Validation of Thermal Processing Using Time-Temperature Indicators as Process Probes

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Thermal processing is a very common technique in the food industry. Commonly foods are overprocessed to ensure safety, but this may give excessive loss of quality attributes. Thermocouples are commonly used to obtain temperature-time profiles, but for many products it is not feasible to use them. Time-temperature indicators (TTIs) are small particles containing a reactive species that can be passed through a process and then assayed to measure the process effects. A series of tests have been carried out to determine the feasibility of using TTIs to validate thermal processes, using a pilot scale process vessel. TTI results could show the effects of local wall heating and distinguish between different heating regimes. Particle paths can be quantified using Positron-Emission Particle Tracking (PEPT); for viscous fluids of the kind found in food applications there is very close match between the flow of the fluid and of the TTI. Suggestions for the use of TTIs are made.

Key words: Thermal processing, time-temperature integrators, batch vessels, thermal modelling

1. INTRODUCTION

1.1 Thermal processing

Thermal processing is the basis for the majority of the operations of the food industry, with processes such as canning being the basis for large industry sectors. The aim of thermal processing is to reduce the bacterial load in the food to a level that allows it to be safe over the proposed shelf life of the material. In addition, reactions that lead to the creation of acceptable taste and flavour (such as the gelatinisation of starch, the denaturation of proteins, and Maillard reactions) should be promoted, whilst those which lower quality (such as, for example, the loss of vitamins or the creation of carcinogens such as acrylamide) minimised.

The practical problem is that the need for process safety is paramount – the damage done to a brand by the need to recall product, or a food poisoning outbreak, is so severe that processes are routinely overdesigned. The result is lowered product quality, which in turn makes the material less attractive to the consumer. The job of the food engineer is to ensure that the quality of the food is maximised while maintaining safety. In part this is an optimisation process and can be highly mathematical [1–3]; however in practice the critical issue is in validating the process. Such validation requires that there is an effective measurement method which can show the level of thermal treatment which the material has received.

In some cases, it is possible to measure the temperature-time profile of the food accurately, and thus infer the amount of processing it has received using equations such as:

\[ P \text{ or } F = \int_{t}^{10^2} \frac{T(t) - T_{ref}}{dt} \]  

(1)

where \( T(t) \) is the product temperature (°C), \( T_{ref} \) is the reference temperature for the \( D_t \) value (°C), \( t \) is the process time (min) and the \( z \) is the number of degrees centigrade needed to bring about a ten-fold change in decimal reduction time.

To obtain the \( F \) value in an industrial context, thermocouples are commonly used. However, this is not always possible, such as in rotating retorts and in continuous processes. Alternative methods, such as temperature probes that are portable [4, 5], although these devices are still bulky, and beads containing spores which can be assayed after the process [6], can be used. Time Temperature Integrators are a further alternative to thermocouples;
the work that has been done; further details can be found in Mehauden et al [7, 8] and in Yang et al [9].

1.2 Time-temperature indicators

TTIs are devices which contain a thermally labile substance. Under a heat treatment, the encapsulated substance will undergo irreversible changes which can be quantified as a F or P value [4, 6, 10–13]. TTIs present some advantages over thermocouples; they are small, and can be made neutrally buoyant and from materials with the same thermal conductivity as food particles.

TTIs can be classified according to the substance that they contain, which can be microbiological, enzymatic, chemical or physical. Enzymatic TTIs are the most commonly used since microbiological TTIs present safety risks and few physical and chemical reactions have been identified so far in the range of z values used in heat treatment of food products [13]. Enzymatic TTIs are based on the quantification of the activity that remains after thermal treatment. Their high thermostability allows them to be used over a very wide range of temperatures (pasteurisation, sterilisation) [14–19]. Numerous enzymes have been identified for pasteurisation temperatures [10, 18, 19]. However, finding an enzyme with the adequate thermostability at sterilisation temperatures (around 121°C) can be difficult; Tucker et al [20] show that amylase from the extremophile Pyrococcus furiosus is suitable for sterilisation monitoring, Pyrococcus furiosus grows at 100°C, and thus has enzymes which are active at that temperature.

Recently, some studies have been reported on the use of TTIs on various industrial processes. Studies have been performed on the pasteurisation of yog-fruits [21] which show that a P value can be obtained with the TTIs where the use of thermocouples is not possible. In addition, Guiavarch et al [6] used TTIs to study continuous rotary processing of canned ravioli.

TTIs are valuable tools in the evaluation of process efficiency. However, literature published currently on their use is limited; there is a lack of knowledge on their accuracy and efficiency. Errors in measurements using TTIs will rise from a number of factors, including variability in the manufacture of the TTIs, errors in determining the final and initial value of activity, as well as non-linearities and variation in the kinetics of the enzyme. The need is to develop TTIs as effective process probes, and to do this some understanding of the inherent accuracy of these devices is needed.

1.3 Positron-emission particle tracking (PEPT)

The technique of positron emission particle tracking (PEPT) has been developed at the University of Birmingham for tracking a single particle accurately and non-invasively for various applications in engineering and science. The technique involves a positron camera, a labelled tracer particle and a location algorithm used for calculating the tracer location and speed. The camera consists of two position-sensitive detectors, each with an active area of 500×400 mm², mounted on either side of the field of view, and are used to detect pairs of 511 keV γ-rays. The tracer particle, which is on the order of 50 μm diameter, is labelled with a radionuclide which decays by β⁺ decay with the emission of a positron. Each positron rapidly annihilates with an electron giving rise to a pair of 511 keV γ-rays, which are emitted almost exactly back to back. The two γ-rays are simultaneously detected in the two detectors and define a trajectory passing close to the source. Therefore, the location algorithm derives from the principle that all the uncorrupted γ-ray trajectories for a given set of events should meet (within the resolution of the camera) at a point in space; this is the location of the particle, the point that minimises the sum of perpendicular distances to the various trajectories.

However, many of the detected events are corrupt, because (i) one or both of the pair of γ-rays has undergone Compton scattering prior to detection, or (ii) the two detected γ-rays were not in fact a pair of 511 keV photons originating from the same positron annihilation event. Thus, the location algorithm is also used to discard these corrupt events, whose trajectories are broadcast randomly in space and do not in general pass close to the true particle location. The location is then recalculated using just the uncorrupted events. From successive locations, the velocity of the labelled particle can be found as it passes through the view of the camera [22–24].

For the past 10 years, PEPT has been used to study a wide range of engineering processes by a number of research groups. As the γ-rays can penetrate consider-
able thickness of material, PEPT offers several advantages for providing an insight into flow and mixing processes inside real-plant equipment without disturbing the process. For example, Bakalis et al [25] used the method to validate a model for viscous flow inside a model stainless steel heat exchanger, in which the tracer acts as an isokinetic flow follower, and the method has also been used to study mixing in canned food [26], granular systems [27, 28], solid liquid flows [29] and liquid expanded beds [30] among others. Recent work has developed methods by which multiple tracers can be followed in the same experiment [9]; this is of relevance to some food flow situations, such as the heat transfer between particles and fluids [31] but has not been used here.

The advantage of PEPT is that it can track materials under realistic conditions, and follow flows to sub-mm accuracy. A PEPT tracer can be placed in a TTI particle, which allows the flow path of the TTI to be followed in time and space; this can then be compared with flow paths for the tracer alone to identify whether the two have the same flow profile.

2. Material and Methods

2.1 TTI preparation

The enzyme used in the TTI preparation was α-amylase (EC 3.2.1.1. Type II-A, Sigma, UK) isolated from Bacillus licheniformis. The enzyme will be denoted as BLA hereafter. This enzyme has a pH activity which ranges from 5.5 to 6.5 with an optimum at 5.9 and its optimum rate of activity is at 65°C. The BLA solution was prepared by dissolving 200 milligrams of BLA powder into 20 ml of 0.05 M tris buffer pH 8. (prepared from Trizma base C4H11N03) giving a final enzyme concentration of 10 mg/ml. The Decimal reduction time, DT, of BLA at 85°C is 29.15±4.7 min and its z value is 10°C±0.8°C. The DT and the z value were determined for isothermal conditions operated with a waterbath. The z value used for the calculation of the P values obtained from the thermocouples and data loggers was the same as the TTIs (10°C). Where temperature is available, therefore, equations (1) and (2) give two values of process P which should be identical within experimental error.

2.2 Measurement of TTI activity

After heat treatment, the amylase activity remaining in the TTIs was measured by a spectrophotometer. Prior to this, amylase was removed from the TTIs using a syringe and diluted with the tris buffer. 1ml of enzyme assay reagent from Randox (Crumlin, Ireland) was added to the diluted enzyme and the reaction started. The Randox amylase test method uses ethylidene-blocked p-nitrophenyl-maltoheptaoside as substrate. This substrate is cleaved by the α-amylases into various fragments. These are further hydrolysed in a second step by α-glucosidase producing glucose and p-nitrophenol. The increase in absorbance represents the total amylase activity in the sample (Randox, 2006). The activity of the TTIs was measured at 405 nm by spectrophotometer (CECIL, Cambridge, UK) at 30°C. The enzyme activity was recorded over 200 seconds and the rate of reaction was determined from this activity.

2.3 Determination of the Pasteurisation value

Equation 2 allows the calculation of the P value from the TTI analysis.

\[ P = D_T \log \left( \frac{A_{\text{initial}}}{A_{\text{final}}} \right) \]  

Where \( A_{\text{final}} \) is the enzyme activity after a specific time temperature history, \( A_{\text{initial}} \) is the initial activity (without any heat treatment) and the DT is decimal reduction time, which depends on the temperature and on the thermoresistance of the enzyme. Where temperature is available, therefore, equations (1) and (2) give two values of process P which should be identical within experimental error.

2.4 PEPT experiments

The PEPT camera is a modified body scanner and is thus limited in the size of equipment that can be used. As a result a scaled down version of the vessel of Fig. 1 was built for use in the PEPT experiments. The vessel had a diameter \( D = 244 \text{ mm} \), height \( H = 174 \text{ mm} \) and the radius \( R \) of the spherical part was equal to 122 mm. The vessel was first filled with fluid and was then positioned between the PEPT cameras. A radioactive tracer particle (ca. 600 μm in diameter) was introduced into the system and an experiment was performed for approximately 45 minutes for each experimental condition.

Particle paths were obtained using specially developed algorithms [33] and consecutively velocity profiles were obtained by differentiating particle paths [25]. Two types of experiment were done, in which (i) tracers were allowed freely to circulate within the fluid, and (ii) tracers
2.5 Use of TTIs in an industrial scale agitated vessel

Experiments were performed on a vertical jacketed mixing vessel manufactured by Giusti Ltd. (Burton on Trent, UK) as shown in Fig. 1 (a) The maximum capacity of this vessel is 250 litres. It is equipped with a horizontal rotating agitator with polytetrafluoroethylene (PTFE) scrapers attached to the steel blades which make contact with the vessel wall. Agitation speeds used range from 3 to 15 rpm.

A calibrated wireless thermocouple (Tracksense pro, Ellab UK Ltd., King’s Lynn, Norfolk UK) with a 50 cm long probe was fixed securely onto the middle of the agitator shaft. The $P$ value at 85°C was obtained from the time temperature history recorded by both the wall and the wireless thermocouple using equation (1) with $T_{ref}=85^\circ$C and $z=10^\circ$C and was used for comparison with the $P$ values given by the TTIs. Fluids used were aqueous solutions of starch at different concentrations (Colflo 67, National Starch & Chemicals, Manchester UK). Further experimental details are given in Mehauden et al (2008).

Different TTIs were used to identify different parts of the process:

- Some were placed in the fluid at the start and were able to move freely around the vessel (F TTIs). However, it is possible that collisions between the TTIs and the hot vessel wall (ca. 130°C) give extra denaturation of the enzyme leading to an abnormally high $P$ value.
The effect of wall collisions was assessed by placing some TTIs inside 4 cm diameter airflow golf balls with widened holes (called GB) or inside a system of 2 tie clips (called TC) shown in Figs. 1 (b) and (c) respectively. Some were added inside the vessel through the aperture of the vessel at the end of the vessel heat up time just before the start of the ‘holding time’ (when the steam is switched off) to identify the effect of the heat up time (when the steam is constantly on) on the TTIs. These are referred to as HT TTIs hereafter.

P values were analysed for significant difference using statistical tests; Mann–Whitney U and the Kruskal–Wallis H tests were used and implemented using SPSS software (SPSS Inc. Chicago USA). The Mann–Whitney U test was used for measurements between two samples. This non-parametric test is used when the t test cannot be applied (variance not equal).

3. Results and Discussion

3.1 Accuracy of TTIs.

Preliminary experiments were conducted in which TTIs were given programmed T-t profiles using a Peltier microscope stage [8] and results compared with thermocouples on the same stage. Results are shown in Fig. 2 for:

- Single heating and cooling conditions (Plots 1-4), in which the shape of the curves is closer to those seen in industrial practice (for examples, see Stoforos and Taoukis [34]).
- Multiple heating and holding conditions, which identify the responses of the TTI for several repetitions of time temperature profiles (Plots 5 and 6).

The graph in the middle of Fig. 2 plots P values from the integrated T-t history of thermocouples against the TTIs P values at 85°C for the experiments of plots (1–6). The data shows good correlation; when the data is fitted into the equation y=x, the R² obtained is 0.8376. Therefore, the data fits the equation x=y and the responses of the TTIs correlate well with the responses of the thermocouples, even over a wide range of time–temperature profiles.

3.2 PEPT experiments.

The data from the PEPT experiments is first found as the trajectory of the tracer particle, and can then be manipulated to find the velocity field. To be acceptable as a process probe, it is necessary that the TTI particle has the same velocity field as the fluid and has the same occupancy, i.e. that the TTI particle travels throughout the fluid, and is not confined to one region of the vessel.

Figure 3 shows the behaviour of the two particles in water, in terms of the velocity field in the central part of the flow. The images are taken along the axis of the mixer.
so the blank region in the centre of the flow pattern is the shaft.

Figure 3(a) shows the velocity field for the TTI particle, whilst Fig. 3(b) shows the free particle behaviour. The TTI particle occupies a much narrower region of the flow than does the free particle, shown by the stripes in the flow pattern. The free particle shows a fairly uniform velocity field, with high velocities in the surface region. The difference between the free and TTI particle velocities is plotted in Fig. 3(c), and the effect of sedimentation can be seen; in the downflowing regions of the fluid the TTI travels faster than the surrounding fluid whilst in the rising parts of the fluid the TTI particle travels slower than the fluid. The magnitude of the difference between the two can clearly be seen in Fig. 3(c) – the sedimentation velocity of the particle is the same order of magnitude as the velocity differences seen. In this case, using TTIs would not be sensible in validation as the difference in flow between the free tracers and the TTIs is too large, and the flow patterns are significantly different.

In contrast, Fig. 4 shows similar views of a flow of a starch solution. Here the difference in the velocities of the two tracers, representing the fluid and the TTI flow, are much smaller, and the TTI tracer covers the whole area of the vessel. In this case the match between TTI and free tracer is such that it is clear that the TTI is following the same paths. Proper statistical analysis is necessary to confirm formally the match between the two, but it is clear that in this case the TTI results would be representative of the behaviour of the fluid.

3.3 TTIs as process probes

The previous section has shown that TTI data for starch is representative of the fluid. Fig. 5 shows experiments performed in the larger vessel with 4% starch at a holding temperature of 83°C. The Figure shows the *P* value calculated from the wall and centre thermocouples, as well as the average value of the *P* values from the four systems. Error bars show one standard deviation – as would be expected, there is a scatter in the data due both to the dif-
Fig. 4  Velocity fields measured by the PEPT camera in the central region of the model vessel of the same shape as Fig. 1, 4% starch as fluid: (a) for the TTI particle, (b) for the free particle. All velocity data in m/s.

Different temperature-time histories of the TTIs and the error in the measurement, so statistical tests were used to determine whether the values were different. The experiment of Fig. 5 (a) was performed with non-gelatinised 4% starch solution. The $P$ value from the centre thermocouple is 9 min (at 85°C), lower than that obtained by the wall.

Fig. 5  Experiments performed with 4% starch and holding temperature of 85°C. (a) Starch not pre-gelatinised. No statistically significant differences between all TTIs. (b) Starch pre-gelatinised. Statistically significant differences between $F$ and $GB$, $F$ and $TC$, $HT$ and $GB$, $HT$ and $TC$ and all TTIs. (c) Starch pre-gelatinised, steam injection used. Statistically significant differences between $F$ and $HT$, $F$ and $GB$, $F$ and $TC$ and all TTIs. (d) Starch not pre-gelatinised, vessel overfilled. Statistically significant differences between $F$ and $HT$ and all TTIs. Error bars show one standard deviation.
thermocouple. $P$ values for the 4 different kinds of TTIs are very similar to that of the centre thermocouple (9 min at 85°C) and there is no significant difference between results from the TTIs. The starch starts to gelatinise at 75°C and until this temperature, the fluid is of similar viscosity to water. Therefore initial mixing is very efficient and fluid reaches the set temperature relatively quickly. Since the fluid temperature is uniform, the starch begins to gelatinise throughout the vessel once 75°C is reached, so, all the TTIs receive a similar thermal treatment due to the efficiency of the initial mixing.

However, when the starch (4%) is already viscous at the start of the experiments (Fig. 5 (b)), results are significantly different. The difference between the $P$ values of the 2 thermocouples is high (10 min at 85°C). During heating, mixing efficiency is lowered due to the fluid being viscous. Therefore the temperature is not homogenous inside the vessel. The free TTIs have high $P$ values, similar to that of the wall thermocouple, and that of the TTIs fitted in the balls are similar to the $P$ values of the centre thermocouple. The GB TTIs and the TC TTIs have lower $P$ values due to the lack of contact with the vessel wall. The results show the results of viscous fluids being difficult to process: hot and cold spots lead to local under- and over-processing.

Figure 5 (c) displays $P$ values for the TTIs and thermocouples for experiments performed with 4% pre gelatinised starch but with steam injection into the vessel. Steam injection seems to improve heating efficiency; the difference between $P$ values of the centre and the wall thermocouples is lower compared with Fig. 5 (b). The $P$ values of the F TTIs are higher than that of the wall thermocouple. F TTIs could have passed close to the steam injection nozzle and therefore received further localised heating. After the heating time steam injection was not in operation most of the time and hence the HT TTIs have lower $P$ values. Values for the GB and TC TTIs are also significantly lower than those of the F TTIs and they appear less affected by steam injection.

Figure 5(d) shows results from experiments performed with 4% starch but for an overfilled vessel. $P$ values from the TTIs are all similar at between 12 and 14 min at 85°C. They are surprisingly closer to the $P$ values of the wall thermocouple (16 min at 85°C) than to those of the centre thermocouple (8 min at 85°C). In this experiment, the fluid path is different to the normally filled vessel (Fig. 5 (a)), here all the TTIs have higher $P$ values (by about 4-5mins) suggesting that fluid mixing in the overfilled case is different.

4. Conclusions: usefulness of TTIs as process probes

The above discussion has shown the type of results that can be achieved using the TTIs. For viscous fluids there is little difference between the flow of the TTIs and of the fluid, so the results are relevant. The design of TTIs using the plastic golf balls allows the effect of local wall heating to be assessed – here the need is to ensure that the path of the TTI and the fluid is not the same.

The data presented here show the kind of results that could be obtained using the method; once it is certain that the TTIs give data that represents the real fluid behaviour then it is possible to use the method to identify the true processing which the food has received. This will make it easier to define an appropriate temperature–time profile in any given situation.

The TTIs are relatively easy to use in practice, but their use on real plant is complex and requires some practice; factors such as extraction of the TTIs from the fluid can make experiments messy to do! The method is probably best suited to initial process start-up, when process schedules are being set, rather than as a day-to-day operation. The techniques developed here can be applied to most food heating and mixing processes and will hopefully aid process design and operation.

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References


