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# Nitrophenyl glucoside hydrolysis as a potential time-temperature integrator reaction

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Hydrolysis of *p*-nitrophenyl β-D-glucopyranoside (NPG) in borate buffer, pH 11.0, has been investigated as a potential chemical time–temperature integrator (TTI) reaction. The yellow nitrophenolate ion produced on hydrolysis was shown to be highly stable and to follow first order kinetics of formation. The temperature coefficient of the reaction rate (z-value) was found to be 21.7 (±1.2)°C over the range 91.0–121.1°C. Glucose formed on hydrolysis was unstable, apparently undergoing caramelisation to brown pigments. The latter compounds were shown not to interfere with the measurement of nitrophenolate absorbance. It was concluded that NPG hydrolysis was a reaction with significant potential for application as a chemical TTI. 

## INTRODUCTION

The chemical constituents of foods that degrade during sterilisation and cooking processes do so at rates that vary with temperature in a similar manner to many other chemical reactions. Suitable reactions can therefore be used as time-temperature integrators (TTI) of quality deterioration of foods during processing. Such reactions have been reviewed by Hendrickx *et al.* (1995). Thiamin degradation (Mulley *et al.*, 1975), dextran hydrolysis (Ellborg and Trägårdh, 1988), Maillard browning (Favetto *et al.*, 1989) and sucrose hydrolysis (Silva *et al.*, 1994) have been evaluated for their potential as chemical TTI reactions. However, many of these are mechanistically complex with side-reactions that influence the level of component being measured. Also, the methods for measuring such components can be relatively complex and time-consuming which could be a major drawback in industrial applications. Chemical TTIs involving simpler reactions, with fast methods for determining the end-products, are therefore required.

The objective of this study was to evaluate the hydrolysis of commercial *p*-nitrophenyl glucoside (NPG) under alkaline conditions as a reaction having properties suited to TTI applications.

## MATERIALS AND METHODS

NPG, almond β-glucosidase and glucose were purchased from Sigma Chemical Company, and sodium borate decahydrate, sodium hydroxide and hydrochloric acid from Merck. All chemicals were used without further purification.

Soda-glass melting point capillary tubes (internal diameter = 1.3 mm) were purchased from Merck. They were cut into 4 cm lengths, washed in boiling de-ionised water, dried and then heat-sealed at one end before use.

### Alkaline hydrolysis

Twenty millimolar borate buffer was prepared by dissolving 3.814 g sodium borate in 500ml de-ionised water. The pH was adjusted to 11.0 with 4 M sodium hydroxide.

Five millimolar NPG solution was prepared by dissolving 0.075 g NPG in 50 ml borate buffer. The solution was vacuum-filled into glass capillaries and then 10 μl solution removed by syringe to allow sufficient space for the open end of the tubes to be sealed without heating the contents. Each tube contained approximately 25 μl of NPG solution.

Kinetic studies were carried out by heating the tubes in a thermostat-controlled glycerol bath at 91.0, 101.1, 111.2 and 121.1°C (±0.1°C). Tubes were also heated for 0.5–5 h at 130°C (±1°C), times greatly in excess of those required for complete hydrolysis. After heating, the tubes were rapidly cooled in water, dried and then opened. The solution (20 μl) was mixed with 980μl of borate buffer, pH 11.0, in a 1.5 ml, 1 cm pathlength silica cuvette. The absorbance at 400nm was measured against a borate buffer blank using a Unicam PU 8700 spectrophotometer. The absorbance of unheated NPG solution at 300 nm was measured after diluting 20 μl with 980 μl of borate buffer.

Standard first order kinetics treatment of the data was carried out. Rate constants were determined from the regression of

$$-\ln\left(1 - A^{t}_{400\,nm}/A^{f}_{400\,nm}\right)$$

on time ($t$)(min). $A^{f}_{400\,nm}$, the final absorbance at 400 nm of the heated NPG solution after complete hydrolysis, was determined by multiplying the absorbance at 300 nm of the unheated solution by 1.66. $D$-values were calculated from the relationship $D = 2.303/\text{rate constant}$. The temperature alteration required to change the rate of reaction 10-fold (z-value) was determined from the regression of $\log_{10} D$ on temperature (°C).

### Enzymatic hydrolysis

$\beta$-glucosidase (1 mg ml$^{-1}$) was added to 5 millimolar NPG in borate buffer adjusted to pH 7.0 with hydrochloric acid. After holding for 30 min at 30°C, the sample was diluted 50-fold with borate, pH 11.0, and the absorbance at 400 nm was measured in a 1 cm pathlength cuvette. The absorbance at 300 nm of untreated NPG, diluted 50-fold with borate, pH 11.0, was also measured.

### Glucose determination

The glucose contents of 5 millimolar NPG and of 5 millimolar glucose, in borate, pH 11.0, heated at 130°C (± 1°C), were assayed enzymatically using a glucose/fructose test kit supplied by Boehringer Mannheim GmbH.

## RESULTS AND DISCUSSION

NPG was readily hydrolysed under alkaline conditions to the yellow nitrophenolate ion with a corresponding spectral shift from 300 to 400 nm (Fig. 1). In 0.1 M NaOH, the reaction took several hours at room temperature to reach completion. However, in borate buffer, pH 11.0, temperatures in excess of 70°C were required to obtain measurable rates.

The alkaline hydrolysis of NPG is presumed to be favoured, mainly due to the resonance stabilisation of the nitrophenolate ion (Fig. 2). The nitrophenolate stability in borate was demonstrated by heating NPG at high temperature (130°C) for times greatly in excess of that required for complete hydrolysis. No significant change in absorbance was found (Fig. 3). As a result of the high nitrophenolate stability, the nitrophenolate level can be assumed to represent the time–temperature conditions actually experienced during the heat process.

The constancy of the absorbance during the long heating times at high temperature was also strong evidence that any side-reactions had not led to coloured products. Thus, the expected caramelisation of the glucose formed on NPG hydrolysis did not occur to such

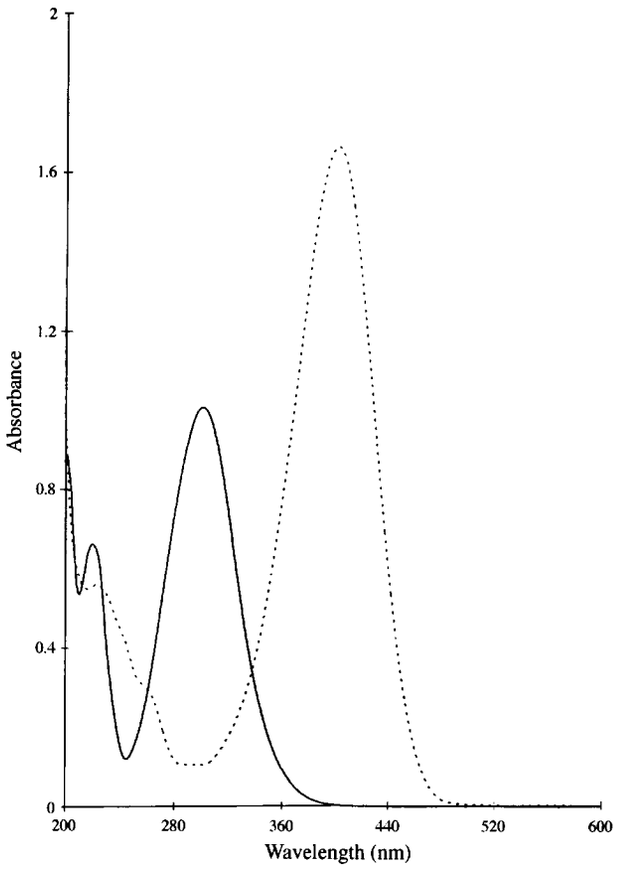


**Fig. 1.** Spectral changes on heating NPG in borate buffer, pH 11.0 (—) no heating, (- - -) 30 min at 130°C.

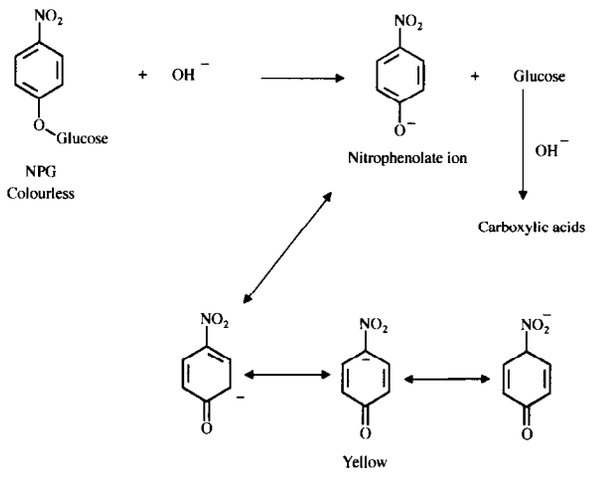


**Fig. 2.** Hydrolysis of NPG in borate buffer, pH 11.0.

an extent that it interfered significantly with the nitrophenolate absorbance measurement. This was confirmed by heating glucose in borate buffer when a pale brown colour was noted that gave zero absorbance at 300 and 400 nm on diluting by the same factor used in measuring nitrophenolate formation from NPG. No glucose was detected by enzyme assay after heating NPG or glucose, presumably due to its facile degradation to carboxylic acids under the alkaline conditions (de Bruijn *et al.*, 1986).

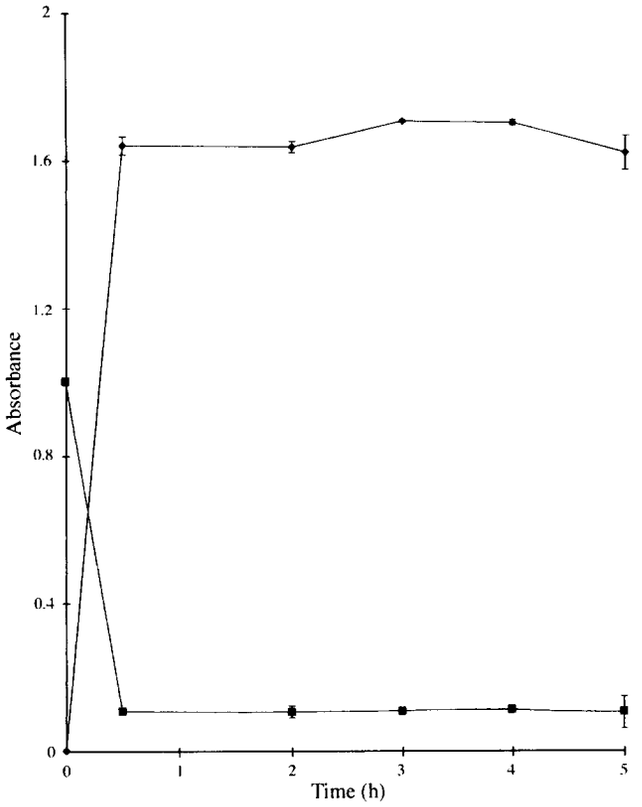


**Fig. 3.** Nitrophenolate formation on heating NPG at 130°C for excessive times. ■, $A_{300\,nm}$; ◆, $A_{400\,nm}$.

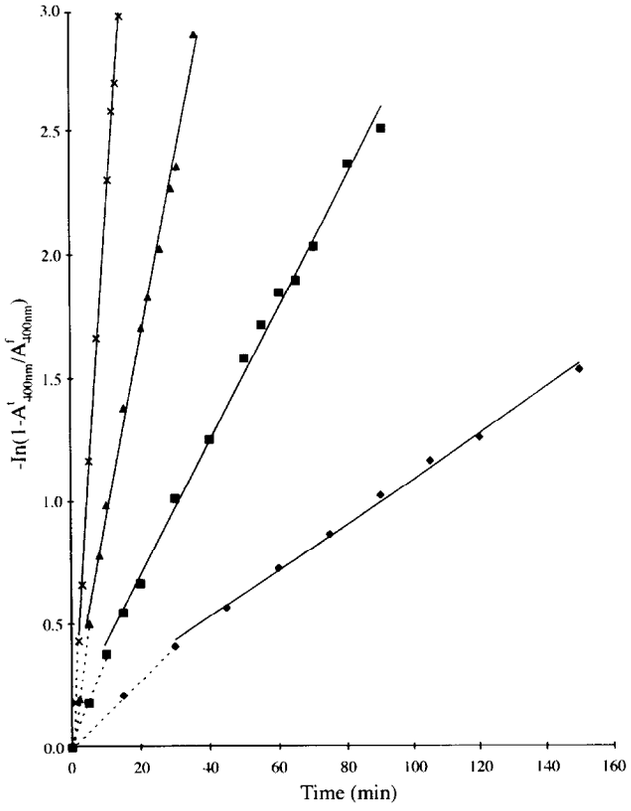


**Fig. 4.** Rates of nitrophenolate formation on hydrolysis of NPG in borate buffer, pH 11.0, in the temperature range 91.0–121.1°C; ◆, 91.0°C; ■, 101.1°C; ▲, 111.2°C; ×, 121.1°C.

In order to apply standard first order kinetic procedures to the hydrolysis of NPG, an accurate estimate was required of the absorbance at 400 nm on completion of the reaction. This was determined in two ways. First, by allowing the reaction to proceed to completion at 111.2 and 121.1°C, a mean ratio of the final absorbance at 400 nm to the absorbance at 300 nm of the unheated NPG ($A^f_{400\,nm}/A^o_{300\,nm}$) was found to be 1.66 (±0.01). Second, treatment of NPG with β-glucosidase was used to catalyse the hydrolysis at 30°C and then the absorbance at 400 nm was measured after adjusting the pH value to 11.0. This method also yielded an absorbance ratio of 1.66, thereby providing further evidence that heating did not influence the final absorbance.

First order kinetics were found for nitrophenolate formation at all temperatures in the range 91.0–121.1°C (Fig. 4). The rate constants calculated from the linear segments of the plots are presented in Table 1 along with the corresponding *D*-values. The temperature coefficient of the reaction rate (z-value) was found to be 21.7 (±1.2°C) (Fig. 5). A greater sensitivity to temperature change was therefore implied than found for many other chemical reactions (z~30°C). This would be an advantage for NPG-based TTIs in situations where temperatures changed slowly such as in foods that heat by conduction.

At 90–110°C, the absorbance values were slightly low at short heating times, presumably due to the temperature inside the relatively thick-walled glass capillary tubes lagging behind the heating bath temperature. Improvements in containment material are evidently required to overcome the thermal lag of glass, and also because glass would be unacceptable in the food industry due to the possibility of contamination of food products with glass fragments. In standard heat processes involving hot water or steam, a chemically inert metal would be preferred because of its high thermal conductivity. In non-standard heating processes, particularly those involving microwaves, a non-metallic, inert containment material would be required with thin walls to reduce the thermal lag effect to a minimum. Preliminary work with teflon capillaries has shown promise though heat-seals can break down on exposure to high temperature.

The application of the NPG hydrolysis reaction in a TTI has yet to be tested in a real food processing context. However, an NPG-based TTI would have an

**Table 1.** The first order rate constants for NPG hydrolysis in borate buffer, pH 11.0 in the temperature range 91.0–121.1°C

| Temperature (°C) | Rate constant ($min^{-1}$) | *D*-value (min) | $\log_{10}D$ |
|---|---|---|---|
| 91.0 | 0.00940 | 245 | 2.39 |
| 101.1 | 0.0273 | 84.4 | 1.93 |
| 111.2 | 0.0756 | 30.5 | 1.48 |
| 121.1 | 0.231 | 10.0 | 1.00 |

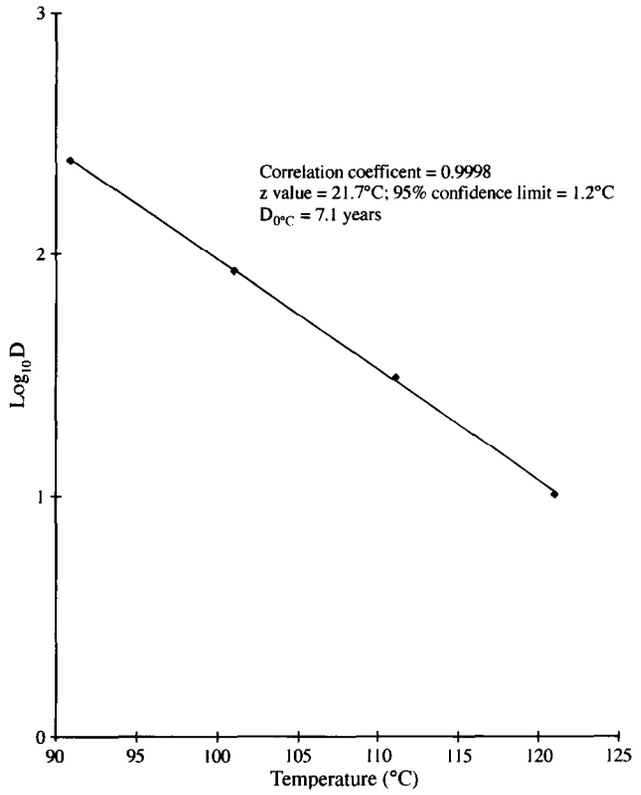


**Fig. 5.** Variation in decimal reduction time with temperature for NPG hydrolysis in borate buffer, pH 11.0.

advantage over many chemical TTIs in being very simple to read, requiring only to be opened, the contents diluted and absorbance at 400nm measured. The measurement could be simplified further by determining the absorbance of the tube contents directly, without dilution. This is feasible using current low volume spectrophotometric cuvettes. However, these have the same pathlength as the higher volume cells, implying that absorbance values would be too high for accurate measurements to be made in most heat-processing situations except when heat-treatments were very mild. This could be overcome by using a more dilute NPG solution.

Storage of NPG-based TTIs before or after heat treatment should be carried out at 0°C. At this temperature, a $D$ value of over seven years is calculated from the intercept of the $\log_{10} D$ on temperature plot. In practice, it is expected that some hydrolysis would have already occurred before the TTI is exposed to the heat process. Measurement of the difference between the initial and final absorbances in duplicate TTI devices would then be required.

## CONCLUSIONS

Hydrolysis of NPG under mildly alkaline conditions has been shown to be a reaction with significant potential for application as a chemical TTI. The yellow nitrophenolate ion produced on hydrolysis was highly stable, following first order kinetics of formation. The temperature coefficient of the reaction (z) was found to be 21.7 (±1.2°C) over the range 91.0–121.1°C, implying a more sensitive response to temperature variation than normally found for chemical reactions.

The glucose formed on hydrolysis was unstable, apparently undergoing caramelisation to brown pigments. The latter compounds did not interfere with the measurement of nitrophenolate absorbance.

## ACKNOWLEDGEMENT

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## REFERENCES

de Bruijn, J. M., Kieboom, A. P. G., van Bekkum, H. and van der Poel, P. W. (1986) Reactions of monosaccharides in aqueous alkaline solutions. *Sugar Technology Review* **13**, 21–52.

Ellborg, A. and Trägårdh, Ch. (1988) Dextran as reactive tracer for thermal time distribution in continuous flow sterilisation. *Proceedings of the International Symposium on Progress in Food Preservation and Processing* **1**, 163–170.

Favetto, G. J., Chiriffe, J., Scorza, O. C. and Hermida, C. A. (1989) Time–temperature integrating indicator for monitoring the cooking process of packaged meats in the temperature range of 85–100°C. U.S. Pat., 4, 834,017.

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

Mulley, E. A., Stumbo, C. R. and Hunting, W. M. (1975) Thiamine: a chemical index of the sterilisation efficacy of thermal processing. *Journal of Food Science* **40**, 993–996.

Silva, C. L. M., Oliveira, F. A. R., Lamb, J., Torres, A. P. and Hendrickx, M. (1994) Experimental validation of model for predicting optimal surface quality sterilisation temperatures. *International Journal of Food Science and Technology* **29**, 227–241.