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Isothermal Calorimetry for Biological Applications in Food Science and Technology

Subtitle: Isothermal calorimetry – the measurement of the heat production rate of processes – is a general measurement technique that has many uses in the food field. We here describe some biological applications concerning tissue respiration, fermentation, and shelf-life.

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1. Introduction

Isothermal calorimetry – the measurement of thermal power and heat at constant temperature – is a general measurement technique as nearly all processes (physical, chemical, biological etc.) produce heat. Such instruments are used in such diverse fields as in the pharmaceutical industry, in cement science, and at defense laboratories. Also in the food science/technology laboratory isothermal calorimetry is a valuable tool for the study of various phenomena, but the use in this field has yet been rather limited. In this paper we discuss biological applications of isothermal calorimetry in food science and technology.

Probably the first food-related calorimetric measurement was made by Dubrunfaut (Dubrunfaut 1856). Although he did not have access to a calorimeter of the type we use today, he studied the energy and heat balance of a wine fermentation vat. Today, isothermal calorimetry is a fairly commonly used instrument for studies of fundamental biology, see for example (Gustafsson 1991; Kemp 2000; Lamprecht 2003) and some food-related microbiological studies have also been made together with a handful of studies of vegetable tissue respiration (references are given later). Few studies have been made on physical processes in food systems.

Isothermal calorimetry has some interesting properties:

- It is a general and unspecific technique that can be used for many different tasks in a laboratory.
- During a calorimetric measurement the thermal power is continuously measured. One can thus monitor processes while they take place.

-It is a non-destructive technique and as heat flows through all materials one can monitor processes taking place inside opaque materials and packages.

The main problem with isothermal calorimetry is related to its generality: it measures heat that can come from a large number of different sources. Instruments and methods therefore have to be designed so that only the heat from the process of interest is measured (Wadsö and Wadsö 2005).

Isothermal calorimetry is an interesting tool for looking at kinetics of all types of reactions and processes. This use is based on that the thermal power P (W) measured is related to the rate ν (g/s) of the process studied, and the produced heat Q (J) (the integrated thermal power) is related to how far the process has proceeded, i.e., what mass m (g) of a sample (or of a part of a sample) that has reacted (this can also be formulated in molar quantities). Both these relations involve the enthalpy change Δh (J/g) as proportionality constant:

$$P = \Delta h \cdot \nu \quad (1)$$

$$Q = \Delta h(m_0 - m) \quad (2)$$

These two equations are the connections between a calorimetric measurement (P and Q) and the corresponding kinetic rate law (ν and m). For processes in foodstuffs and other complex materials enthalpy change and rates are not as easily defined as for simpler reactions, but measured thermal powers (and heats) can still be used to model kinetics of, e.g., degradation processes (Hansen 2000).

Both metabolism of microorganisms, tissue respiration and physical processes like crystallization produces heat that in principle can be measured by isothermal calorimetry. If it is practically measurable depends on the enthalpy and rate of the process, and the specific sensitivity and sample volume in the calorimeter. Biological samples with aerobic respiration always produce about 455 kJ per mol oxygen consumed (or carbon dioxide produced). The same is true for chemical oxidation phenomena. Different types of anaerobic respiration produce much less heat. Typically, much less than 200 kJ per mol carbon dioxide are produced under anoxic conditions. For other types of reactions, enthalpy can vary substantially.

2 The calorimetric instrument

The most common technique to make isothermal calorimetry is the heat conduction calorimetry. The heat produced in the sample is then conducted away from the sample through a heat flow sensor. The thermal conductance of the heat flow sensor should be high enough so that the temperature changes in the sample are negligible. In the applications described in this paper the temperature of the sample changes less than 0.1 K from the thermostat temperature and the measurements can therefore be considered to be essentially isothermal.

All measurements presented here except the last one have been made in TAM Air isothermal heat conduction calorimeters (TA Instruments/Thermometric, Järfälla, Sweden). Each such instrument contains eight calorimeters of the heat flow type that consist of a sample and a reference side. The samples and references are assessable from the top of the instrument. Figure 1 shows a cut-away drawing of the instrument. An air thermostat keeps the calorimeter at (constant)

temperatures between 5 and 90°C by a Peltier heater/cooler. The temperature fluctuations of the air thermostat are less than ± 0.02 K over 24 h.

The samples are placed in 20 ml ampoules, most commonly made of glass or high density polyethylene. The glass ampoules are closed with a Teflon coated septa and an aluminium crimp cap. The plastic ampoules are closed with a screw cap. It is possible to combine isothermal calorimetry with other techniques or to change the conditions of the sample during the measurement. One can therefore design special vessels, e.g., with electrodes or other sensors or with possibilities for, e.g., injection (Wadsö 2005).

All heat conduction calorimeters need to have a reference sample that should have the same heat capacity as the sample, but not produce any heat. The measured signal is the difference between the signals from the sample and the reference heat flow sensors. In this way the noise level is decreased substantially as disturbances will influence the sample and the reference similarly.

The sample and reference ampoules are placed in ampoule holders. The heat flow sensors (solid-state Peltier thermocouple plates) are positioned between the ampoule holders and the surrounding heat sink. The calorimeters are normally operated in a ± 60 mW range where the resolution of the instrument is 1 μ W and a typical baseline noise level is ± 5 μ W at 20°C. From these figures one can see that one needs thermal powers in the order of 10 μ W to differentiate between thermal power and no thermal power, and higher than 100 μ W to get a reliable figure of the thermal power. There are several other calorimeters on the market; the more sensitive ones

are often called microcalorimeters and can be used for measurements on small samples or samples with low heat production rate.

It should be noted that isothermal (heat conduction) calorimetry discussed in this paper is a quite different technique than other, possibly more commonly known, calorimetric techniques like bomb calorimetry (used to determine enthalpies of combustion and calorific/fuel values), differential scanning calorimetry (DSC, in which changes in heat are measured as the sample temperature changes) and solution calorimetry (used to determine enthalpies of dissolution). Note also that isothermal calorimeters commonly have 1000 times higher specific sensitivity (W/g sample) than do DSC instruments run in isothermal mode, mainly because the DSC samples are very much smaller (Hofelich and LaBarge 2002).

3. Applications examples

We have here collected six food-related biological applications of isothermal calorimetry that we have worked with. When no references are given the measurements have been made specifically for this paper.

Wound respiration

Fresh vegetables and fruits generally do aerobic cell respiration that produce about 455 kJ of heat per mol oxygen consumed. Many types of tissue can also do unwanted anaerobic metabolism, at least for short periods of time, with lower heat produced per carbon dioxide produced. Because of

this, calorimetric measurements on tissue respiration can give similar information as respiration measurements and already in 1912 Langworthy and Milner of the US Department of Agriculture published a study on banana respiration (Langworthy and Milner 1912). We have used isothermal calorimetry to investigate the effect of different unit operations on cut vegetables (Gomez, Toledo, Wadsö, Gekas and Sjöholm 2004; Wadsö, Gomez, Sjöholm and Rocculi 2004; Gómez Galindo, Rocculi, Wadsö and Sjöholm 2005; Rocculi, Romani, Dalla Rosa, Gómez Galindo, Sjöholm and Wadsö 2005; Gómez Galindo, Sjöholm, Wadsö, Rasmusson, Widell and Kaack 2006; Peredes Escobar, Gómez Galindo, Wadso, Ruales Nájera and Sjöholm 2007; Rocculi et al. 2007). Smith et al. (Smith et al. 2000) used a combination of calorimetry and respiration measurements to study aging potato slices. Such techniques are further discussed by Hansen and co-workers (Hansen et al. 1995; Hansen, Hopkin and Criddle 1997; Hansen, MacFarlane, McKinnon, Smith and Criddle 2004).

It is well known that cutting through a vegetable causes cell damage (wounding) that sets of several different protection and repair process in the non-wounded cells. Measured in a calorimeter, samples with increased surface area produce more heat than less wounded tissue and this increase is approximately proportional to the area of wounded tissue (Wadsö, Gomez, Sjöholm and Rocculi 2004). One example of this is shown in Fig. 2. Excised cylinders (diameter 9.5 mm) of one carrot (*Daucus carota L*) were either used as whole cylinders or cut transversally so that the different samples had different surface areas (wounding). They were then placed in sealed glass ampoules and inserted into the calorimeter.

In the calorimetric result seen in Fig. 2a one can see that it takes about 40 minutes before the signal is stable after the samples have been inserted. It is also seen that the signals are not

constant, which is rather typical of calorimetric measurements on biological materials. This shows that the wounding reactions are dynamic processes, but the result can also be influenced by other factors, for example the change in gas composition in the closed vial. At the end of the measurement the samples were taken out and baselines measured with inert vials.

The specific thermal powers measured after 240 min were plotted as a function of the specific surface area to yield Fig. 2b. It is seen that there is a linear relationship that makes it possible to separate the wound thermal power (the slope of the line) from the bulk thermal power (at zero specific surface area). One can use this to investigate wound induced processes and how the rates of these can be reduced, e.g., to prolong shelf life and decrease off-flavor. It should be noted that different individual, e.g., carrots can have rather different specific thermal power, so it is advisable in this type of studies to mainly compare results from measurements on tissue from each individual separately.

Tissue wounding has typically been studied by measuring the concentrations of specific wound-induced compounds (see for example (Reyes, Villareal and Cisneros-Zevallos 2007)). A non-specific calorimetric measurement is an excellent complement to such techniques. In studies of aerobic systems, calorimetry is similar to respirometry, but gives an output with higher resolution as the heat production *rate* is measured, while respirometers measure gas concentration (essentially the integral of the heat production rate).

Cell Death from Blanching

We have used calorimetry to quantify cell damage that occurs during blanching. Increasing intensity of the heat treatment progressively reduces the number of viable cells in a tissue sample and results in a corresponding reduction in the rate of metabolic processes (Gómez Galindo, Toledo and Sjöholm 2005). We have studied this by immersing carrot slices (thickness 7 mm, diameter about 3 cm) in boiling water for different times and then rapidly cooling them in ice-water to minimize over-processing. Similarly as in the previous example we chose a time at which to determine thermal powers and have drawn these as a function of time in Fig. 3. As the decrease in thermal power is caused by that increasing fractions of the samples are dead, these calorimetric results can be combined with calculations of the increase in temperature in the samples during blanching to find the temperature at which the cells are killed (Gomez, Toledo, Wadsö, Gekas and Sjöholm 2004). This makes it possible to optimize blanching processes. A factor that may complicate the analysis is that an un-blanching sample has wound respiration, so these may not be suitable as no-treatment references for thermal calculations. The analysis can still be made, but only with treated samples in which the surface has been heat treated to prevent wound respiration.

The effect of blanching has for example been studied by texture measurements (see for example (Sebok, Bontovics and Bleszkan 1999)) or with vital staining (see for example (Gomez, Toledo, Wadsö, Gekas and Sjöholm 2004)). Calorimetry nicely complements these techniques as it assesses a quite different aspect of the effect of blanching.

Milk Fermentation

Fermentation of milk and other products such as beer, wine and pro-biotic foods can be conveniently studied by calorimetry as the microbiological fermentation produces heat. One can for example map the properties of microbial cultures, e.g., assess differences between different cultures, measure their doubling time, look at the influence of additives etc.

We have here used pasteurized milk (3% fat) and two Swedish cultured buttermilk products as start cultures: “filmjök” and “A-fil”. The first is a culture of different strains of *Lactococcus lactis*, while the second is a mixed culture of *Lactobacillus acidophilus* and *Lactococcus lactis*. The samples were prepared by adding 0.25% starter culture to 20 ml milk in glass ampoules. The samples were then mixed and placed in the calorimeter. Measurements were made at 20 and 23°C. The result is seen in Fig. 4. Integration of the measured heats (from 4 h to 40 h) gave about 7.2 J/g at 20°C and 6.6 J/g at 23°C, possibly indicating that the microorganisms metabolize more substrate at the lower temperature. As the thermal powers measured are proportional to the rates of the studied processes, it is possible to calculate how much the processes are accelerated by the temperature increase, i.e., the Arrhenius activation energy or the Q_{10} -value. From the present measurements we found that both cultures had factors k in $A \cdot \exp(kt)$ during the exponential growth phases that were about 10% higher at 23°C than at 20°C.

It is seen in Fig. 4 there is a delay before the main peak comes, possibly this marks the end of an initial aerobic phase. As isothermal calorimetry gives a continuous output one can see details like this that would be difficult to find with other techniques; however, other more specific techniques often need to complement the unspecific calorimetry to elucidate the true nature of the processes seen. One such technique – that is commonly used to follow milk fermentation – is to

monitor the decrease of pH during the fermentation (see for example (Gernot, Goelling, Klimant, Schneider and Heinzle 2003)). To follow both the heat production rate and the pH simultaneously, a pH-sensor can be incorporated into a calorimetric ampoule ((Johansson and Wadsö 1999)).

Microbiological spoilage prevention

The general goal of preventing microbial spoilage can be reached by many methods, e.g., natural and synthetic chemical preservatives, and thermal treatments. Isothermal calorimetry can be used to study the effect of such methods by studying samples treated with different methods or measuring the thermal power of microorganisms before and after the application of a method. It has been used for studying the effect of various inhibitors on microbial growth (Kawabata, Yamano and Takahashi 1983; Okuda, Takahashi, Fukada, Nitta, Nakao and Kirihata 1996; Antoce, Antoce, Takahashi, Pomohaci and Namolosanu 1997; Alyabyev et al. 2004).

Fresh carrot juice is an extremely perishable food-stuff as it has a neutral pH, high sugar content and also contains many soil microorganisms. We here describe an experiment we made using carrot juice and three additives: green tea, orange juice and the well known preservative sodium benzoate. The measurements were made by taking fresh carrot juice and mixing it with the three additives, placing 15 mL of each mixture in glass sealed vials, and measuring the thermal power in a calorimeter. We also measured on a sample of 15 mL pure fresh juice. The result (shown in Fig. 5) indicates that green tea is not effective in reducing the growth of the microorganisms in the carrot juice. Mixing with orange juice is a slighter better option. Probably because of the lowered pH the curves look quite different when orange juice is added, but the initial increase in

thermal power takes place at about the same time as for the pure carrot juice, so its use to prolong shelf life is doubtful. The high dose of sodium benzoate significantly delays the onset of growth, but it does not prevent all microorganisms from growing. What types of microorganisms that are present in the juice cannot be inferred from the calorimetric measurements, but calorimetry is in many cases an ideal method to start a study with as it gives a general overview of how fast processes take place and what factors that control the process rates.

Thermal Treatment

We have here made an accelerated study of how much shelf life of carrot juice is prolonged by mild thermal treatment. The measurement was made by heating about 500 mL of fresh carrot juice and charging ampoules with 20 ml juice samples taken when the carrot juice reached 50, 60 and 70°C. The temperature was measured with a thermocouple in the stirred juice. The calorimetric measurements were then made at 20°C.

The result is seen in Fig. 6. The measured thermal power is the heat from the microbiological activity in the sample. It is seen that the lower treatment temperatures gave only slightly lower thermal powers, but that the 70°C treatment gave a substantially delayed signal. If a threshold thermal power level can be established the effect of a treatment can be quantified. When we, rather arbitrarily, choose 0.1 mW as threshold in the present study we find that the fresh juice and the juice treated at 50 and 60°C give this thermal power after 22 h, while it takes the 70°C sample 35 h to reach it. At 20°C the shelf life was thus increased by more than 50% by the 70°C treatment. This may not be true for normal storage temperatures where other microorganisms are

active, but the same study can be made at any temperature wanted, possibly with a more sensitive calorimeter at lower temperatures.

Shelf Life

Isothermal calorimetry is potentially a useful tool for shelf life predictions. Several attempts have been made in this direction. For example have isothermal calorimetry been tested as a method to detect microbiological spoilage of meat (Gram and Sögaard 1985), milk (Berridge, Cousins and Cliffe 1974; Nunomura, Ki-Sook and Fujita 1986), carrot juice (Alklint, Wadsö and Sjöholm 2005), pineapple (Iversen, Wilhelmsen and Criddle 1989) and salads and eggs (Riva, Fessas and Schiraldi 2001). Lampi et al. (Lampi, Mikelson, Rowley, Previte and Wells 1974) have also described a calorimeter to detect microbiological growth in canned foods.

We will here discuss some results from (Alklint, Wadsö and Sjöholm 2005) that used a sensitive isothermal microcalorimeter and plate counts to quantify microbial spoilage of carrot juice. At present, plate counts is the standard method and regulatory limits give how many colony-forming units of microorganisms that are allowed in different food stuffs. As microbial growth produces heat, calorimetry could possibly be used as a complement to plate counts, for example to check for microbial growth in packed foods as industrial quality control and for studies of the efficiency of preservatives and other anti-microbial treatments.

Alklint et al. (Alklint, Wadsö and Sjöholm 2005) packed pasteurized carrot juice in cartons that were stored at 17°C. At certain times cartons were opened and CFU counts were made and the results were fitted to a Gompertz equation. In parallel the pasteurized juice was at the start of the

measurements placed in sealed vials that were measured on in an isothermal calorimeter. The result (Fig. 7) shows that when the CFU-counts increase, an increased thermal power is also seen in the calorimetric measurement. A curve looking more like the CFU-curve can be made by integrating the thermal power to get the produced heat, but by integrating a curve its “sharpness” is reduced and we find that the thermal power shown gives the best early warning that microbial growth is occurring. From the result it is clear that calorimetry can be a sensitive tool to early detect microbiological processes in food stuffs.

4. Concluding remarks

Isothermal calorimetry is an extremely general technique as it measures heat production rate, which accompanies nearly all physical, chemical and biological processes. From the above examples it is clear that also in the food field isothermal calorimetry can be used to study biological processes. We have in our work seen that isothermal calorimetry is an excellent tool for initial exploratory studies. As it is often a sensitive instrument for biological samples, one can usually with only a few measurements increase one’s knowledge of a system under study. Typically, one can assess approximate reaction rates and the associated kinetics, and study what factors (oxygen, nutrients, temperature etc.) that influences the process. In a second step one often complements the calorimetry with other more specific analytical techniques.

We envisage that isothermal calorimetry will become more important in the microbiological field in the future. Although it is not possible with a calorimeter to determine what types of organisms that are growing in, e.g., a food-stuff, its sensitivity and generality makes it possible to very early detect microbial growth. It is also a much less work-demanding technique than plate counting, so

it could be an alternative in food industries to detect faulty heat treatments. As heat is conducted through all materials, this can also be made on whole cartons and cans using special calorimeters.

A related future use can be as a tool in predictive microbiology. As a calorimeter measures the heat from all microbiological processes in a sample – also from inside the sample – it is a much more general technique than the often used surface cultures where one measures radial growth etc. Measurement of thermal power is also a way to assess the activity of a mixed microbial culture; a parallel to the use of calorimetry to quantify the total biological activity of soils (Barros, Feijóo and Fernández 2003).

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Figure captions

Figure 1. A cut-away drawing of the instrument with the top insulated lid removed. Three of the eight calorimeters are shown (1-3); one with part of the heat sink removed so that one can see the ampoule holders for the sample and the reference (1). The temperature of the calorimeter is set on a temperature regulator (4) and regulated by a Peltier heater/cooler (5). The thermostat is protected from the ambient temperature with an insulation (6). The bottom part of the unit contains the amplifier, the data logger and power supplies (7).

Figure 2. Results of experiment with wound respiration (Wadsö, Gomez, Sjöholm and Rocculi 2004). The left diagram shows the results of calorimetric measurements on eight samples excised from a carrot, some of which have been further cut to increase the wound area. “BL” are the baselines after the measurement. The right diagram shows the specific thermal power at 240 min as a function of the specific wound area.

Figure 3. Results of an experiment with blanching of carrot samples (Gomez, Toledo, Wadsö, Gekas and Sjöholm 2004). The measured thermal powers are drawn as a function of blanching time.

Figure 4. The thermal power from the fermentation of milk at two temperatures using two different buttermilk cultures (F is “filmjök” and A is “A-fil”).

Figure 5. The effect of different additions on carrot juice spoilage. The thermal power seen is from the microbiological growth.

Figure 6. Thermal treatment of carrot juice resulting in increased shelf life only at the highest treatment temperature.

Figure 7. Results from parallel measurements on carrot juice with plate counts and isothermal calorimetry (adapted from (Alklint, Wadsö and Sjöholm 2005)).

Figures

Figure 1.

(TAMAirfig.emf)

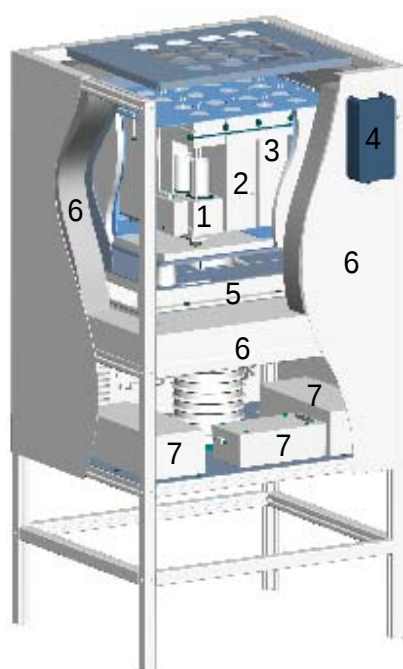


Figure 2 Tissue wounding

(Fig. 1 from c:\measure3\food\ApplPaper\wound\Pietro\figwoundcarb.m)

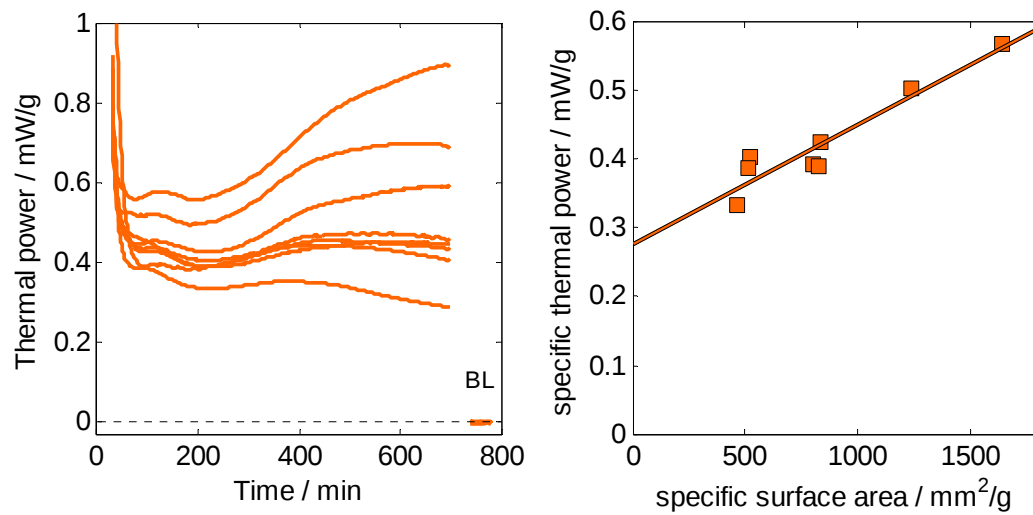


Figure 3. blanch

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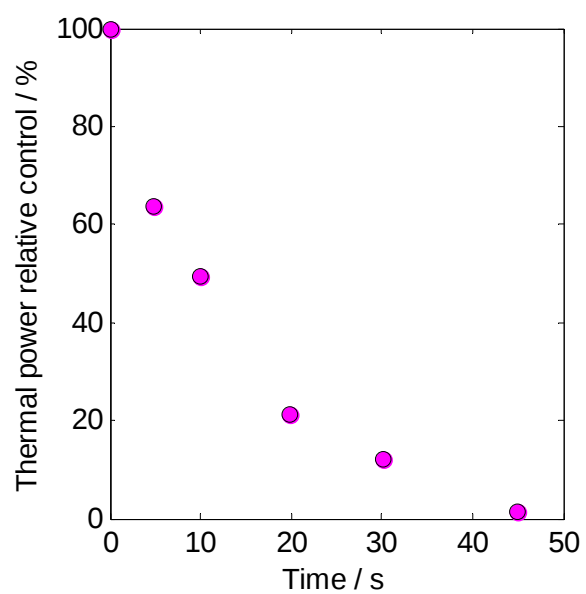


Figure 4. Fermentation

(Figure 1 from c:\measure3\food\ApplPaper\milk\milkferm.m)

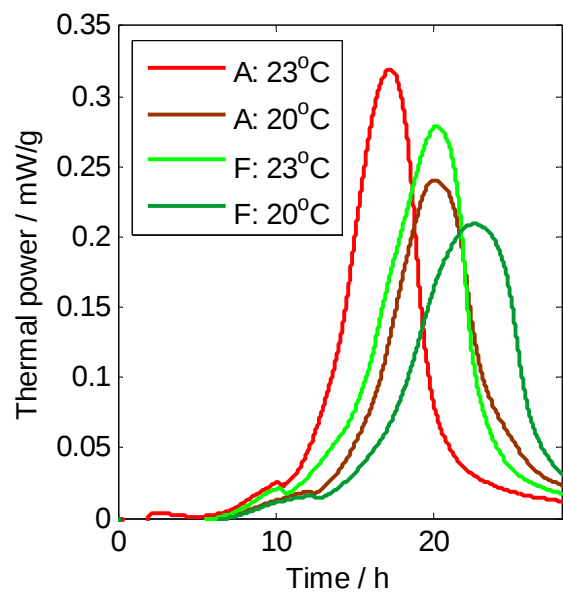


Figure 5. Carrot juice + things

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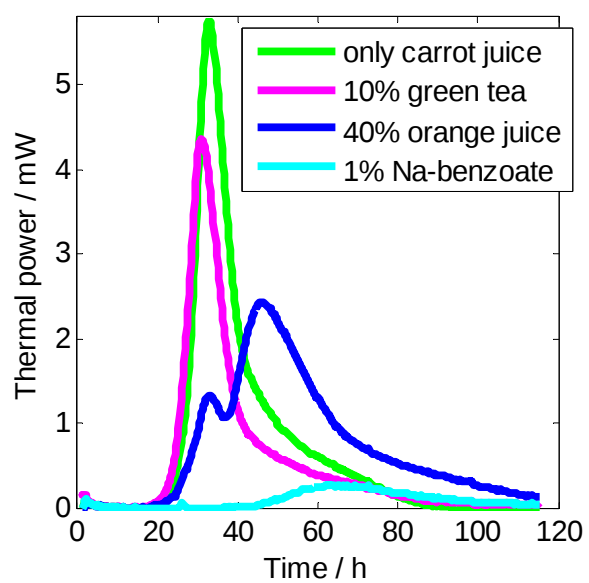


Figure 6. Thermal treatment

(Fig. 2 from c:\measure3\food\ApplPaper\juiceFG)

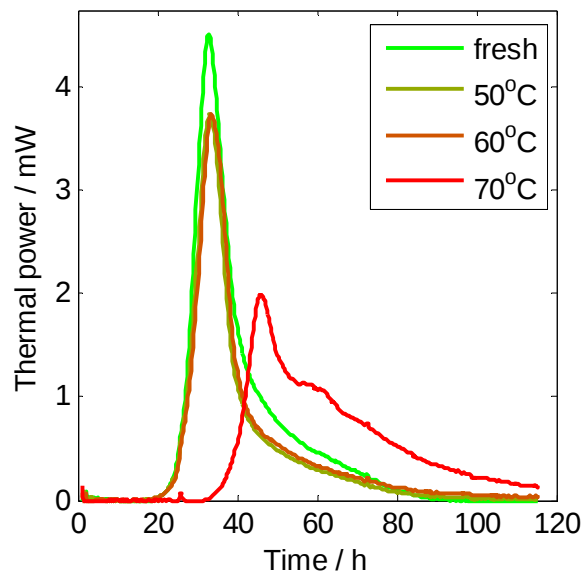


Figure 7. Alklint

(Fig 1 from c:\measure3\food\ApplPaper\juiceCA\CAfig1x.m)

