauce

4.1.1 Methods

TTI particles sized 5 and 10 mm cubes were prepared containing the enzyme Bacillus *licheniformis* α-amylase. The heating and cooling tests were done in a saucepan, using the contents of a 2.4 kg bottle of sweet & sour sauce as the food product. This contained various particulates with sizes up to 10 mm (pineapple pieces). The 5 mm cubes were intended to heat up at a similar rate to the smaller particulates (e.g. peppers, onions) and the 10 mm cubes similar to the pineapple. The objective was to evaluate the effectiveness of these TTIs with a high viscosity product containing a variety of particulates.

Two trials were performed using TTI cubes and three Ellab Tracksense units to measure temperatures in the sauce, to compare the P-values with those fiom the cubes. In order to identify the cubes between the trials, some were marked with a blue cross on 2 surfaces and others with a green cross; this also helped to identify them as they were being recovered fiom the sauce.

(a) No hold period

11 x 5 mm and 8 x 10 mm cubes were added with the 3 x Tracksense loggers to the sauce, stirring continuously while heating over a gas flame. A hand-held Comark thermometer was used to monitor sauce temperatures so that the heating could be stopped when 93.3 °C had been reached. On reaching this temperature, the sauce was cooled by standing the saucepan in cold water and stirring until the temperature had dropped to below 70 °C. At this teniperature, the loss in amylase activity was considered to be minimal.

(b) 5 minute hold period

12 x 5 mm and 9 x 10 mm cubes were used, with a 5 minute hold period at 93.3 °C before cooling. All other conditions were as for the first trial.

4.1.2 Results and Discussion

(a) No hold period

P-values for the 5 mm cubes ranged from 0.9 to 2.5 minutes with a mean of 1.8 minutes, and for the 10 mm cubes from 1.2 to 2.5 minutes with amean of 1.6 minutes (Table 9a). These compared with P-values of 7.9, 8.6 and 8.6 minutes for the Tracksense loggers (T_{ref} 93.0 "C, z 9.1 C°).

93°C No hold				93°C, 5 min hold			
Activity (abslmin)	P-value for D_{93} (min)	Activity (abs/min)	P-value for D_{93} (min)	Activity (abslmin)	P-value for D_{93}(min)	Activity (abs/min)	P-value for D_{93}(min)
5mm cubes		10 mm cubes		5mm cubes		10 mm cubes	
0.897	0.9	0.732	1.7	0.253	5.6	0.346	4.4
0.908	0.9	0.587	2.5	0.237	5.8	0.419	3.7
0.705	1.8	0.768	1.5	0.243	5.7	0.483	3.1
0.795	1.4	0.825	1.2	0.144	7.7	0.534	2.7
0.597	2.5	0.731	1.7	0.234	5.9	0.348	4.4
0.604	2.4	0.775	1.5	0.197	6.5	0.405	3.4
0.586	2.5	0.818	1.3	0.148	7.6	0.421	3.6
0.670	2.0			0.264	5.4	0.219	6.1
0.664	2.1			0.321	4.7	0.292	5.0
0.668	2.0			0.187	6.7		
Thermocouple P-values (min.)				Thermocouple P-values (min.)			
7.9	8.6	8.6		16.7	14.1	13.2	

Table 9. P-values from TTIs processed: a) heating and cooling with no holding period and b) heating to 93°C and holding for 5 minutes before cooling.

(b) 5 minutes hold period

P-values for the 5 mm cubes ranged from 4.7 to 7.7 minutes with a mean of 6.1 minutes and for the 10 mm cubes from 2.7 to 4.4 minutes with amean of 3.5 minutes (Table 9b). P-values measured by Tracksense loggers gave 16.7, 14.1 and 13.2 minutes (T_{ref} 93.0 "C, z 9.1 C°).

For both trials, the P-values estimated with the amylase cubes were less than those calculated from the Tracksense temperature measurements. This was because of the conduction lag introduced with the silicone encapsulation of the amylase and also the location of the Tracksense loggers at the base of the saucepan. Temperatures recorded with the loggers reached 100 °C, whereas those from the hand-held thermometer only reached 95 "C maximum. The amylase cubes were free to move around in the sauce, as dictated by the stirring action, and were unlikely to stay at the hotter base of the saucepan.

4.2 Oven Cooking of a Meat Product

4.2.1 Method

Twenty TTI tubes were filled with *Bacillus amyloliquefaciens* α-amylase (D_{72} 200 minutes, z 8.6 C°), for insertion directly into 8 mm thick meat products or sandwiched in the folded-over product to represent a worst case for pasteurisation. The meat samples were cooked in a moving-belt oven with low clearance height and chilled with liquid nitrogen, which made it difficult to use wire-based probes and loggers. The objectives were to evaluate the feasibility of using the TTI tubes in this cooking environment and to measure the range of P-values. The target process was to exceed an equivalent of 2 minutes at 72 "C, a process target based on achieving at least 6-log reductions of aerobic non-spore formers such as *Listeria monocytogenes* and *Salmonella* (CCFRA, 1992). This was substantially lower than the minimum measurable with the TTI, but the degree of over-processing or safety margin was thought to be considerable. Identification of the cooked product containing a TTI was achieved by segregating a section of belt and destructively searching through the entire product in this section.

4.2.2 Results and Discussion

The objectives of this trial were achieved, in that the *Bacillus amyloliquefaciens* amylase had suitable kinetics to estimate the P-values achieved during the cooking process, and the PTFE tubes were an effective method of enclosing the amylase that did not interfere with the heat transfer. Although this work was done before the problem of leaking tubes was identified, it is assumed that no dilution had occurred in this case as the product was generally solid and minimal liquids were involved.

1 mm diameter tubes			
Activity	P-value	P-value	P-value
(abs/min)	For D_{72}	for D_{82}	for D_{85}
	(min)	(rnin)	(rnin)
0.296	82.0	5.6	2.5
0.293	82.9	5.7	2.6
0.204	114.4	7.9	3.5
0.102	174.7	12.0	5.4
0.149	141.7	9.7	4.4
0.098	178.2	12.2	5.5
0.021	312.3	21.5	9.6
0.515	33.8	2.3	1.0
0.093	182.8	12.6	5.6
0.089	186.6	12.8	5.7

3 mm diameter tubes			
Activity	P-value	P-value	P-value
(abs/min)	for D_{72}	for D_{82}	for D_{85}
	(min)	(min)	(min)
0.301	80.5	5.5	2.5
0.492	37.8	2.6	1.2
0.394	57.1	3.9	1.8
0.336	71.0	4.9	2.2
0.285	85.3	5.9	2.6
0.254	95.3	6.6	2.9
0.241	99.9	6.9	3.1
0.322	74.7	5.1	2.3
0.048	240.3	16.5	7.4

Table 10. Pasteurisation values measured in a meat product cooked in an oven process.

The P-values measured with the TTIs during continuous cooking of meat pieces showed a minimum value of 33.8 minutes. Using 1 mm o.d. tubes gave a mean P-value of 148.9 minutes, with a maximum of 312.3 and minimum of 33.8 minutes. Using 3 mm o.d. tubes gave a mean P-values of 93.5 minutes, with a maximum of 240.3 and a minimum of 37.8

minutes. These wide ranges were expected because of the variation in thickness of the individual pieces, many of which were folded or in contact during cooking. These results showed that the cooking was adequate to achieve the target value equivalent to *72* °C for *2* minutes and all of the P-values exceeded this with a substantial safety margin.

4.3 Sprayed Water Processing of Pickled Cucumber Slices in Glass Jars

4.3.1 Method

TTIs were used to measure pasteurisation values (P-values) achieved in 480 g jars of pickled cucumber slices processed in a sprayed water tunnel. The TTI was an amylase from *Bacillus amyloliquefaciens* encapsulated inside the *2.5* mm i.d. silicone tubes.

The tubes were inserted into the pickle slices with some kept aside as controls. Small pieces of the pickle were removed with a scalpel and the TTI tubes were pressed into the space left. The slices were then replaced into jars so that the TTIs were arranged as shown in Table 11. The jars were then re-filled with the vinegar pickling solution and lidded on the normal production line. The fill weight of cucumber slices was given as *255* g with a net weight of 480 g.

Jar No	TTI Arrangement
1	*5* TTIs each in different slices, positioned in the geometric centre of the jar.
2	*5* TTIs each in different slices, positioned in slices in different locations.
3 – 19	2 TTIs in one slice of cucumber positioned in the centre of each jar.
20	*5* TTIs in one slice of cucumber positioned in the geometric centre of the jar.

Table 11. Arrangement of TTI tubes *(Bacillus amyloliquefaciens* amylase) in jars of sweet pickle slices.

Two jars were also prepared with Ellab Tracksense probes, measuring the environment conditions and the temperatures inside the jar. All of the prepared jars were processed in a sprayed water tunnel set at 90 °C. The heating time was *25* minutes and the cooling time was

5 minutes, giving a total process time of 30 minutes. After processing, the TTIs were removed and analysed following the standard procedure.

4.3.2 **Results and Discussion**

The P-values measured in jars 1, 2 and 20 are shown in Figure 14. Where the TTIs were inserted into different slices of cucumber, the P-values showed variations, and where TTIs were in one slice located in the geometric centre of the jar, there was acceptable agreement.

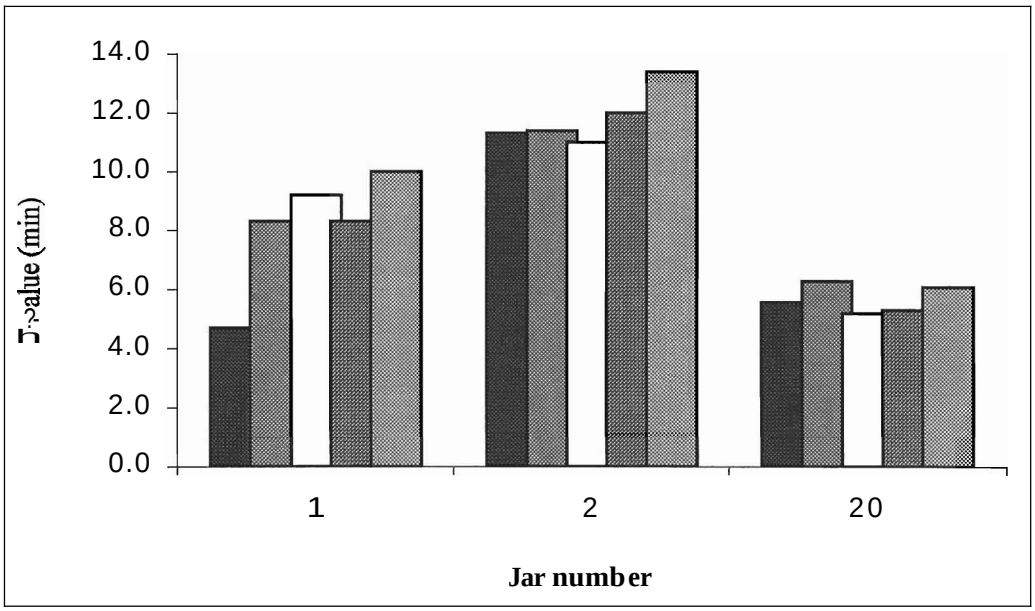

Figure 14. P-values measured for sliced pickled cucumber, with TTIs in Jars 1 and 2 in different slices within the jar, and 5 TTIs in Jar 20 in one slice in the jar centre.

For the replicate jars where two TTIs were positioned in the centre in one slice, it was found that within each jar the TTIs gave consistent P-values but between jars there were significant differences. Only a small variation was expected due to the nature of the product and the conditions in which they were processed. Figure 15 shows these results.

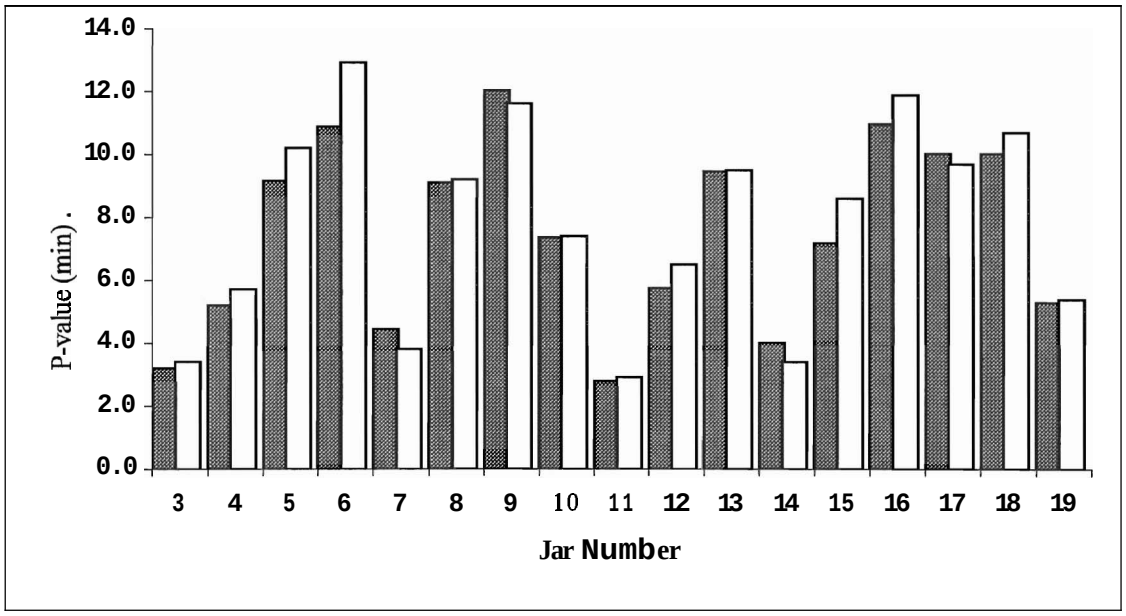

Figure 15. P-values measured for pickled cucumber slices, with two TTIs in one slice of cucumber in the centre of each jar.

The Ellab Tracksense probe gave a P-value of **32.3** minutes for the probe placed inside the jar of cucumber slices. This jar could not be lidded under vacuum and it was not possible to position the probe in the centre of a cucumber slice. All of the **TTI** tubes gave P-value results that were much lower than this Tracksense value.

Having already demonstrated in the feasibility studies that there was generally good agreement in measured process data between thermocouples and **TTIs,** other possible reasons for this difference were proposed:

- **Jars** with Tracksense loggers were lidded without a vacuum whereas those with TTIs were lidded under vacuum. The vacuum will act to pull the cucumber slices together, thus minimising the liquid that can circulate between the slices. Under a vacuum, the slices will behave more as a conduction heating pack.

- When inserted into the product, the Tracksense probe can push the cucumber slices apart, allowing the vinegar solution to move between the slices more easily. This effect would be increased because the jars were not lidded under vacuum.

- Thermocouple conduction along the probe may account for a small increase in temperature measured at the tip.

- The probe measuring tip may not have been at the geometric centre and is likely to move during the process in response to movement in the lid.

Evidence suggested that the vacuum was the most significant factor affecting the results although each of the above effects was likely to have occurred. Thus, the TTIs probably gave a closer measure of the true process that the cucumber slices received and the Tracksense measured the process received by the vinegar solution. This conclusion was critical to the success of using TTIs to measure P-values in food products.

4.4 Ohmic Heating of Fruit Preparations

4.4.1 Method

A significant application of TTIs has been for the validation of an ohmic heater process with a strawberry fruit preparation. The process was challenged to its operational limits by stepwise increases in the flowrate of product through the process in order to optimise the process within the boundaries of microbial safety.

For these trials, the TTIs were constructed from Sylgard cubes of similar shape and dimensions to the diced strawberry pieces. Due to the process being challenged at the higher flow rates, the process P-values were likely to lie at either end of the measuring range of the enzymes. Therefore, both enzymes *(Bacillus licheniformis* and *B. amyloliquefaciens* amylase) were used because of their different heat stabilities.

The TTI particles were incorporated randomly into the feed tanks, which contained approximately 400 kg of strawberry pieces in a high viscosity starch-based carrier liquid. Four runs were completed at various flow rates, starting with the typical production rate of 750 kg/h and working upwards to over 1,000 kg/h. It was hoped to complete 4 runs at flow rates of 800, 900, 1,000 and 1,100 kg/hr, but operational problems were experienced on the ohmic plant, causing high currents that made these rates impossible to achieve. The compromise was to operate runs 2 to 4 at flowrates that varied slightly, albeit achieving the

stepwise increases, as shown in Table 12. The final run (run 4) at the highest flowrate used 20 TTIs containing B. *licheniformis* enzyme and 20 TTIs containing B. *amyloliquefaciens* enzyme, to ensure that the process could be measured if it was close to the minimum of 5 minutes at 85°C.

	Flowrate (kg/h)	**Details of TTI particles added to the feed batches**
Run 1	750	30 *B. licheniformis*
Run 2	750 – 800	20 B. *lichenformis*
Run 3	815 – 850	20 *B. licheniformis*
Run 4	1,015 – 1,050	20 *B. lichenformis* + 20 *B. amyloliquefaciens*

Table 12. Details of process flowrates and TTIs for batches of a strawberry fruit preparation processed in a 75 kW ohmic heater.

After processing, the batches were sorted by hand to recover the TTI particles and the enzymes assayed following the standard procedure.

4.4.2 Results and Discussion

Run 1 - Although the process was set at a flowrate of 800 kg/h, problems occurred with the ohmic heater that caused this to fluctuate and the average flowrate was closer to 750 kg/h. This is reflected in the results where the measured P-values ranged between 62.4 minutes and 195.3 minutes, as shown in the frequency distribution of Figure 16. Not all of the particles were recovered, the remaining ones were assumed to have washed down the drain as the plant was cleaned out. All of the particles that had been collected showed they had been processed well above the target process.

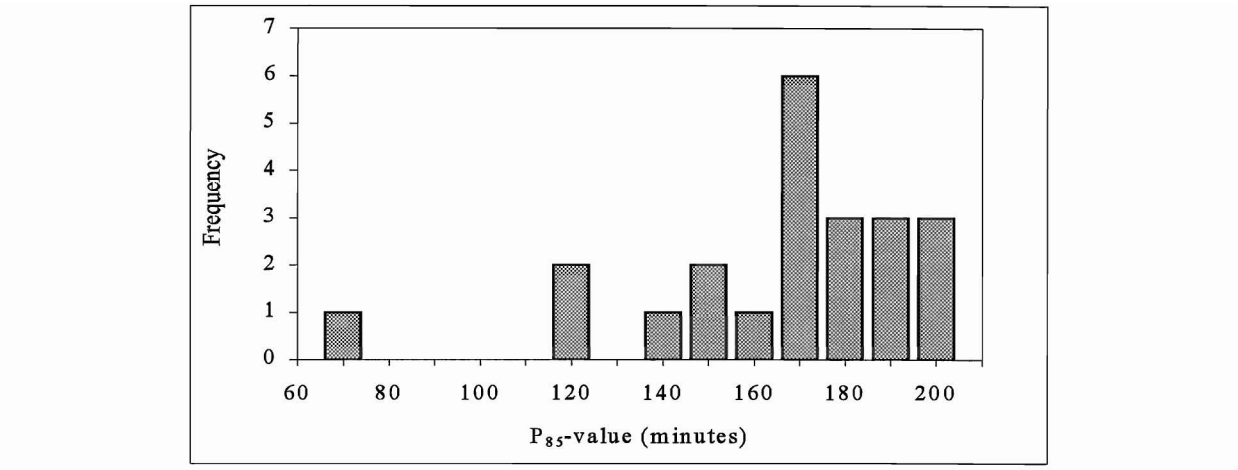

Figure 16. Frequency of P-values measured by B *lichenifomis* amylase in a strawberry fruit preparation processed by ohmic heating with a flow rate of 750 kg/h.

Run 2 - This run was set at the normal production flowrate of 750 kg/h, but again the rate was unsteady, fluctuating between 750 and 800 kg/h. 18 out of the 20 particles were recovered and all showed P-values that exceeded the target process. The P-values ranged from 32.8 minutes to 164.4 minutes.

Run 3 - It was possible to achieve an increased flowrate of between 815 and 850 kg/h by allowing the ohmic column outlet temperature to fall slightly. The screen displays at the control panel indicated that the product Pu values remained above 6 minutes. Actual P-values measured with the TTIs ranged between 11.6 minutes and 135.1 minutes, with the frequency distribution shown in Figure 17.

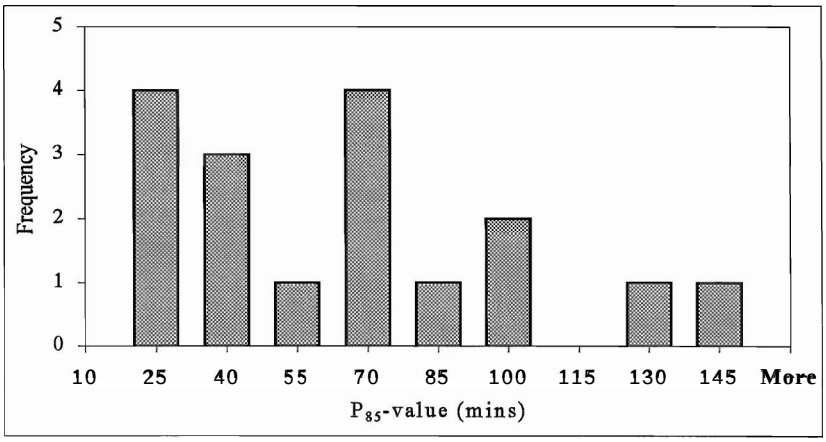

Figure 17. Frequency of P-values measured by B *lichenifomis* in a fruit preparation processed by ohmic heating with a flow rate of approximately 815-850 kg/h.

Run 4 - The flowrate for this run was between 1,010 and 1,050 kg/h, which was achieved by allowing the ohmic exit temperature to fall below 95 "C. 24 of the particles were recovered from this batch. The particles containing B. *licheniformis* amylase gave P-values of between 5.3 and 38.4 minutes; however, the enzyme's relatively high heat resistance at 85 "C meant that at this flowrate only a small percentage of its activity was lost. More reliable results were measured by the B. *amyloliquefaciens* amylase particles whose P-values ranged from 4.8 to 10.7 minutes, within the one to two log reduction range of activity where the measurements were at their most accurate. At this flowrate one of the P-values, at 4.8 minutes, was outside of the target and so the fruit would not have been adequately processed.

The P-value results fi-om the silicone TTI particles allowed the normal production flowrate to be increased safely from 750 to 800 kg/h, without changing the product temperature on exit from the ohmic column. Further increases in flowrate to 1,000 kg/h are under investigation, with plans submitted to purchase additional lengths of holding tube so the ohmic column can operate at a reduced exit temperature.

5. Conclusions and future work

The TTI particles were found to be a reliable and alternative method for measuring the therrnal process delivered to products where conventional probe-based validation techniques were not suitable. The industrial application trials using *Bacillus amyloliquefaciens* and *Bacillus licheniformis* amylases were successful in all cases and showed that the pasteurisation treatments being applied were in general substantial, and over-processing was taking place in most cases.

The TTI encapsulation method was adapted for each product and process tested, and could be made to represent a realistic worst case for process validation purposes. For particulate products, the enzyme was incorporated into silicone cubes of similar shape and dimensions to the critical food particulate, and for products that were mainly solids or liquids, the TTI was encapsulated inside a tube which was inserted into the centre of the product.

Future work will continue as part of this LINK funded project in four areas:

- Further applications of the *Bacillus amyloliquefaciens* and *licheniformis* amylases to measuring pasteurisation values for products that cannot be easily measured using wire-based systems.
- Development of a multiple TTI particle containing three different TTIs with z-values of approximately 10, 20 and 30 C°. This will allow the time temperature history of the entire thermal process to be predicted from the three TTI F-values. The TTIs being studied for use in this particle are the *Bacillus* amylase enzymes, horse radish peroxidase (HRP) and nitrophenol glucoside (NPG).
- Development of a TTI that can be used to monitor processes that operate at sterilisation temperatures. Drying *Bacillus subtilis* amylase to controlled moisture levels has shown potential to increase the heat stability without altering the z-value.
- The possibility of using a colorimeter as a portable piece of equipment to do the enzyme assays.

References

Adams, J.B. (1996). Determination of $D_{80°C}$ for a - amylase inactivation. Campden & Chorleywood Food Research Association. C&B/rep/12598/1, May 1996

CCFRA (1992). Food pasteurisation treatments. CCFRA Technical Manual No.27, Campden & Chorleywood Food Research Association.

De Cordt, S., Hendrickx, M., Maesmans, G. and Tobback, P. (1992). Immobilised α-amylase from *Bacillus licheniformis*: a potential enzymic time–temperature integrator for thermal processing. International Journal of Food Science and Technology 27, 661 – 673.

Department of Health. (1994). Guidelines for the Safe Production of Heat Preserved Foods. ISBN 0-11-321801-X. HMSO.

Hendrickx, M., Maesmans, G., De Cordt, S., Noronha, J., Van Loey, A. and Tobback, P. (1995). Evaluation of the integrated time–temperature effect in thermal processing of foods. Critical Reviews in Food Science and Nutrition, 35 (3), 231 - 262

Hendrickx, M., Weng, Z., Maesmans, G. and Tobback, P. (1992). Validation of a time temperature integrator for thermal processing of foods under pasteurisation conditions. International Journal of Food Science and Technology 27, 21 - 31

McKenna and Tucker G.S. (1991). Computer modelling for the control of particulate sterilisation under dynamic flow conditions. Food Control 2(4): 224-233.

Maesmans, G., Hendrickx, M., De Cordt, S., Van Loey, A. Noronha, J. and Tobback, P. (1994). Evaluation of process value distribution with time temperature integrators. Food Research International 27, 413 - 423

Taoukis, P.S. and Labuza, T.P. (1989). Reliability of time temperature integrators as food quality monitors under non-isothermal conditions. Journal of Food Science 54 (4), 789-792.

Tomazic, S.J. and Klibanov, A.M. (1987). Mechanisms of irreversible thermal inactivation of *Bacillus* α-amylases. Journal of Biological Chemistry. 263 (7), 3086 – 3091

Tucker, G.S., Noronha, J.F. and Heydon, C.J. (1996). Experimental validation of mathematical procedures for the evaluation of thermal processes and process deviations during the sterilisation of canned foods. Transactions of the Institution of Chemical Engineers, 74, Part C, 140-148.

Tucker, G.S. (1999). Application of time temperature integrators for validation of thermal processes. R&D Report No.77. Campden & Chorleywood Food Research Association.

Tucker, G.S. (1999). A novel validation method: Application of time temperature integrators to food pasteurisation treatments. Transactions of the Institution of Chemical Engineers, 77, 223 - 231

Van Loey, A., Hendrickx, M., De Cordt, S., Haentjens, T. and Tobback, P. (1996). Quantitative evaluation of thermal processes using time – temperature integrators. Trends in Food Science & Technology, 7, 16 – 26.

Van Loey, A., Arthawan, A., Hendrickx, M., Haentjens, T. and Tobback, P. (1997). The development and use of an α-amylase based time temperature integrator to evaluate in pack pasteurisation processes. Lebensmittel – Wissenschaft und Technologie, 30, 94 – 100

Van Loey, A., Hendrickx, M., Ludikhuyze, L., Weemaes, C., Haentjens, T., De Cordt, S, and Tobback, P. (1995). Potential *Bacillus subtilis* a-amylase based time temperature integrators to evaluate pasteurisation processes. Journal of Food Protection, 59 (3), 261-267.

Van Loey, A., Hendrickx, M., Haentjens, T., Smout, C. and Tobback, P. (1997). The use of an enzymic time temperature integrator to monitor lethal efficacy of sterilisation of low-acid canned foods. Food Biotechnology, 11 (2), 169-188

Van Loey, A., Ludikhuyze, L., Hendrickx, M., De Cordt, S and Tobback, P. (1994). Thermal consideration on the influence of the z-value of a single component time/temperature integrator on thermal process impact evaluation. Journal of Food Protection, 58 (1), 39-48.

Williams, A. and Adams, J.B. (1997). A time-temperature integrator to quantify the effect of thermal processing on food quality. Engineering and Food at ICEF 7, 2, K9-K12.