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Microcalorimetry: A Useful Tool for Solving Shelf Life Problems in the Food Industry

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Instrument: *2277 Thermal Activity Monitor*

Field of Application: *Biology*

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Introduction

Food items are often perishable and have a limited shelf life. Legislation requiring a declaration of "Best before" date and the interest of the manufacturer to assure the quality of his products within the declared time makes a firm control of the adulterating reactions in the product highly desirable. A thorough knowledge of the aging mechanism allows the manufacturer to take proper actions to prevent rapid decay.

Microcalorimetry is a very useful tool for the measurement of the degree of aging of a product and also for the elucidation of the aging mechanism. This can sometimes be a formidable task in such a complex matrix as a food product.

The main mechanisms for food deterioration are:
Bacterial growth
Mould or yeast growth
Enzymatic oxidation reactions
Chemical oxidation reactions
Hydrolytic reactions
Condensation reactions like the Maillard browning reaction.

The degree of instability of food products is determined by its water activity (a_w) (Ref 1). Approximate water activities and the expected shelf life for some common foods are shown in table 1. Labuza et al (Ref 2) showed that the water activity to a great degree determines the reaction rate of many spoilage reactions as shown in fig 1. We usually expect a dry food like biscuits to last longer than moist foods like fresh vegtables and meat, since most of the spoilage reactions are favoured by a high a_w. Bacterial growth requires a high a_w value, typically 0.90. However, different bacterial types have different limits for growth (Ref 3).

Food item	Approx a_w	Expected shelf life
Fruit Vegtable, Milk, Fish, Meat	0.99	Week
Bread	0.98-0.93	Week
Sausage, ham, jam	0.96-0.93	Month
Marmalade	0.87-0.60	Month-Year
Dried fruit	0.60	Year
Biscuits	<0.50	Year

Table 1. *Water activity and the expected shelf life for a variety of foods.*

Experimental

A 2277 multichannel Thermal Activity Monitor (TAM) of the isothermal heat conduction type was used in these studies. The TAM can be equipped with upto four different calorimetric units each with a combination of functions. Samples were placed in sterilised glass ampoules under the appropriate atmospheric conditions at 37°C, sealed and then lowered into the measuring position in two stages to allow efficient equilibration.

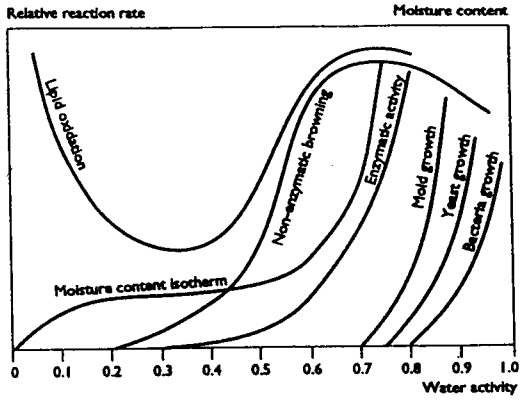

Fig 1. *Stability map of foods as a function of water activity.*

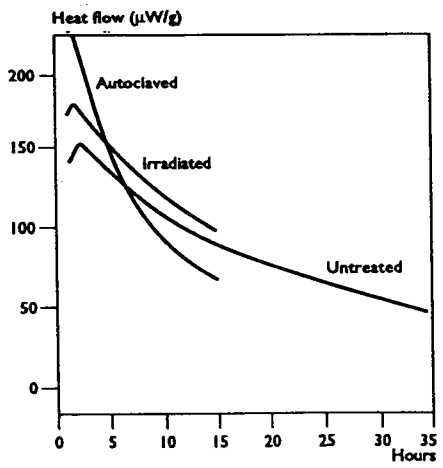

Fig 2. *Heat flow curves of autoclaved and irradiated tobacco samples.*

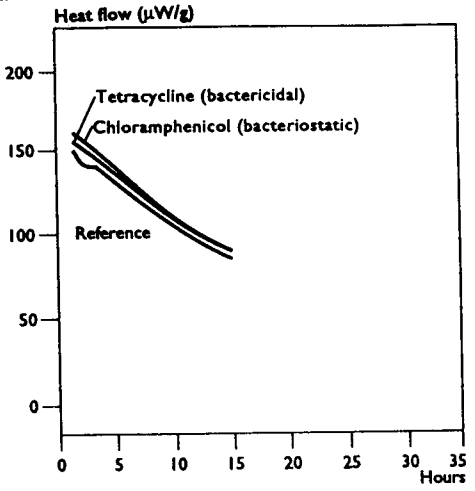

Fig 3. *Heat flow curves of antibiotic sterilised tobacco samples.*

Results and Discussion

By measuring the heat flow from a food sample under conditions that favour or inhibits one or more reactions, information can be gained on the reaction mechanism that causes the spoilage. We applied this technique for solving an instability problem observed in moist tobacco products. However, the same strategy is applicable to many other food products of vegetable origin. After a three month storage of moist tobacco products (a_w 0.90-0.93), the pH decreased by about 1 unit and the aroma had changed notably.

The preliminary microcalorimetric investigation indicated a vigorous activity in the moist tobacco. Immediately after moistening and pH adjustment (pH 8-9), the heat output was in the order of 300-400 μW/g. On storage at room temperature, it decreased to 100-150 μW/g after 4 days and to 10-15 μW/g after 15 weeks. This high activity is not unexpected since in the manufacturing process we have increased the moisture content to a level similar to a green leaf.

The first question we asked was if the aging reaction was microbial or chemical. Four different experiments were designed to answer this question. Comparison of the heat flow from a reference sample with one which had been autoclaved or gamma-ray sterilised (fig 2), or one with added antibiotics (tetracycline or chloramphenicol) (fig 3), showed that the activity was not caused by bacterial growth. Neither of these treatments decreased the heat flow. Autoclaving and irradiation both increased the heat flow, probably by triggering the chemical reactions.

The rapid decline in activity with time indicated that the substrate and the oxygen were consumed. The latter was confirmed by comparison of a sample which had been sealed for one week in an alumina foil pouch with one exposed to air. The pouch sample (measured in the pouch) produced only about 8 μW/g, while the one exposed to air gave 100 μW/g. Furthermore, when the air in the ampoule was changed to nitrogen, the heat flow rapidly decreased to less than 10 μW/g within a few hours (fig 4). Measurement of a freshly prepared sample in a perfusion vessel with continuous air flow also showed a decline in heat flow, indicating that the substrate was consumed. In addition, the pH dependence was determined, both on the stored and freshly prepared samples as shown in figures 5 and 6. The heat flow increased dramatically at pH values above 7. Thus, increasing the starting pH before packing in order to compensate for the drop during storage was not fruitful since this increased the oxidation process. Oxygen consumption was measured in separate experiments using sealed alumina foil bags. The average hourly oxygen consumption during the first day after packing was about 1ml/100g of tobacco. The rate was twice

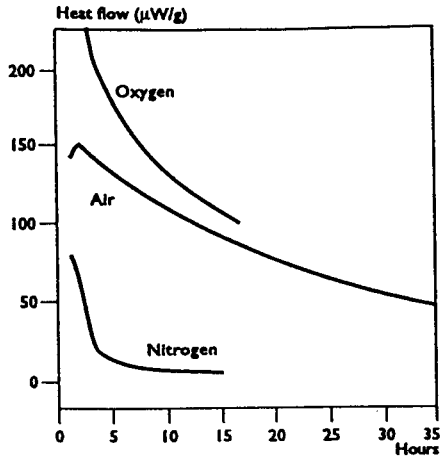

Fig 4. *Heat flow curves of tobacco samples. under different atmospheric conditions.*

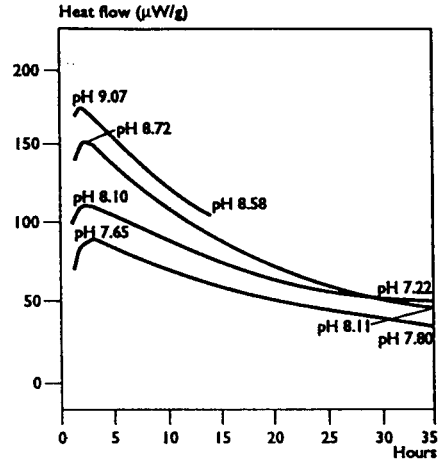

Fig 5. *Heat flow curves of tobacco samples at different pH.*

as high in the first few hours. Thus, in a sealed airtight package, all available oxygen is consumed within the first three days.

The main reaction is a base catalysed oxidation reaction. Chemical analysis of fresh and stored tobacco samples showed a large decrease in the amounts of phenols having a catechol or pyrogallol structure on storage.

Thus, chlorogenic acid and rutin, major polyphenols in many plants were completely absent in the stored samples. The content of other substances like guiacol and syringol and their alkyl substituted homologues decreased to less than one tenth of the original value after only 4 days at room temperature. These substances produced by wood smoke, were deposited on tobacco during the "smoke fire-curing" applied to some of the tobacco types used in making the product.

Review of the literature showed that lignine, present in plant material, was easily oxidised by air in alkaline solution (Ref 4). Lignine is considered to be built from phenols of the guiacol and syringol type. Lignine, while being oxidised produces a drop of pH. The mechanism for this reaction has been elucidated for a number of model substances which make up the backbone of the polymer (Ref 5). The formation of a substituted benzoic acid and carbon dioxide explain the drop in pH.

The obvious solution to the instability problem is to chose an airtight package, possibly also using an inert atmosphere like nitrogen. The microcalorimetric results also demonstrated the importance of storing the product at low temperatures. The reaction rate increases 10-fold for a 20°C rise in temperature.

In conclusion the first rapid reaction is due to the oxidation of monomeric soluble phenols, while the slower reaction is caused by oxidation of insoluble polymeric material like lignine. This was confirmed by the observation of substantial heat generation from the solid matrix remaining after water extraction of the tobacco.

It is well known that the oxidation of polyphenols, guiacol and syringol is also catalysed by the plant enzymes polyphenoloxidase and polyphenolperoxidase. These enzymes are also present in tobacco and have optimum activity at about pH 6. The fairly constant rate observed in the pH range 5-7 (fig 6) and the steep increase at pH 7 suggests that the enzymatic reaction dominates at the lower pH range and the pure chemical base catalysed reaction dominates at higher pH.

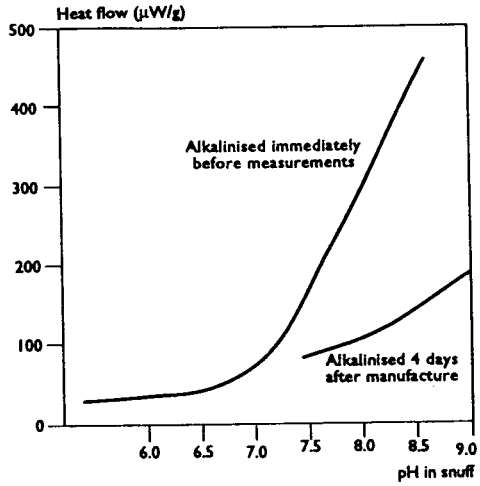

Fig 6. *Heat output of tobacco as a function of pH.*

The same strategy as described above was applied to another moist tobacco product. In this case, the instability problem was due to growth of bacteria requiring relatively low water activity. Addition of tetracycline lowered the heat output by 3-5 μW/g. By adding small amounts of water to increase the a_w by 0.01 units, bacterial growth produced a 40-50 μW higher heat flow after a few days than the sample with slightly lower a_w. The tobacco was found to have an a_w value close to the growth limit for the resident bacterial flora.

Although our work was focused on tobacco products, similar aging problems are expected to occur in other food products. Polyphenols are ubiquitous in plant material. Plants also contain other substances with similar structure, for example Coniferylalcohol, Sinapylalcohol, Ferulic and Syringa acids. Smoke flavour phenols are present in smoked fish and meat products. Even spices like clove contain easily oxidised phenols like Eugenol.

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