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NO. 77

Applications of time-temperature integrators for validation of pasteurisation processes

1999

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APPLICATIONS OF TIME-TEMPERATURE INTEGRATORS FOR VALIDATION OF PASTEURISATION PROCESSES

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SUMMARY

A process validation method was developed using a *Bacillus amyloliquefaciens* α-amylase time-temperature integrator injected into the centre of silicone particles. These amylase particles were used to validate pasteurisation processes for production scale yogfruit batches of whole 10-12 mm strawberry, 10 mm pineapple and 17 mm apricot. The kinetic factor (z-value) for the amylase was 9.7 ± 0.3 C° over the temperature range 70 - 90 °C, which was within the range of values common to many pasteurisation treatments. Amylase activity before and after processing was converted to P-values using the appropriate D_T value. For most of these high acid products, the target P-value was equivalent to 5 minutes at 85°C (T_{ref}= 85°C, z = 10 C°). Minimum P-values measured using the α-amylase were 8.3, 6.0 and 9.7 minutes respectively, thus demonstrating the microbiological safety of these processes. A feasibility study was also conducted on an industrial ohmic plant using 10-12 mm whole strawberries as the yogfruit product. Encapsulated 5 mm amylase bubbles were sealed into the centres of 15 strawberries, added to the feed batch and allowed to pass through the ohmic process unhindered. On retrieval, the amylase activities were assayed and a minimum P-value calculated of 160 s at 90 °C (T_{ref}= 90 °C, z = 10 C°); this compared with the target of 90 s.

This technique was developed and demonstrated on continuous pasteurisation processes but can be applied to almost any process for foods that contain solid particles. It has advantages over conventional temperature probe systems for foods in which the sensor creates an interference with the temperature response and where heat conduction along the probe is an issue. There are economic advantages for using TTIs compared with microbiological methods in addition to the more rapid analyses that allow processes to be evaluated within hours of removal.

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1. INTRODUCTION AND BACKGROUND

Thermal processing of food products containing discrete particles in sauces is a very important industry sector, with the market for cook-in-sauces, soups and preserves expanding. The demand is for products of ever increasing quality to compete with the quality of chilled products but with the advantage of an extended shelf life. In order to achieve this goal, the delivered thermal process should be the minimum that gives a safe product. The process delivered to the centre of the largest, and/or slowest heating particle is therefore critical in order to ensure that the products are safe to eat, have a long shelf life and are of high quality. The key to achieving this is to use accurate and reliable process validation techniques that give confidence in the scheduled processes. Without the ability to measure process values accurately and repeatedly it is not possible to optimise thermal processes.

Most commercial thermal processes for mixtures of solids and liquids are delivered to the product after they have been filled into an hermetically sealed container. Temperature probe systems, such as thermocouples, can be used to take temperature measurements from which the process time and temperature is established. The technology for continuous processing has been less readily accepted by the food industry despite the potential benefits it offers to product quality and plant throughput. Part of the reason for this is the difficulty in establishing the process, because thermocouples cannot be used here and therefore relatively expensive microbiological methods are required (Brown et al, 1984).

There are a number of commercially available heating systems for process suspensions of solids and liquids; these include scraped surface heat exchangers, tubular heat exchangers and direct steam injection systems. When using these processes for liquid foods, only the thermal process contribution made within the holding tube is taken into account. However, thermal treatment of the food occurs throughout the entire heat, hold, cool process, and as a result the product quality can suffer by not accounting for this. Validation data that allows the total heat treatment, and hence quality changes, to be measured accurately would be extremely useful and possibly encourage food processing companies to seriously investigate continuous thermal processing.

The final step, before a thermal process can be commercially applied and the products marketed, is to validate the pasteurisation or sterilisation achieved in the slowest heating part of the product. This is a complex task for continuous processes and one that is further complicated by the presence of particulates.

The objective of this work was to develop a method that used a time-temperature integrator (TTI) to measure the pasteurisation value (P-value) achieved at the centre of a moving food particle. Results from case studies are presented for the application of this method to commercial processes for three yogfruit products: whole 10-12 mm strawberries, a 10 mm pineapple and passion fruit product, and a 17 mm apricot product. The method was developed within a continuous processing project but, with minor modifications, is equally applicable to in-pack processing of mixtures of solids and liquids.

1.1 Time-Temperature Integrator Systems

The use of TTIs as an alternative means of process evaluation to either temperature or microbial systems has received considerable attention recently (Maesmans et al, 1994; Hendrickx et. al, 1995; Van Loey et al, 1996). The intention of this work was to identify TTIs that could be used in the temperature range associated with pasteurisation treatments (60 - 105 °C). The kinetics of

the two TTI systems chosen for this work were both investigated during an EU funded project led by the Katholieke Universiteit te Leuven (Van Loey et al, 1997a). Hydrolysis of nitrophenyl glucoside (NPG) and denaturation of *Bacillus amyloliquefaciens* α-amylase were the TTIs chosen for their ease of measurement. Within this EU project, the kinetics had been proven for non-isothermal conditions and reliable analysis methods developed for use in measuring integrated process values (see table 1).

The respective TTI reaction rates were minimal at ambient temperatures and almost non-existent at chilled conditions. This simplified the methods required to transport the TTI particles from the laboratory to the food processing environment, but to ensure that there was as little activity loss as possible the TTI particles were kept chilled before and after processing.

Table 1: Key Attributes of the Nitrophenyl Glucoside (NPG) and *Bacillus amyloliquefaciens* α-amylase TTI Systems

	NPG	α-amylase
Operating principle	Formation of the yellow nitrophenolate ion	Reduction in enzyme activity
Measurement method	Colour change	Enzyme assay
Active temperature range (°C)	70-130	60-100
Kinetic factor, or z-value (C°)	23.0±1.2	9.7±0.3
Decimal reduction time (min)	$D_{100} = 88.2$	$D_{80.7} = 18.7$
Process value	'cook-value'	'pasteurisation-value'
Sample size (mL)	0.02	0.02

1.1.1 Nitrophenyl glucoside (NPG)

Hydrolysis of p-nitrophenyl β-D-glucopyranoside (Sigma Chemical Company) in borate buffer (pH 11.0) was used as a chemical TTI reaction (Adams and Langley, 1996; 1998). The yellow nitrophenolate ion produced on hydrolysis was shown to be highly stable and to follow first order kinetics of formation. The z-value was found to be 23.0 ± 1.2 C° over the range 91.0 to 121.1 °C, with a D_{100} of 88.2 minutes.

A spectral shift from 300 to 400 nm was observed during formation of the yellow nitrophenolate ion. In order to apply standard first order kinetics to the hydrolysis of NPG, an accurate estimate was required of the colour absorbance at 400 nm on completion of the reaction. The final absorbance at 400 nm (A_{final}) of the heated NPG solution after complete hydrolysis was determined by multiplying the absorbance at 300 nm of the unheated NPG solution by 1.66. Cook values (C) were calculated from the absorbance readings using equation 1 (Williams and Adams, 1997).

$$C = D_T[\log (A_{final}) - \log (A_{final} - A_t)] \quad (1)$$

where, D_T is the decimal reduction time (min)
A_{final} is the final absorbance for NPG at 400 nm calculated from the absorbance at 300 nm for unheated NPG x 1.66
A_t is the absorbance for NPG at 400 nm after a specific time-temperature history

The measurements of absorbance were simple and required approximately 5 minutes per TTI sample. The disadvantage was that the z-value of 23.0 ± 1.2 C° was not in the typical spore destruction range but representative of cooking reactions. NPG was therefore an indicator for estimating cook values.

1.1.2 *Bacillus amyloliquefaciens* α-amylase

Inactivation of α-amylase enzyme from *Bacillus amyloliquefaciens* (EC 3.2.1.1 Type II-A, Sigma A-6380) was used as a TTI (Adams, 1996). To measure the enzyme activity, 0.02 mL of α-amylase solution was added to 1 mL of amylase reagent equilibrated at 30 °C, mixed by inversion, and the rate of increase of absorbance at 405 nm measured between 1.5 and 2.0 minutes after insertion into a spectrophotometer (Unicam PU8700). The z-value was found to be 9.7 ± 0.3 C° over the range 74.0 to 83.0 °C, with a $D_{80.7}$ of 18.7 minutes.

P-values were calculated from the initial and final activities using equation 2.

$$P = D_T[\log (A_{initial}) - \log (A_{final})] \quad (2)$$

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

The measurements of enzyme activity required a reaction rate gradient to be calculated, and hence were more involved than for the NPG but still only required approximately 10 minutes per TTI sample. The advantage of α-amylase over NPG was that the z-value of 9.7 ± 0.3 C° was in the typical spore destruction range for pasteurisation organisms. The α-amylase TTI was thus relevant to the measurement of P-values, hence a technique was developed for encapsulating α-amylase to measure P-values achieved during continuous processes.

1.2 Pasteurisation Treatments

There are numerous recommended pasteurisation treatments (CCFRA, 1992) for thermally processed foods, the severity of which are dependent on the likely spoilage organisms present and the contamination levels (see examples given in table 2).

Table 2: Selected Pasteurisation Treatments Recommended for Thermally Processed Foods

Process Type	pH	Time/Temperature Equivalent	T_{ref} (°C)	z-value (C°)
Cook-chill	>4.5	2 minutes at 70.0 °C	70.0	10.0
Sous Vide	>4.5	40 minutes at 70.0 °C	70.0	10.0
'Psychrotrophic bot'	>4.5	10 minutes at 90.0 °C	90.0	10.0
Acid Fruits	3.7 - 4.2	5 minutes at 85.0 °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

The α-amylase z-value was 9.7 ± 0.3 C°, therefore it was applicable to all of the pasteurisation treatments highlighted in table 2. The decimal reduction time at 85 °C was 6.95 minutes and at 90 °C was 2.05 minutes. Measurement of the residual activity after processing gave accurate results within two log reductions in activity. Hence, P-values can be measured up to 14 minutes equivalent at 85 °C and 4 minutes equivalent at 90 °C. This excluded its use for the two recommended processes for tomato products (pH>4.3) and for the 'psychrotrophic bot' process.

2. MATERIALS AND METHODS

2.1 Particle Construction

Sylgard 184 silicone (Dow Corning Ltd) was used for encapsulating the α-amylase because it was transparent, robust, safe to handle, chemically inert and could be moulded into particle shapes. The physical properties of the silicone had been tested in previous work and shown to be of a similar order of magnitude to those of food products (McKenna and Tucker, 1991). The density of the silicone was 1,050 $kg.m^{-3}$ resulting in a final density of the particles similar to that of the carrier fluids used (ca. 1,000 $kg.m^{-3}$). Polycarbonate particle moulds were used to manufacture 50 particles at the same time. The manufacturing process required many steps, finally resulting in ~ 0.04 mL of the TTI injected into an air bubble at the particle centre, of which exactly 0.02 mL was required for the analysis.

The particle moulds used for the trials described in this report were for cubic particles with dimensions of 10 or 14 mm. 50 particles were manufactured in each batch in order to allow replicates to be processed that ensured the results were representative of the distribution expected during production. Manufacture of a particle with a small quantity of TTI at the centre involved a number of stages:

- Mix the silicone base and curing agent in the ratio 10:1 and pour into a shallow tray
- Allow the small air bubbles introduced by the mixing to dissipate
- Use a hypodermic syringe to inject a number water bubbles of ~ 0.04 mL volume into the silicone, avoiding overcrowding that may lead to bubble coalescence
- Allow the silicone to cure overnight, or use an oven at 70°C to speed up the curing time to a few hours
- Cut out the water bubbles with a sharp knife to give cubes of ~ 5 mm size
- Mix 50 g of silicone and one third fill each of the 50 particle moulds

- Cure the silicone until the surface is tacky, using an oven at 70 °C to speed up the curing time to 1 hour
- Position one bubble cube into the centre of each mould and fill with the remaining silicone to fully enclose the bubble
- Fully cure the silicone using an oven at 70 °C to speed up the setting time to 2 hours
- Remove the cured particles, puncture each bubble with a hypodermic syringe and remove as much water as possible
- Heat the particles in an oven at 110 - 120 °C for 60 - 120 minutes in order to evaporate the remaining water so that it escapes as steam through the needle entry hole
- Allow the particles to cool to ambient temperature, ready for use
- Use a hypodermic syringe to fill the bubble with the TTI
- Seal the needle entry hole by cutting away a small part of the silicone and filling with uncured silicone
- Heat the particles in an oven at 40 °C for 120 minutes to fully cure the silicone
- Keep the particles chilled until ready for use

2.2 Particle Thermal Properties

Thermal diffusivity ($\alpha = k / \rho.C_p$) is the measure of how fast the centre temperature of a solid body responds to a step change in ambient temperature. Sylgard 184 silicone ($\alpha = 1.02 \times 10^{-7}$ $m^2.s^{-1}$) has a thermal diffusivity approximately 25 % lower than a typical high water content food particle ($\alpha = 1.4 \times 10^{-7}$ $m^2.s^{-1}$). Silicone particles of the same size as food particles would therefore heat up more slowly, resulting in lower and unrepresentative amylase activities. Thermal processes established on this basis would err significantly on the safe side from a microbiological standpoint. However, from the quality perspective, the data could not be used to analyse the distribution of process values and therefore process optimisation studies would not be possible. In order to study the distribution of process values, it is necessary to match the thermal characteristics of the silicone particles to those of the target food particles.

The correct size and shape of the silicone particles can be calculated using a series of simple expressions (see Table 3) so that they heat up at the same rate as the food particles. These expressions use the logarithmic heating rate (f_h) as the factor to equate the heating rate of solid bodies of varying shape, size and dimensions. They were developed for the canned foods industry (Ball and Olsen, 1957) for use with conduction heating foods. Their application to the heating of particles is valid because solid food particles will heat mostly by conduction.

Table 3: Heating Factor Equations for Regular Geometries

Geometry	Heating factor
Infinite slab (1D)	$\alpha.f_h = 0.933 / a^2$
Finite cylinder (2D)	$\alpha.f_h = 0.398 / (1/a^2 + 0.427/b^2)$
Brick (3D)	$\alpha.f_h = 0.933 / (1/a^2 + 1/b^2 + 1/c^2)$
Rectangular rod (2D)	$\alpha.f_h = 0.933 / (1/a^2 + 1/b^2)$
Infinite cylinder (1D)	$\alpha.f_h = 0.398a^2$
Sphere (1D)	$\alpha.f_h = 0.233a^2$

where, α is the 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)

Selection of the 'target' food particle is essential when sizing the silicone particles to represent the slowest heating particle. This requires a compromise between the food particle dimensions and thermal diffusivity value for the food, and is not necessarily the largest particle. The key is to calculate the particle with the largest heating factor, from which the silicone particles can be sized. It is best that the same geometry is used for the food and silicone particles in order to ensure similarity in the temperature lag experienced at the particle centre and in the heat transfer conditions at the surfaces.

For example, a 10 mm Sylgard particle ($\alpha_{Sylgard} = 1.02 \times 10^{-7}$ $m^2.s^{-1}$) has an f_h of 76 s which equates to a typical food particle of 11.7 mm in size ($\alpha_{food} = 1.4 \times 10^{-7}$ $m^2.s^{-1}$). This conversion can be carried out for different particle sizes and thermal diffusivities, having first selected the target food particle.

2.3 Feasibility Studies at CCFRA Using Particles Immersed in a Boiling Water Bath

A series of trials were conducted to assess the accuracy of the integrated pasteurisation (or cook) values achieved using the TTI method. This was considered a necessary step in order to lend weight to the argument that the TTI encapsulation method was a viable means to validate thermal processes. The tests used 10 mm silicone cubes with ~ 0.04 mL of the TTI injected into an air bubble at the cube centres. All of the tests were conducted in a vigorously boiling water bath with data recorded at 15 s intervals using a Grant Squirrel logger (Grant Instruments). On removal of the particles from the bath they were immersed into cold water to stop the TTI reactions from proceeding.

2.3.1 Cook Values Measured with NPG Particles and Thermocouples

Cook values were measured for 7 particles injected with NPG and 4 with thermocouples at the centres. Following heating, 0.02 ml of NPG was extracted using a hypodermic syringe and analysed using the procedure outlined in section 1.1.1.

2.3.2 Distribution of Pasteurisation Values from Amylase Particles Heated under Controlled Conditions

The distribution of P-values (T_{ref} = 85°C, z = 10 C°) was measured using 43 particles injected with α-amylase. The mean, maximum and minimum values were compared with those from 0.2 mm type K thermocouple measurements at the centres of 4 particles. Extraction was as outlined above and analysis of the α-amylase followed the procedure outlined in section 1.1.2.

2.4 Feasibility Studies to Validate a Commercial Batch-Continuous Fruit Process for a Strawberry Yogfruit

The first commercial test for the TTI particles was to validate a process for a strawberry yogfruit product. Figure 1 shows a schematic layout of the key heat transfer and shearing operations for the fruit processing line. The trial used a 400 kg batch prepared with frozen 10-12 mm strawberries in a Colflo 67 carrier liquid (National Starch, Manchester). The 10 mm TTI particles, 12 with amylase and 12 with NPG, were added to the cold batch and allowed to move within the product according to the mixing patterns. The batch was heated to 90 °C in a steam jacketed vessel (Giusti) with horizontal scraped surface agitation at about 15 rpm, and held at the target temperature for 5 minutes before being cooled to 35-45 °C in a 5-pass 50 mm Spiraflo (Tetra Pak). The product flowrate during cooling was 1,200 $L.h^{-1}$. Figure 2 shows the heating and holding profile of the carrier liquid, taken by manual temperature sampling at the batch cold spots. The TTI particles were retrieved from the 400 kg tank at the end of the process using a course sieve and the product re-used for the next trial.

The objective was to test the integrity of the silicone particles through an industrial process and thus to gain a measure of the level of pasteurisation achieved. The target thermal process for the strawberry yogfruit was to exceed the equivalent of 5 minutes at 85 °C (T_{ref} = 85 °C, z = 10 C°).

The second trial used the reclaimed (once-processed) strawberry batch but with the addition of 30 kg of Colflo 67 slurry (2:1, water:Colflo) to increase its viscosity. The TTI particles, 12 NPG and 12 amylase, were added to the process vessel and the batch heated until the target of 90 °C was reached.

The objective here was to assess the progress of the pasteurisation by stopping the agitator every 5 minutes and removing a TTI particle. On finding a TTI particle, it was removed and cooled in cold water to slow down the chemical reactions; the batch temperature was taken together with the time of removal (Figure 3). At the end of the Spiraflo cooling process the remaining particles were retrieved from the product disposal bins using the sieve and labelled as representing the final batch P-values.

Figure 1: Schematic layout of the fruit processing line used for production of two batches of a strawberry yogfruit product

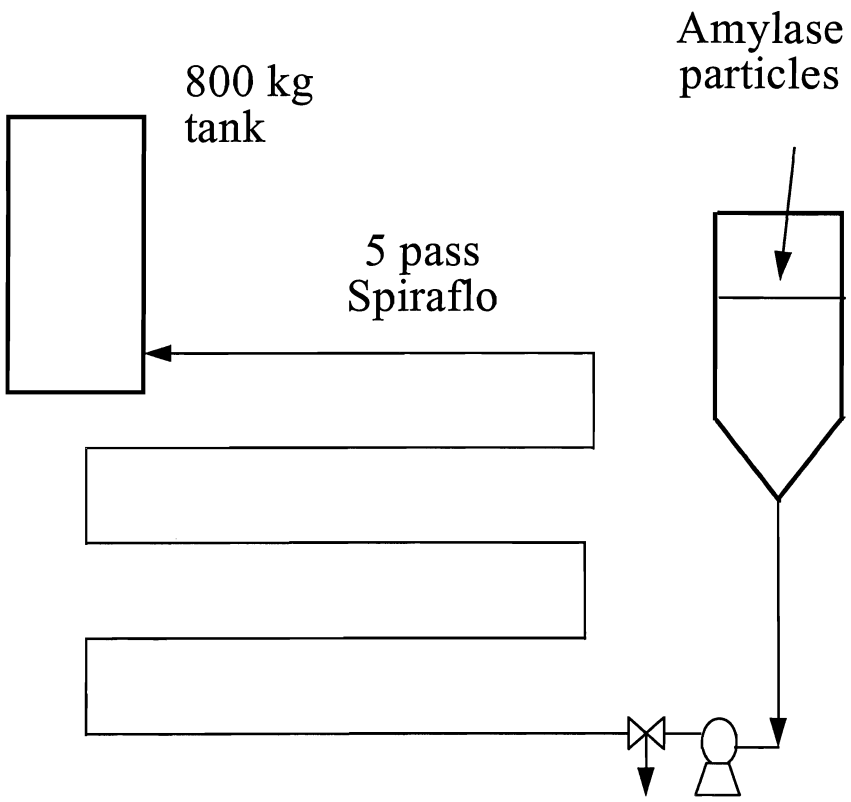


Figure 2: Heating and holding temperature profile of the strawberry carrier liquid for batch 1: taken by temperature sampling of the batch cold spots

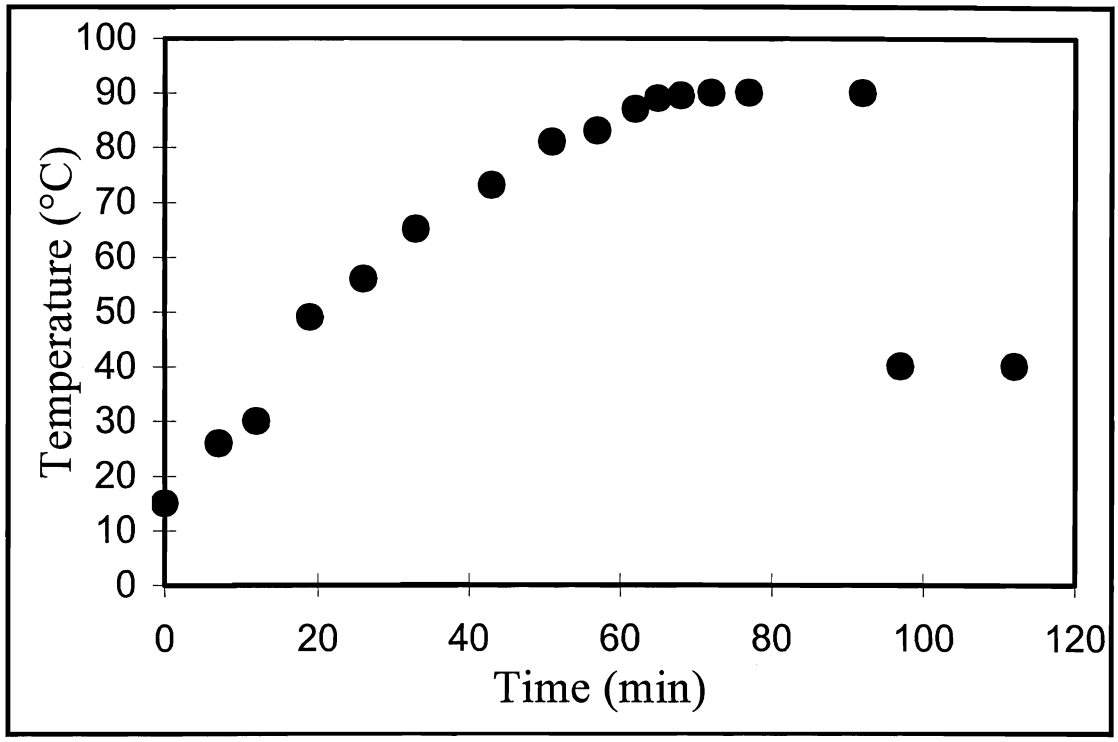
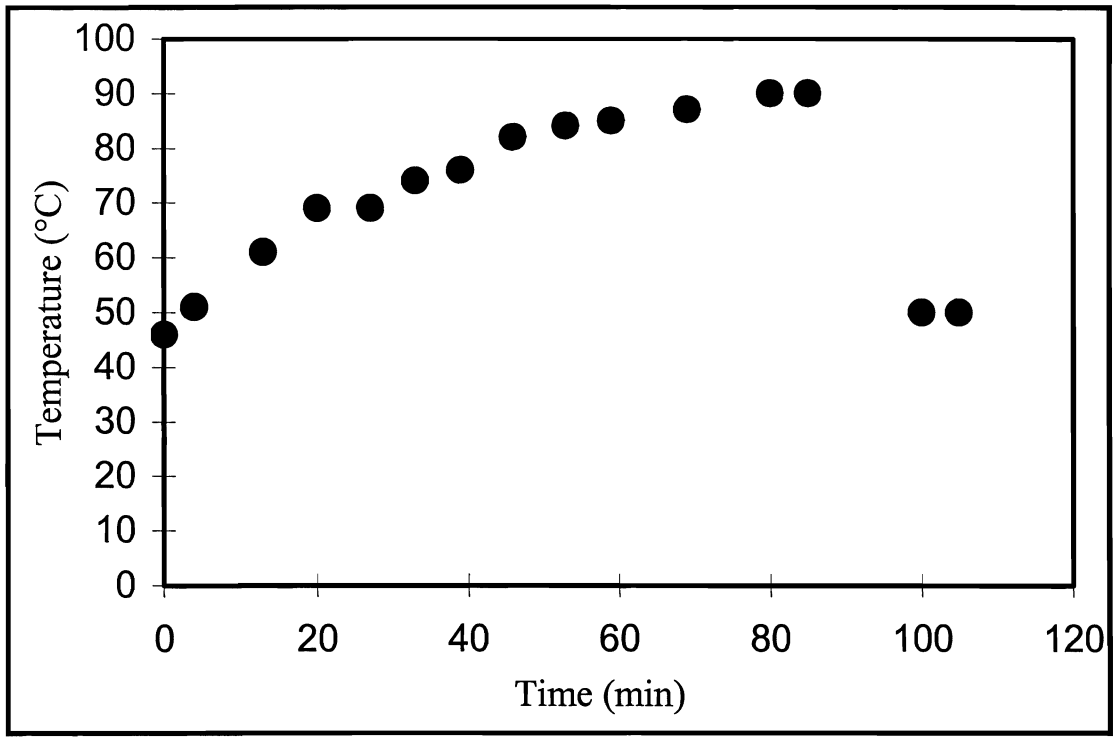


Figure 3: Heating and holding temperature profile of the thickened strawberry carrier liquid for batch 2: taken by temperature sampling of the batch cold spots



2.5 Validation of a Commercial Batch-Continuous Process for a Pineapple and Passion Yogfruit

Improvements to the methods used for particle construction were made following the strawberry process validation trials in order to strengthen the silicone particles. The procedure given in section 2.1 was the result of experience in the trials at CCFRA and in the fruit processing plant.

The fruit product chosen for the pasteurisation validation trials was a pineapple and passion fruit mixture, with 10 mm diced pineapple. This was chosen so that the same 10 mm particle moulds could be used and more replicate particles incorporated into the batches. The processing method was the same as that described in section 2.4 but with batch sizes of 500 and 430kg respectively. Temperature sampling of these batches was not done until they were close to 90 °C, otherwise too much heat would have been lost and the heating times would have been unnecessarily long.

The objective of these two trials was to use the amylase particle method to validate the efficacy of the delivered thermal process. This included contributions in the processing vessel during heating and holding, but also during cooling in the tubular heat exchanger.

The product pH target was in the range 3.2-3.5, with a target thermal process to exceed the equivalent of 5 minutes at 85 °C (T_{ref} = 85 °C, z = 10 C°). The product composition was 26.6 wt% of 10 mm pineapple cubes, with various thickeners (0.36 wt% pectin, 0.23 wt% LBG, 2.75 wt% starch) and sugar, water, flavourings and colourings.

For the first trial, 75 amylase particles were added to the cold product and the batch processed according to the normal production schedules. This number of particles was chosen so that the distribution of P-values could be assessed.

For the second trial, 45 amylase particles were added to the cold batch and the vessel operators instructed to give the batch the minimal process. This was a challenge to the pasteurisation in that the 430 kg batch size was the minimum that would be run in normal production and as such would heat up more rapidly than the larger batches up to 800 kg. The reasons for choosing 45 particles to define the P-values for this batch of 430 kg are given in section 2.5.1 below.

2.5.1 Statistical Requirements for Sample Replicates

The number of amylase particles required to fully define the P-values for the 430 kg batch of pineapple and passion fruit mixture was determined using the sampling plans given in BS 6001: Part 1: 1991. These guidelines are intended for quality sampling but the underlying thought process for safety sampling is similar and they provide a rationale for selecting a sample population.

In statistical terms it is impossible to be 100 % sure that no particle has a P-value less than the target of 5 minutes since statistical theory assumes that defects will occur and therefore an acceptable level for defects has to be chosen. Sample sizes become larger and unmanageable as the degree of confidence increases, and for this reason the Acceptable Quality Level (AQL) for this product was chosen to be 95 % (or 5 % of defective units). This may seem to have been an unacceptably high defect level since the sampling regime was used for safety assessment; however, the amylase particles incurred a significant safety margin because they were the same size as the target pineapple pieces.

The 'defective unit' as defined in this example was taken to be the end product for this processed yogfruit, that was a 150 g pot of yoghurt containing 25 % of the pineapple and passion fruit mixture. A special inspection sampling plan was used with the S-3 level because this represented one of the more intensive sampling regimes. The calculation of sample numbers first required a calculation of the number of pots of yoghurt that could be produced from this 430 kg batch. This gave 11,460 pots, for which the sampling plan was designed to ensure that less than 5 % of pots contained a pineapple with a P-value less than 5 minutes. For lot or batch sizes between 3,201 and 10,000, the number of samples to be randomly selected at special inspection level S-3 was 32.

To ensure that no P-values were less than 5 minutes in the production process it was necessary to build in a safety margin in designing the amylase particles. This was achieved by utilising the thermal properties of the silicone encapsulating material that were approximately 25 % less than those of the fruit products. Manufacturing the amylase particles to be the of same size as the target fruit pieces ensured that the process would be measured safely.

The number of particles to be used will vary with different processes and careful consideration must be given to this issue and the subsequent use of the results.

2.6 Validation of a Commercial Batch-Continuous Process for an Apricot Fool Yogfruit

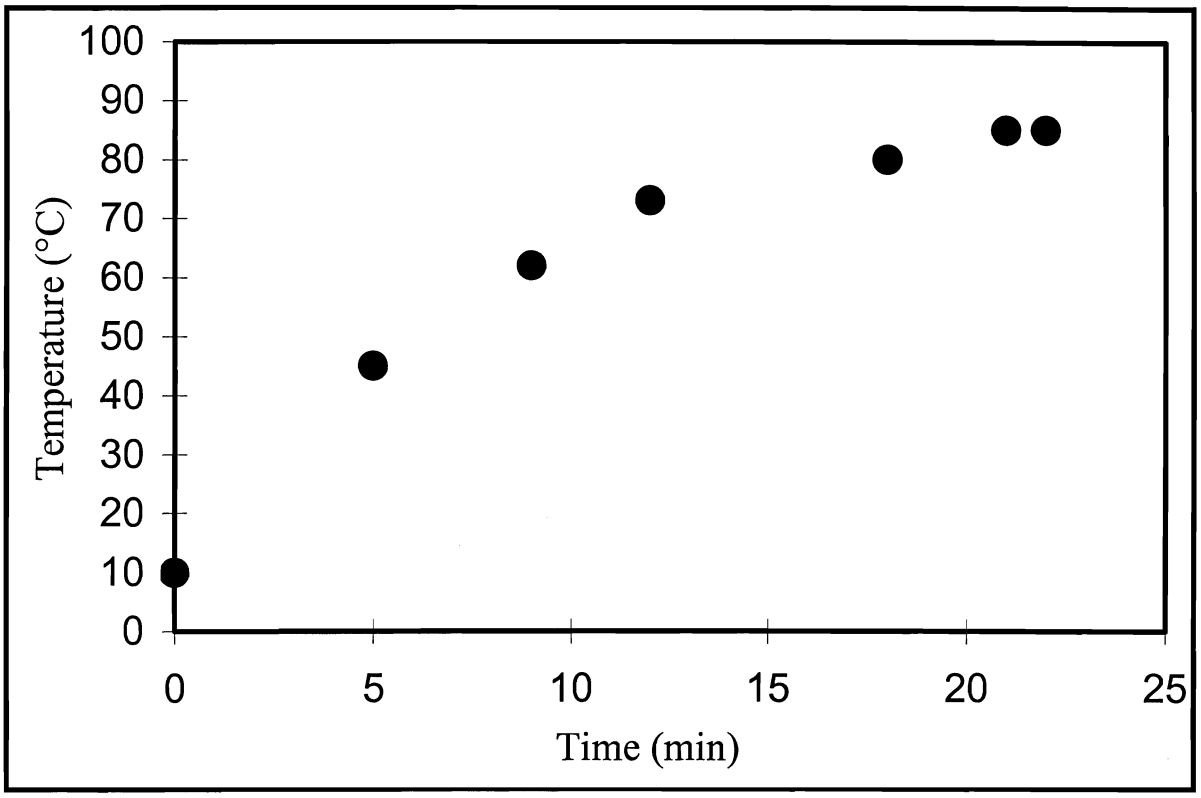
Having improved on the method for manufacturing the 10 mm amylase particles, a trial was conducted to validate the pasteurisation achieved at the centre of a larger fruit piece. The product chosen was an apricot fool that contained roughly chopped apricot given as a nominal 20 mm. On sorting through several kilos of apricot it was decided that the largest and therefore the target particle was equivalent to a 17 mm cube of apricot. The silicone moulds were constructed for 14 mm cubes in order that the P-values measured with the amylase particles were representative of the target apricot particles, albeit with a small oversize margin for safety. The calculation procedure used to determine the appropriate silicone particle size is given in section 2.2, with the thermal diffusivities of the silicone and pineapple as before.

The heating methodology was the same as that described in sections 2.4 and 2.5, but with a batch size of only 325 kg, the time to reach 90 °C was reduced. 45 amylase particles were added to the cold batch and the vessel operators instructed to give the batch the minimal process as a challenge to the delivered pasteurisation. Temperature sampling of the processing vessel was done at infrequent intervals until the temperature was close to 90 °C (see Figure 4). When processed, the product was hot filled into 5 kg Pergall bags directly from the vessel and the bags cooled rapidly in a forced convection chiller.

The objective of this trial was to use the amylase particle method to validate the efficacy of the delivered thermal process.

The product pH target was in the range 3.3-3.8, with a target thermal process to exceed the equivalent of 5 minutes at 85 °C (T_{ref} = 85 °C, z = 10 C°). Product composition was 44 wt% of 17 mm apricot, 8.9 wt% of Clearam CH20 starch thickener added as a 1:2 slurry, and sugar, water, flavourings and colourings.

Figure 4: Heating and holding temperature profile of the apricot fool carrier liquid: taken by temperature sampling of the batch cold spots

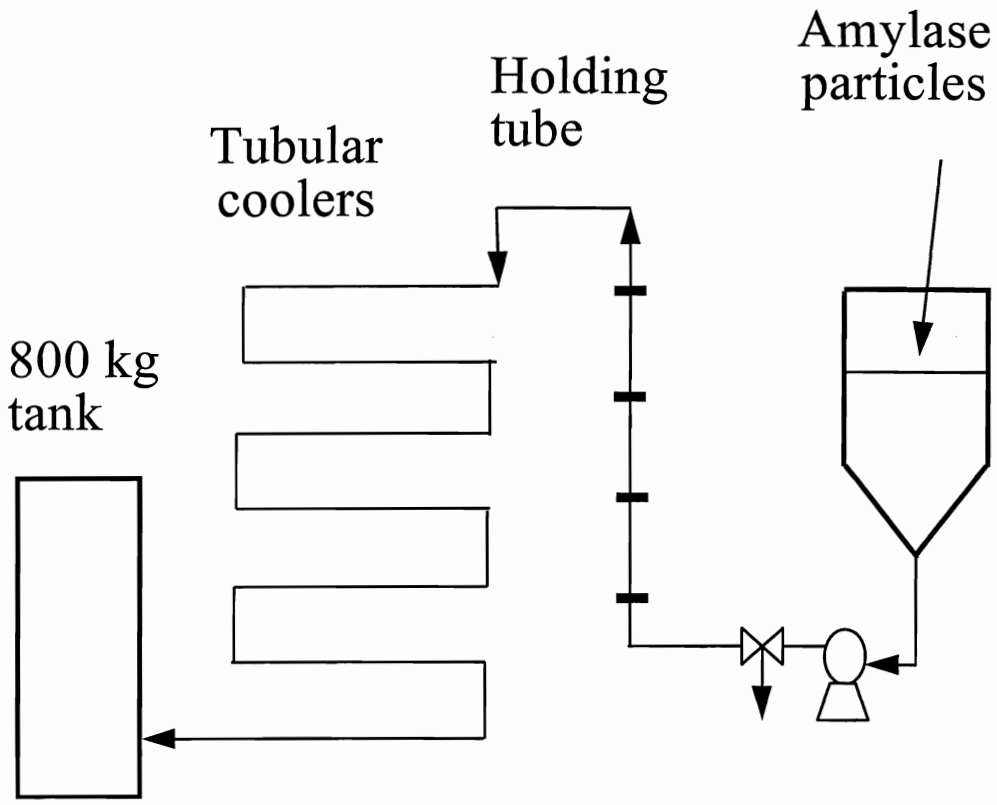


2.7 Validation of a Commercial Ohmic Process for a Strawberry Yogfruit

A feasibility trial was conducted on a commercial scale ohmic heater (APV Ltd). The 75 kW ohmic heater used four PTFE lined electrodes which gave a capacity of 750 $kg.h^{-1}$ (see Figure 5). Heating of the strawberries took place via volumetric resistance heating and at a faster rate than the surrounding carrier liquid. This was controlled using the electrical conductivity of the batch, which was measured at around 0.24 $S.m^{-1}$. Cooling was achieved using a series of water cooled tubular heat exchangers. A gelatinised starch solution of the same conductivity as the strawberry yogfruit was pumped through the ohmic column to recover as much of the strawberry product from the pipework as possible before having to divert to drain. Processed product was filled into a 400 kg bulk tank at temperatures between 25 and 50 °C.

The objective of the trial was to test the 5 mm silicone beads containing the amylase TTI as a validation method for the ohmic process. For this trial the small silicone enclosed air bubbles, containing the amylase, were not made up into larger silicone particles but put directly into the strawberries. A 250 kg batch of re-worked strawberry product was used as the feed batch with a strawberry size quoted as nominal 10-12 mm.

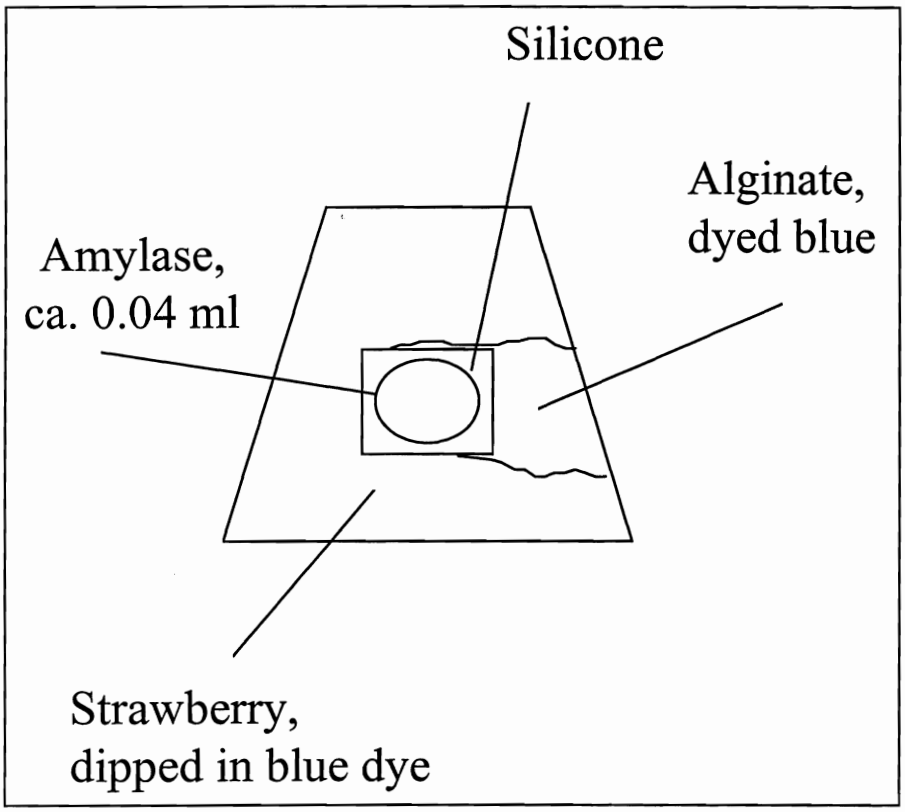
Figure 5: Schematic layout of the fruit processing line used for production of two batches of a strawberry yogfruit product



The 'amylase strawberries' were constructed from whole frozen strawberries that had been allowed to thaw for one hour before insertion of the silicone encapsulated amylase. Approximately 0.04 mL of amylase solution was injected into 3 mm diameter silicone bubbles that were held within 5 mm silicone cubes. The method for incorporating the bubble was given in section 2.1 of this report. The silicone cubes were inserted through a hole to the centre of the strawberries, and sealed with a calcium alginate gel. Blue dye was allowed to soak into the 'amylase strawberries' for 1 hour in order that they could be located in the 250 kg of processed batch. Figure 6 shows the completed strawberry in schematic form. A total of 15 whole 'amylase strawberries' were constructed and added to the 250 kg feed batch.

The target thermal process to achieve a commercially pasteurised product was equivalent to 90 °C for 90 seconds.

Figure 6: Schematic of the construction of the 'amylase strawberries' from a nominal 10-12 mm whole strawberry



3. RESULTS AND DISCUSSION

3.1 Feasibility Studies using Particles Immersed in a Boiling Water Bath

3.1.1 Cook Values Measured with NPG Particles and Thermocouples

The final NPG colour absorbance (A_{final}) was calculated from the initial absorbance at 300 nm for two unheated NPG samples, giving values of 1.55 and 1.63 with a mean of 1.59. Table 4 presents the cook values calculated from these absorbance values for the NPG extracted from the silicone particles. One of the particles contained insufficient NPG for the test.

Table 4: Cook values calculated from final absorbance values for the 10 mm silicone particles injected with NPG

Sample number	A_t at 400 nm	Cook-value (minutes)
1	0.062	1.5
2	0.074	1.8
3	0.082	2.0
4	0.079	2.0
5	0.079	2.0
6	0.078	1.9
7	0.078	1.9

The cook values calculated from the particle centre temperature data were 2.9, 2.9, 3.3 and 3.0 minutes, with a mean of 3.0 minutes. A reference temperature of 100°C and z-value of 23 C° were used in the calculations. The mean of 3.0 minutes compared with a mean cook value of 1.9 minutes from the NPG particles. Obtaining an exact match of cook values is difficult because of the uncertainty with temperature measurements in particles of small size. Errors can arise due to conduction errors (McKenna and Tucker, 1991), capillary action of the hot water along the thermocouple hole and in the precise location of the measuring junction at the centre. The type T thermocouple cable is comprised of a copper and constantan pair of wires and it has been suspected that significant heat conduction can occur along the copper wire. Some doubts were also present in these trials concerning the seal between thermocouple wire and the silicone. Each of these errors will artificially increase the cook values calculated from thermocouple measurements.

3.1.2 Distribution of P-Values from Amylase Particles Immersed in a Boiling Water Bath

The initial amylase activity was measured using 10 samples, giving a mean value of 0.969 minutes. The P-values presented in Table 5 and in Figure 6 were calculated from the final amylase activities for 35 of the particles. It was found that for 8 of the particles there was insufficient enzyme activity to measure with sufficient accuracy.

The mean P-value calculated from thermocouple measurements was greater than that from the amylase activities but there was a less significant difference than with the first set of cook value trials using NPG. This was because the thermocouples were type K (chrome - alumel) which suffered less from thermal conduction than type T and they were set into the silicone rather than pushed to the centre through a hole. The agreement between the two methods was considered to be good, given the potential for discrepancy.

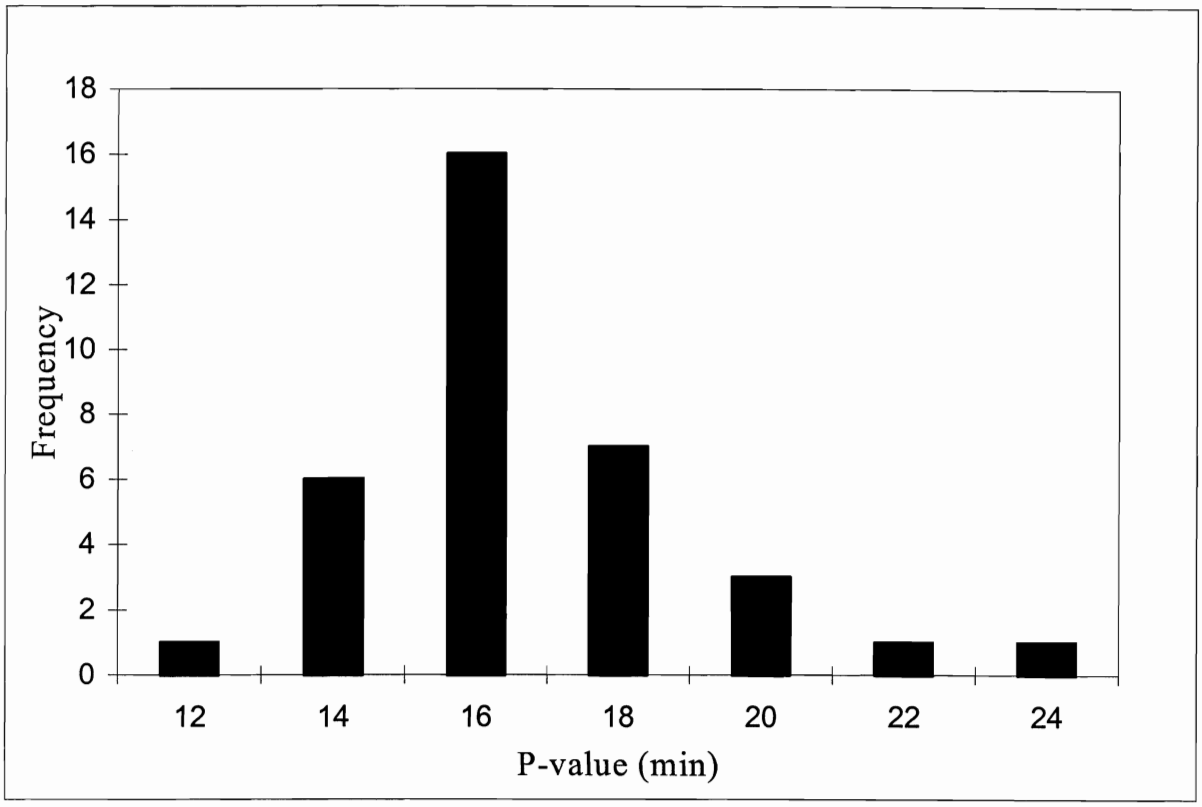
Table 5: Final amylase activity and P-values (T_{ref} = 85 °C, z = 10 C°) for the 10 mm silicone particles immersed in a boiling water bath

A_{final} (min)	P-value (min)	A_{final} (min)	P-value (min)	A_{final} (min)	P-value (min)	A_{final} (min)	P-value (min)	A_{final} (min)	P-value (min)
0.013	13.1	0.001	21.1	0.003	17.9	0.007	14.9	0.006	15.6
0.011	13.6	0.004	16.5	0.002	19.0	0.009	14.2	0.012	13.2
0.004	16.9	0.011	13.6	0.002	19.2	0.007	15.1	0.007	15.0
0.003	17.3	0.011	13.6	0.005	16.0	0.003	17.5	0.008	14.5
0.008	14.6	0.005	15.8	0.009	14.2	0.025	11.0	0.004	17.0
0.007	14.8	0.007	14.8	0.009	14.3	0.009	14.1	0.009	14.1
0.009	14.2	0.001	22.8	0.006	15.5	0.001	19.7	0.010	13.8

The high P-values calculated from amylase activities of 0.002 and less were at the limit of the spectrophotometer measuring accuracy. The correlation coefficients for the measured gradients in the tests were less than 0.91 for values below 0.002, whereas for values above 0.004 they were above 0.98.

In practice it was not possible to measure activity much in excess of two log reductions with the present amylase assay method. At 85 °C this gave a maximum P-value of 14 minutes, with values significantly in excess of this having questionable accuracy. However, if the method was used for process validation it would be possible to deduce that the P-values had exceeded 14 minutes but it would not be possible to measure by how much.

Figure 6: P-value (T_{ref}=85 °C, z=10 C°) distribution for the 10 mm amylase particles immersed in a boiling water bath



The comparison of mean, high and low P-values between the amylase and thermocouple results are given in Table 6. The P-values were similar but with those calculated using amylase giving consistently lower values, for the reasons explained in sections 3.1.1 and 3.1.2. This provided further evidence that the amylase particle method was acceptable to use as a validation tool for selected pasteurisation processes.

Table 6: Summary of the P-values (T_{ref} = 85 °C, z = 10 C°) for the 10 mm silicone particles heated in a vigorously boiling water bath, as calculated from amylase and thermocouple measurements

	Mean (min)	High (min)	Low (min)
Amylase (43 particles)	15.7	22.8	11.0
Thermocouples (4 particles)	17.7	20.9	15.3

3.2 Feasibility Studies to Validate a Batch-Continuous Strawberry Yogfruit Process

In the first 400 kg strawberry trial, 12 amylase and 12 NPG particles were introduced to the batch, with 10 amylase and 11 NPG particles recovered intact at the end of the process. Evidence of particle breakdown was found for the remaining particles. The results from the colour analysis for NPG were converted to cook values (see table 7) and the results from the amylase assays were converted to P-values for reference temperatures of 80.7, 85.0 and 93.3 °C (see table 8). The P-values for a reference temperature of 85 °C were compared with the target of 5 minutes equivalent at 85 °C for this product.

Table 7: Cook value results from the NPG trials on a batch-continuous process for a commercial strawberry product

Sample	Absorbance at 400 nm	Cook-value (min)
1	0.285	6.2
2	0.217	4.6
3	0.210	4.5
4	0.272	5.9
5	0.207	4.4
6	0.267	5.8
7	0.272	5.9
8	0.277	6.0
9	0.264	5.7
10	0.257	5.6
11	0.251	5.4

Table 8: P-value results from the amylase trials on a batch-continuous process for a commercial strawberry product

Sample number	Absorbance at 405 nm	P-value for $D_{80.7}$ (min)	P-value for $D_{85.0}$ (min)	P-value for $D_{93.3}$ (min)
1	0.003	45.9	17.1	2.5
2	0.011	35.3	13.2	2.0
3	0.011	35.3	13.2	2.0
4	0.007	39.0	14.5	2.2
5	0.005	22.3	8.3	1.2
6	0.054	22.4	8.4	1.2
7	0.003	45.9	17.1	2.5
8	0.003	45.9	17.1	2.5
9	0.002	49.1	18.3	2.7
10	0.003	45.9	17.1	2.5

The P-value results confirmed that the target of 5 minutes equivalent at 85 °C was achieved: the safety criterion for this product. The range of P-values was from 8.3 to 18.3 minutes. This wide range was anticipated because of the difficulty in heating large batches of viscous products that experience a range of vessel temperatures. Heat transfer efficiency from the vessel wall to the starch-based carrying liquid and subsequently to the strawberries cannot be controlled uniformly throughout the batch. The end result was a distribution of P-values during heating, that was further complicated because of the continuous nature of the cooling process. At the end of the 5 minute hold at 90 °C, hot product remained in the vessel for between 0 and 40 minutes before being pumped through the Spiraflo cooler. The product temperature was not maintained at 90 °C in the vessel during the Spiraflo cooling process, thus some product experienced up to an extra 40 minutes of heating at temperatures that started at 90 °C and fell to 70 °C in the 40 minutes.

In the second 400 kg strawberry trial (with 30 kg of Colflo 67 slurry added), 12 amylase and 12 NPG particles were introduced to the batch, with 8 amylase and all 12 NPG particles recovered intact. Table 9 presents the cook values calculated from colour absorbance with the NPG and Table 10 shows the P-values from amylase activity.

This showed that the level of pasteurisation was minimal until the later stages of the process. There were insufficient amylase particles removed from the batch to plot the increase in P-value with heating time. However, with a batch temperature of 84 °C after 53 minutes of heating, the centre particle P-value had only reached 1.2 minutes. Therefore, these results show that it was imperative that the batch temperature reached 90 °C in all locations within the process vessel before the particle centres were adequately pasteurised.

Table 9: Cook value results from the NPG trials on a batch-continuous process for a commercial strawberry product, with 30 kg Colflo 67 slurry added to the re-worked product

Sample removal number	Elapsed time (min)	Absorbance at 400nm	Product temperature (°C)	Cook-value (min)
1	0	0.069	46.0	1.4
4	20	0.063	69.0	1.3
5	27	0.067	69.0	1.4
6	33	0.069	74.0	1.4
10	59	0.093	85.0	1.9
11	69	0.205	87.0	4.4
12	80	0.277	90.0	6.0
-	end of cooling	0.369	-	8.3
-	end of cooling	0.367	-	8.2
-	end of cooling	0.331	-	7.3
-	end of cooling	0.318	-	7.0
-	end of cooling	0.329	-	7.3

Table 10: P-values from the amylase trials on a batch-continuous process for a commercial strawberry product, with 30 kg Colflo 67 slurry added to the re-worked product

Sample removal number	Elapsed time (min)	Absorbance at 400nm	Product temperature (°C)	P-value for $D_{80.7}$ (min)	P-value for $D_{85.0}$ (min)	P-value for $D_{93.3}$ (min)
2	4	0.882	51.0	-0.2	-0.1	0.0
3	13	0.922	61.0	-0.5	-0.2	0.0
7	39	0.764	76.0	1.0	0.4	0.1
8	46	0.633	82.0	2.5	0.9	0.1
9	53	0.573	84.0	3.3	1.2	0.2
-	end of cooling	0.003	-	45.9	17.1	2.5
-	end of cooling	0.002	-	49.1	18.3	2.7
-	end of cooling	0.003	-	45.9	17.1	2.5

3.3 Validation of a Commercial Batch-Continuous Process for a Pineapple and Passion Fruit Product

The two batches were filled into separate 800 kg tanks and transferred to the fruit sorting area for locating the amylase particles. In the first batch, 71 out of 75 processed 'amylase particles' were recovered intact and in the second batch 44 out of 45. From these intact silicone cubes and the 4 control cubes per trial, 0.02 mL of amylase solution was extracted for the assays. In the first trial the method for sealing the hypodermic entry hole was insufficient and it was possible to extract 0.02 mL from only 21 of the 71 particles. The sealing method was further improved for the second batch and all 44 particles retained their amylase solution.

Figures 6 and 7 present the distributions of pasteurisation values as measured from a reduction in amylase activity. Pasteurisation values were calculated from equation 2 using $D_{85} = 6.95$ minutes. Both figures showed mean P-values in excess of the 5 minutes minimum. The 500kg batch required longer to process and therefore the mean P-value was higher (16.2 minutes) than that for the 430kg batch (12.4 minutes). This was caused by the increased heat load of the 500kg batch. The minimum values of 6.0 and 10.1 minutes were thought to represent particles that cooled immediately through the Spiraflo, whereas the maximum value of 15.0 and 18.2 minutes were for those that remained in the process vessel until the last portions of the batch were cooled.

Optimisation of the batch cooking processes can be achieved by considering these distributions. For the small batch sizes (e.g. 430 kg) the process time was close to the optimal value, giving P-values at the particle centres of 6 minutes. For the batch sizes greater than this there is scope to reduce the process time in order to move the minimum P-value closer to 5 minutes.

Figure 6: Distribution of pasteurisation values for the 500 kg batch of 10 mm pineapple yogfruit, calculated with D_{85} = 6.95 minutes. Sample size was 21.

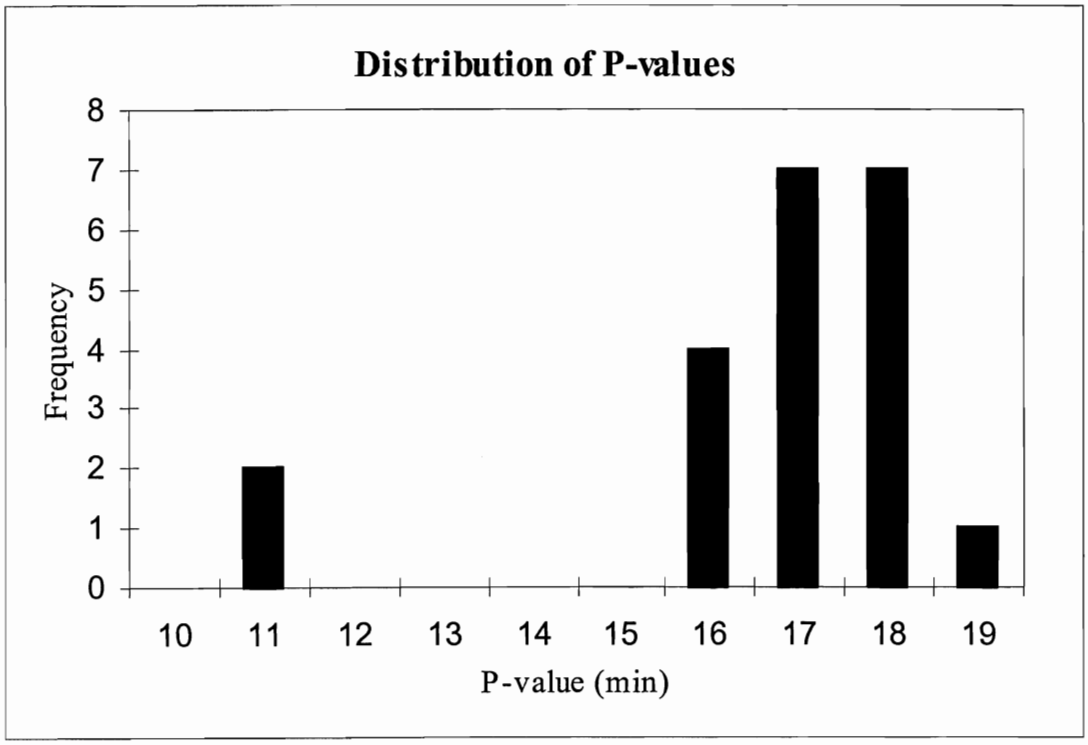
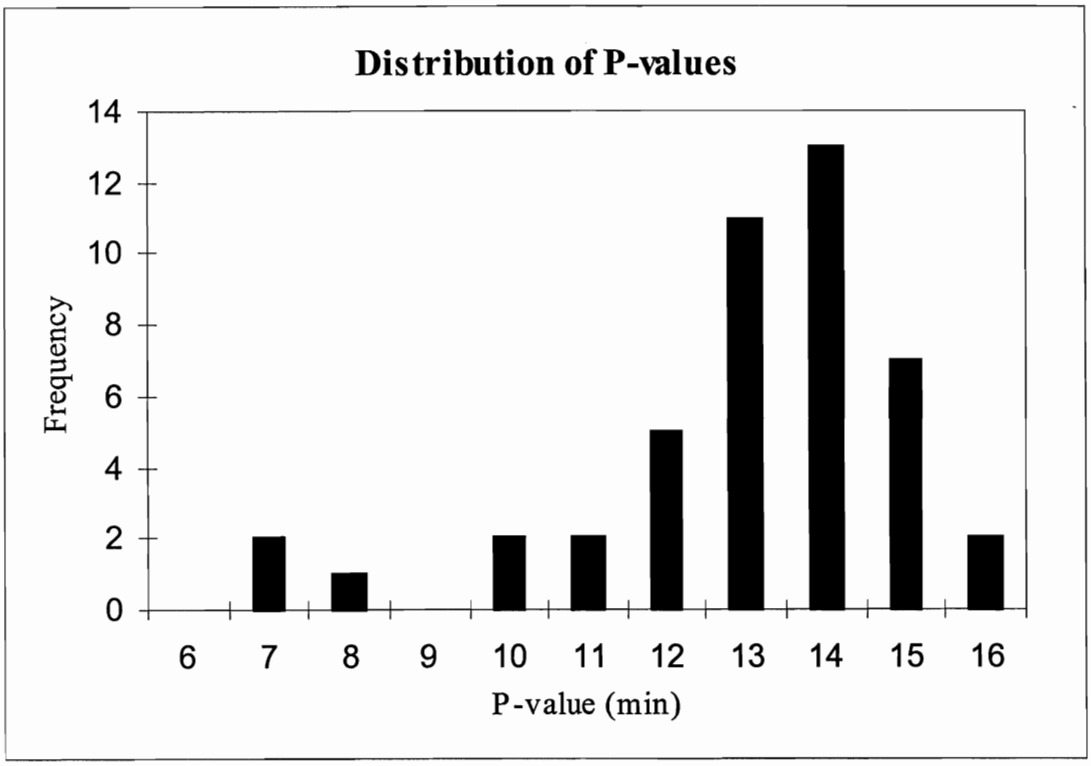


Figure 7: Distribution of pasteurisation values for the 430 kg batch of 10 mm pineapple yogfruit, calculated with D_{85} = 6.95 minutes. Sample size was 44.



3.3.1 Rheological Measurements and Calculation of the Tube Reynolds Numbers

Measurement of the rheological properties of foods containing high concentrations of large particulates is very difficult and requires large scale techniques such as tube viscometry. This is a method that correlates the pressure drop over a straight pipe length with flowrate and converts these measurements to the traditional rheological terms of wall shear stress and wall shear rate respectively (Heydon et al., 1996). Such data was available from pressure sensor readings at the entrance and exit from the Spiraflo used to cool the yogfruit products. Each Spiraflo tube was 6.0 m with additional 2" diameter pipe from the equivalent lengths for the bends and entrance and exit pipes. For the first two strawberry batches, a 5-pass set-up was used, with the total length of 2" pipe between pressure sensors estimated at 33 m. For the first 500 kg pineapple and passion fruit batch a 7-pass set-up was used, with the total length of 2" pipe between pressure sensors estimated at 42 m. An example of the spreadsheet of calculations is given in Figure 8 for this batch of product. For the second 430 kg pineapple and passion fruit batch, a 9-pass set-up was used, with the total length of 2" pipe between pressure sensors estimated at 59 m. The increased number of Spiraflo cooling tubes between the first and second pineapple and passion fruit trials was used to impose the fastest cooling rates for the product in order that the entire heating and cooling process was the minimum that this product was likely to experience.

Tube Reynolds numbers were calculated for these products, assuming that a power law relationship existed between the wall shear stress and wall shear rate (see equation 3). The power law has been used for these types of products (Bolmstedt, 1998). To confirm that the flow conditions were laminar, the calculated Reynolds numbers should be less than 2,100. If the n value of 1 is inserted into equation 4, the familiar tube Reynolds number is obtained ($Re = \rho.D.v / \eta$).

$$\sigma = k.\gamma^{n} \qquad \text{.....(3)}$$

$$Re = \frac{\rho.D^{n}v^{2-n}}{k}\left[\frac{6n+2}{n}\right]^{1-n} \qquad \text{.....(4)}$$

Table 11: Tube Reynolds numbers taken during cooling in a 5-pass 2" diameter Spiraflo, at a mean velocity of 0.0625 $m.s^{-1}$, for a mean product density of 1,050 $kg.m^{-3}$

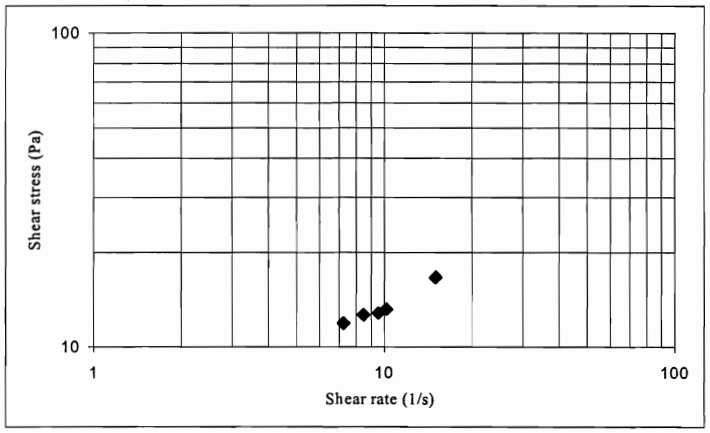
Product	Apparent viscosity at a shear rate of 10 s^{-1}(Pa.s)	Reynolds number
Strawberry batch 1, 400 kg	6.50	0.5
Strawberry batch 2 (thickened), 430 kg	8.60	0.4
Pineapple and passion fruit batch 1, 500 kg	1.35	2.4
Pineapple and passion fruit batch 1, 430 kg	1.30	2.5

Figure 8: Tube viscometer data taken for the first batch of pineapple and passion fruit product, during cooling in a 5-pass 2" Spiraflo

Tube viscometer data during cooling

Pipe diameter:	0.05	m
Pressure readings:	45.0	m length between sensors
Product temperature in:	80-90	°C
Product temperature out:	35-45	°C

P1 (psi)	P2 (psi)	Delta P (N/m^2)	Flowrate (l/h)	Velocity (m/s)	Shear Rate (1/s)	Shear stress (Pa)	Viscosity (Pa.s)	(cP)
13.2	6.3	47576	448	0.0634	10.14	13.22	1.30	1303
15.2	6.5	59987	662	0.0937	14.98	16.66	1.11	1112
12.9	6.2	46197	421	0.0596	9.53	12.83	1.35	1347
12.7	6.1	45507	375	0.0531	8.49	12.64	1.49	1489
12.3	6.1	42749	320	0.0453	7.24	11.87	1.64	1639



Flow behaviour index (95% CIs)	
n =	0.47
n_{min} =	0.30
n_{max} =	0.64

General consistency coefficient (95% CIs)	
k =	4.58 $Pa.s^n$
k_{min} =	3.07 $Pa.s^n$
k_{max} =	6.83 $Pa.s^n$

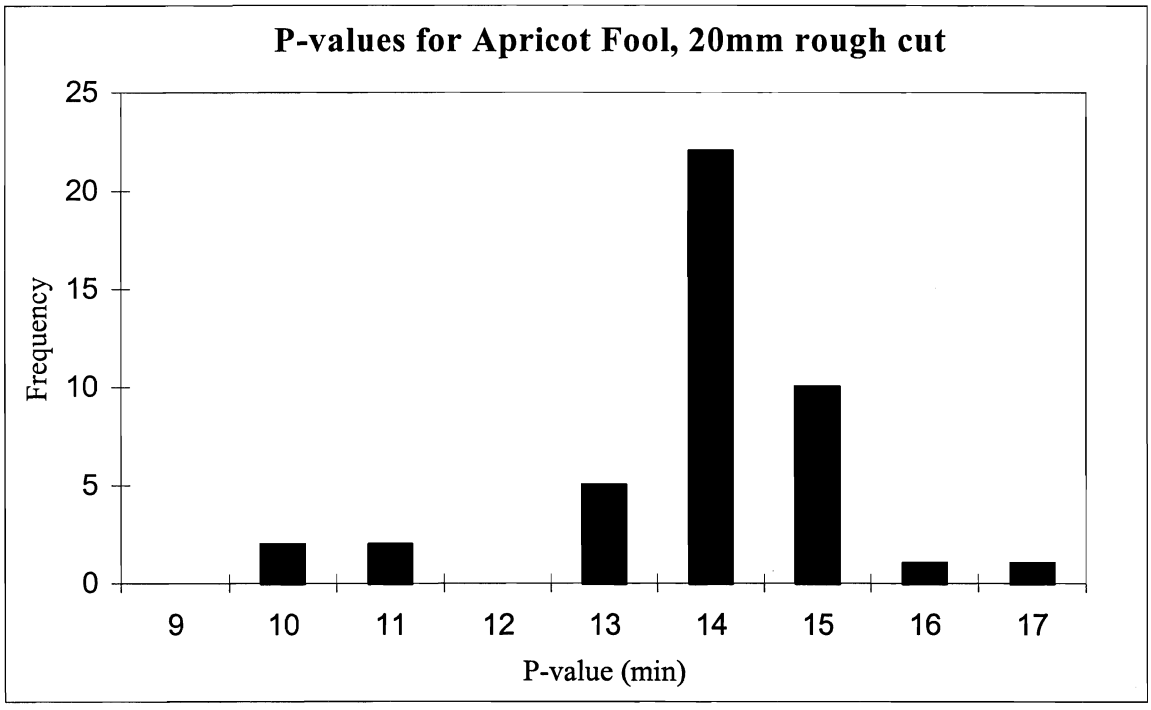
Apparent viscosity at shear rate = 10.0 s^{-1} = 1.35 Pa.s
1,350 cP

3.4 Validation of a Commercial Batch-Continuous Process for an Apricot Fool Yogfruit

44 out of the 45 processed amylase particles were recovered intact from the fruit sorting area, with the other particle found in two halves. Each particle had retained its amylase solution so that 44 assays were completed. This was a satisfactory outcome from a process that contained much potential for shear damage to the large particles during the heating, pumping and filling stages.

Figure 9 presents the distributions of pasteurisation values as measured from a reduction in amylase activity. Pasteurisation values were calculated from equation 2 using D_{85} = 6.95 minutes. No P-values were less than 9.7 minutes, and with the target being to exceed 5 minutes this was a safely processed batch. The batch size was the minimum that would be processed during normal production, and with all of the other processing vessels in operation during the trial it was concluded that the temperature rise of this batch was the fastest that could be achieved.

Figure 9: Distribution of pasteurisation values for the 325 kg batch of 17 mm apricot fool yogfruit, calculated with D_{85} = 6.95 minutes. Sample size was 45.



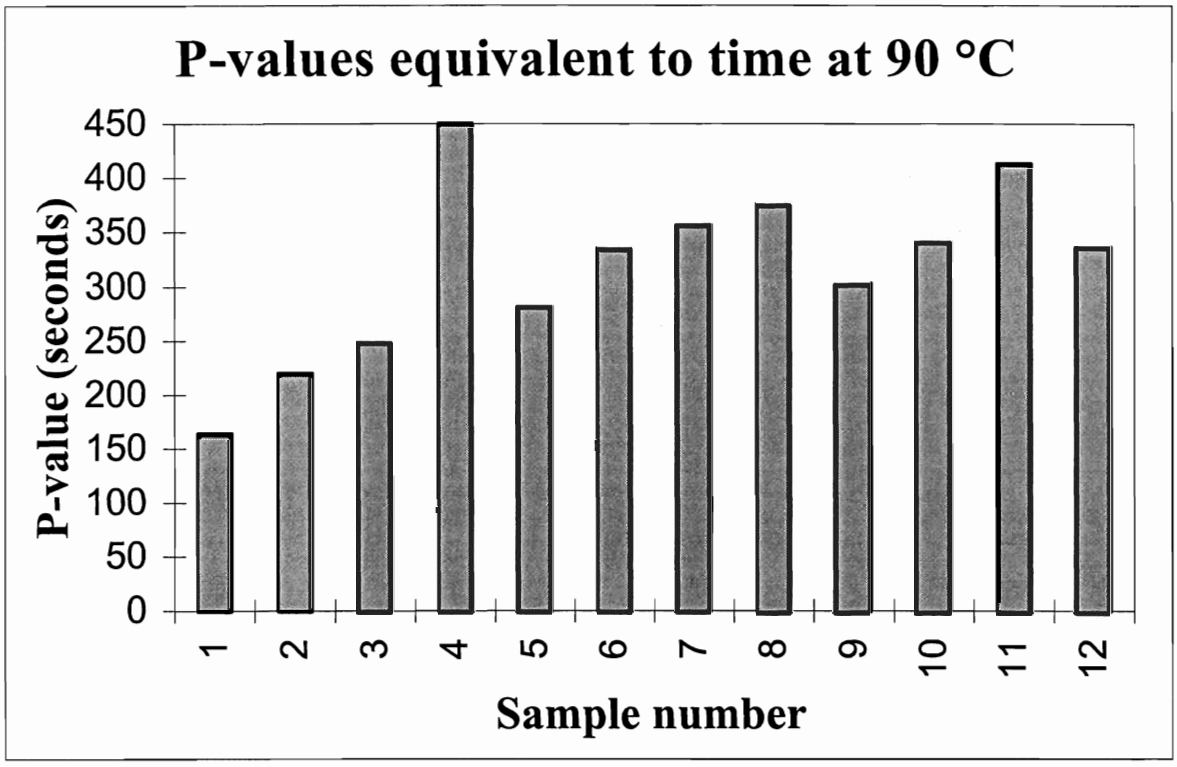
3.5 Validation of a Commercial Ohmic Process for a Strawberry Product

For the purpose of these validation trials, the target temperature at the holding tube inlet was run at 90 °C. This compared with the normal operating temperature that ranged between 92-95 °C. To achieve this temperature, the electrical conductivity figure was manually adjusted as the controller input. Confirmation that the strawberries absorbed electrical energy at a faster rate than the carrier liquid was supported by the 10 C° rise in holding tube temperature from inlet to outlet.

The holding tube was 3" diameter and 16.4 m in length, with a small vertical rise from inlet to outlet. At 750 $kg.h^{-1}$ the minimum holding tube residence time was 180 s. Calculations of pasteurisation values were done on line within the plant software. For a minimum temperature of 90 °C at the holding tube inlet the requirement of an equivalent to 90 seconds at 90 °C was easily achieved. Most product received at least double the minimum requirement due to fluctuations in temperature from 90 to 92 °C.

The 250 kg batch was filled into a 400 kg tank and transferred to the fruit sorting area for locating the 'amylase strawberries'. 12 processed 'amylase strawberries' were recovered intact, with 1 found at the product/starch interface. Remnants of the other two were found but the amylase was not extracted because there was doubt that they had remained inside the strawberries for long enough to have been adequately processed. From the 12 intact silicone cubes and the 4 control cubes 0.02 mL of amylase solution was extracted. Figure 9 presents the individual sample pasteurisation values measured from a reduction in amylase activity. Pasteurisation values were calculated from equation 2 using a D_{90} = 123 s.

Figure 10: The individual sample pasteurisation values for an ohmically heated 250 kg batch of strawberry yogfruit, measured from a reduction in amylase activity, calculated with D_{90} = 123 s.



The assay method was able to measure enzyme activity up to two log reductions from the initial value. This gave confidence in the P-values between 0 and 240 seconds, but values above this have insufficient activity for accuracy in the measurement. It was certain, however, that the P-values had exceeded two log reductions, but it was less certain by how much.

The poor electrical conductivity properties of the silicone insulated the amylase from the electrical heating effects (see Table 12 of conductivity values); therefore the reduction in amylase activity was solely due to heat conduction from the surrounding strawberry. This

resulted in conservative P-values. However, they represented cumulative totals during the heating, holding and cooling stages, and not values for the holding tube. There was also a likelihood that the strawberry in the immediate vicinity of the silicone cube did not receive any electrical heating effect. Each of these factors contributed towards pasteurisation values that erred significantly on the safe side.

Table 12: Electrical conductivity values of foods
(* taken from Fryer, 1995)

Food / material	Electrical conductivity (S/m)	Temperature range (°C)
Sylgard 184	5×10^{-19}	25
Strawberry	0.24	25
Potato *	0.04 - 0.32	10 - 100
Pork meat *	0.09 - 0.08	10 - 100
Beetroot *	0.04 - 0.75	10 - 100

Having proven that the 'amylase particle' method could work for an ohmic process, the next step would be to use greater numbers of 'amylase strawberries' in order to build up data on the distribution of P-values.

4. GENERAL DISCUSSION AND CONCLUSIONS

There are numerous food pasteurisation and sterilisation processes that are difficult, or impossible, to validate with conventional temperature probe systems. The main categories include solid products cooked in continuous belt hot air ovens or in continuous belt fryers (e.g. poultry, chicken nuggets, burgers, bread), cook-chill products with discrete lumps cooked in steam jacketed agitated vessels (e.g. ready meals, soups, cook-in-sauces, yogfruits), and products with particulates processed in continuous heat exchangers (e.g. cook-in-sauces, yogfruits).

The current industry approach to validating these products and processes can be divided into two paths:

(i) The aglinate spore technique is sometimes used whereby spores of a non-pathogenic organism (e.g. *Clostridium sporogenes, Bacillus stearothermophilus*) with similar heat resistance to the target pathogen (e.g. *Clostridium botulinum*) are embedded into an alginate bead (Brown et al, 1984). The beads are made to thermally represent the food particles and pass through the process with the food. However, this method is expensive, involves the deliberate introduction of bacterial spores into the process equipment, requires several days for the incubation of surviving spores and counting colonies, and is subject to the inherent biological variability of microorganisms.

Alternatively,

(ii) No validation is attempted, with the process safety being assumed from temperature probing of the bulk product. Gross over-processing is then allowed in order that the thermal process delivered to the core of the target particulate is deemed sufficient. Products of lesser quality can result with this approach, leaving tremendous scope for process improvement.

The applications for TTI particles will be to obtain accurate measurements of the delivered thermal processes for such processes where trailing thermocouple wires cause a hazard (Tucker, 1998 a, b). The processing technologies highlighted above represent a significant proportion of the present food production within the UK. With a growing trend towards cook-chill and cook-freeze products that are fully pasteurised it is imperative that the food industry has access to appropriate process validation techniques that lead to improved process control.

The process validation method using *Bacillus amyloliquefaciens* α-amylase was successfully applied to pasteurisation processes for yogfruit batches of 10-12 mm strawberry, 10 mm pineapple and 17 mm apricot. For these high acid fruit products, the target P-value was equivalent to 5 minutes at 85 °C (T_{ref} = 85 °C, z = 10 C°); this compared with minimum P-values measured using the amylase of 8.3, 6.0 and 9.7 minutes respectively.

The microbiological safety of an industrial ohmic process for 10-12 mm whole strawberry yogfruit was also demonstrated. This required the 5 mm encapsulated amylase bubbles to be sealed into the centres of 15 strawberries, added to the feed batch, and allowed to pass through the ohmic process unhindered. On retrieval, the amylase activities were assayed and a minimum P-value calculated of 160 s at 90 °C (T_{ref} = 90 °C, z = 10 C°); this compared with the target of 90 s.

This technique was developed and demonstrated on continuous pasteurisation processes but can be applied to almost any processes for foods that contain solid particles. A batch-continuous process and an ohmic process were used as case studies because they were examples of

processes that had previously required microbiological methods for their validation. Conventional temperature probe systems could not be used in these processes. Both of these yogfruit processes were successfully validated with the TTIs. The key step in making this method applicable as a validation tool was in the TTI encapsulation, ensuring that the enzyme was isolated from the surrounding food but could be injected and extracted with a hypodermic syringe. After several improvement stages to the methods used in constructing the amylase particles, the damage loss of particles was almost eliminated.

For these fruit processes, there were advantages of the TTI method over the alginate spore technique. For example, the preparation and assaying of the amylase solutions took only a few minutes as opposed to days, and therefore the total cost of the TTI method was less and the results were available sooner. Also, transportation of the amylase particles to the plant location did not require as much caution as with spores, because the decimal reduction time of amylase is very large at ambient and chilled conditions, and unlike spores there is no issue with out-growth. Potential applications for encapsulated TTIs as a process validation method will thus be considerable.

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