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Properties of Brussels Sprouts Thioglucosidase

M. B. Springett & J. B. Adams

Campden Food and Drink Research Association,
Chipping Campden, Gloucestershire GL55 6LD, UK

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ABSTRACT

Some important properties of Brussels sprouts thioglucosidase have been studied using ammonium sulphate precipitates. The enzyme activity followed Michaelis–Menten kinetics with sinigrin as substrate. Ascorbic acid was shown both to activate and to inhibit the thioglucosidase with an optimum activity at 10^{-2}M ascorbate. Two pH optima were observed at pH 6–6·5 and pH 8, possibly indicating the presence of different isoenzymes. Thioglucosidase was completely inhibited on freezing in aqueous conditions at −6°C and −12°C but was active at these temperatures in 35% (w/w) glycerol. A temperature optimum activity at about 50°C was found and, at higher temperatures (60°C–90°C), the enzyme was thermally inactivated by a complex mechanism.

Using extracts from different regions of Brussels sprouts, it was found that the majority of the thioglucosidase activity was in the outer leaves.

INTRODUCTION

The hydrolysis of glucosinolates in brassica vegetables is catalysed by the enzyme thioglucosidase (systematic name, thioglucoside glucohydrolase, EC 3.2.3.1; common name, myrosinase) yielding glucose and unstable aglycones. The latter undergo rearrangement to a range of compounds including isothiocyanates, thiocyanates and sulphur-free nitriles which may contribute significantly to the characteristic flavour of the fresh vegetable (MacLeod, 1976). On cooking or blanching prior to freezing, the enzyme is

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thermally inactivated and this arrests the formation of any further flavour compounds. In order to suggest processes which can be utilised by the freezing industry to maximise such compounds, it is important to establish the properties of thioglucosidase and, in particular, those factors which control activity and the rate of inactivation of the enzyme.

The most highly characterised thioglucosidases, to date, are those from seeds of white mustard (*Sinapis alba* L.) and rape (*Brassica napus* L.) and little information is available on the enzyme in brassica vegetables processed by the frozen food industry. The work reported in this paper is concerned with the properties of a thioglucosidase preparation from Brussels sprouts (*Brassica oleracea* L. var. *bullata* subvar. *gemmifera*) and considers the implications of these properties for the flavour of the frozen product.

MATERIALS AND METHODS

The Brussels sprouts used in this study were either bought locally or grown by the Agriculture Department at Campden Food and Drink Research Association. Sinigrin (2-propenyl glucosinolate, purity >98%) and bovine serum albumin were supplied by Sigma Chemical Co. Ltd, Poole, UK and were used without further purification. Glucose test kits were supplied by Boehringer Corporation (London) Ltd, Lewes, UK. All other reagents were supplied by BDH Chemicals plc, Poole, UK and were 'AnalaR' grade where available.

Thioglucosidase determination by enzyme-linked assay

The enzyme-linked assay for thioglucosidase activity was similar to that reported by Wilkinson *et al.* (1984*a*) except that the enzymes and cofactors of the Boehringer test kit were used directly. One millilitre of NADP/ATP reagent, 0·02 ml HK/G6P-DH reagent, 0·5 ml 0·012M sinigrin in 0·1M sodium phosphate buffer (pH 7·0) and 1 ml 0·01M ascorbic acid in 0·1M sodium phosphate buffer (pH 7·0) were placed in each of two 3 ml glass cuvettes (1 cm light path). After equilibration for 5 min at 30°C, 0·1 ml enzyme extract was added to the sample cuvette and the increase in absorbance at 340 nm due to the formation of NADPH measured. Thioglucosidase activity was expressed as the initial, linear rate of absorbance increase (A_{340nm} min^{-1}).

Preparation of crude enzyme extract

All procedures were carried out at 4°C. Two hundred and fifty grams of Brussels sprouts were blended with 750 ml 0·1M sodium phosphate buffer

(pH 7·0) containing 0·01M mercaptoethanol. The extract was filtered and after centrifugation for 30 min at 19,200*g*, the supernatant was 90% saturated with ammonium sulphate (GPR grade). The resultant precipitate was centrifuged and then the pellet dissolved in 50 ml 0·01M sodium phosphate buffer (pH 7·0) containing 0·001M mercaptoethanol. After dialysis against the same buffer—mercaptoethanol mixture, the extract was held at 4°C prior to use.

Protein determination

Protein determinations were carried out using the commercial form of the dye binding method of Bradford (1976). The assays were performed as recommended by the supplier (Bio-Rad). A standard curve was constructed using bovine serum albumin in the concentration range 0·2–1·4 mg ml^{-1}.

Distribution of thioglucosidase in Brussels sprouts

In order to investigate the distribution of thioglucosidase in different parts of Brussels sprouts, a total of 10 g of tissue was collected from a number of sprouts of similar size (cv. Roger), covering the central region, inner leaves, outer leaves and the stalk (no leaves attached). Each part was extracted with 30 ml of 0·1M sodium phosphate buffer containing 0·01M mercaptoethanol and an ammonium sulphate precipitate prepared and dialysed as described above. Thioglucosidase activity was determined using the enzyme-linked assay and expressed as A_{340nm} min^{-1} per gram of fresh tissue. Two assays were performed on each extract.

Effect of sinigrin and ascorbic acid on thioglucosidase activity

Sinigrin concentrations investigated were 1, 2·5, 5, 10, 15 and 20 × 10^{-3}M and ascorbic acid concentrations were 0, 1, 5, 10, 20 and 50 × 10^{-3}M. Crude enzyme extract (0·2 ml, prepared from Brussels sprouts cv. Cavalier) and 0·4 ml ascorbic acid in 0·1 M sodium phosphate buffer (pH 7·0) were mixed and equilibrated for 5 min at 30°C. At the 50 × 10^{-3}M ascorbic acid concentration, 0·2M phosphate buffer was required to maintain the pH value at 7·0. The reaction was initiated by adding 0·4 ml sinigrin and terminated, after the appropriate time interval, by vigorously mixing in 0·2 ml 18% metaphosphoric acid. Glucose concentration was determined by means of the Boehringer glucose test kit. Thioglucosidase activity was taken as the slope of the regression line of glucose concentration against time (M × 10^{-3} glucose min^{-1}). Two determinations were performed for each sinigrin and ascorbic acid concentration.

K_m and V_{max} values were computed using the iterative, nonlinear regression procedure of Duggleby (1981), assuming constant standard deviations and applying bisquare weighting.

Effect of pH on thioglucosidase activity

The pH range 4–10 was covered by means of 0·025M sodium borate–KCl buffer (pH 8·6, 9·0, 9·6 and 10·0), 0·1M sodium phosphate buffer (pH 6·0, 6·6, 7·0, 7·6 and 8·0) and 0·2M sodium acetate buffer (pH 4·0, 4·6, 5·0 and 5·6) prepared according to Dawson *et al.* (1986). Buffer (2·5 ml), 0·5 ml 0·04M ascorbic acid and 1 ml crude enzyme extract (prepared from Brussels sprouts cv. Cavalier) were mixed and equilibrated for 5 min at 30°C. The reaction was initiated by adding 1 ml 0·018M sinigrin in the same buffer at 30°C and stopped, after the appropriate time interval, by vigorously mixing in 1 ml 18% metaphosphoric acid. Glucose concentration was determined using the Boehringer glucose test kit. Thioglucosidase activity was taken as the slope of the regression line of glucose concentration against time ($M \times 10^{-3}$ glucose min^{-1}). Two replicate experiments were performed.

Changes in thioglucosidase activity with temperature

Temperatures in the range −12°C to +50°C were investigated. 0·5 ml 0·04M ascorbic acid, 2·5 ml 0·1M sodium phosphate buffer (pH 7·0) and 1·0 ml crude enzyme extract (prepared from Brussels sprouts cv. Roger) were mixed and equilibrated for 5 min at the temperature under investigation. The reaction was then started by the addition of 1 ml 0·018M sinigrin at the same temperature and stopped after the desired time, by adding 1 ml 18% metaphosphoric acid. To study temperatures below freezing, the enzyme preparation and reagents were mixed at 4°C and immediately poured into liquid nitrogen. The frozen mixture was then held at the appropriate temperature for up to 10 months. The reaction was stopped by adding 1 ml of boiling 18% metaphosphoric acid and shaking vigorously until all of the ice had melted (approximately 60 s).

A similar experiment was carried out in which 35% (w/w) glycerol was included in the reaction mixture.

Glucose determinations were performed in duplicate for each temperature using the Boehringer glucose test kit.

Rate constants(k) for sinigrin hydrolysis were calculated from the linear regression slopes of the first order plots of glucose concentration against reaction time. Arrhenius activation energies were determined from the slopes of the linear regression plots of $\log_e k$ against reciprocal temperature over the appropriate range.

Thermal inactivation of thioglucosidase

Thermal treatment of the crude enzyme preparation was carried out in the temperature range 50–90°C using soda glass capillary tubes as described by Adams (1978). A crude enzyme extract was prepared from Brussels sprouts cv. Cavalier. Thioglucosidase activity was determined using the enzyme-linked assay as soon as possible after heating and cooling.

RESULTS AND DISCUSSION

Distribution of thioglucosidase activity in different parts of Brussels sprouts

Thioglucosidase activity was 4–5 times higher in the outer leaves of the Brussels sprouts than in other regions (Table 1). This would be expected if the glucosinolates or their degradation products act as insect attractants as others have suggested (Thorsteinson, 1953; David & Gardiner, 1966; Coaker, 1969).

By blanching Brussels sprouts to leave some residual thioglucosidase, a more acceptable flavour may be achieved if the enzyme is able to catalyse the breakdown of glucosinolates during the remainder of the freezing process. However, as the thioglucosidase activity is highest in the outer regions of the sprout, it is likely that a much reduced heat treatment would be required and this would risk leaving other active enzymes which may be detrimental to product quality.

TABLE 1
Distribution of Thioglucosidase Activity in Brussels Sprouts

Sample	*Thioglucosidase activity*[a] (A_{340nm} min^{-1} g^{-1})
Centre	0·025 (±0·003)
Inner leaves	0·032 (±0·002)
Stalk	0·030 (±0·003)
Outer leaves	0·133 (±0·030)

[a] Range of values in parentheses.

Activation and inhibition of thioglucosidase by ascorbic acid

The thioglucosidase activity of the crude enzyme extract (protein concentration, 2·5 mg ml^{-1}) was investigated as a function of sinigrin and ascorbic acid concentrations. From plots of mean rate versus ascorbic acid

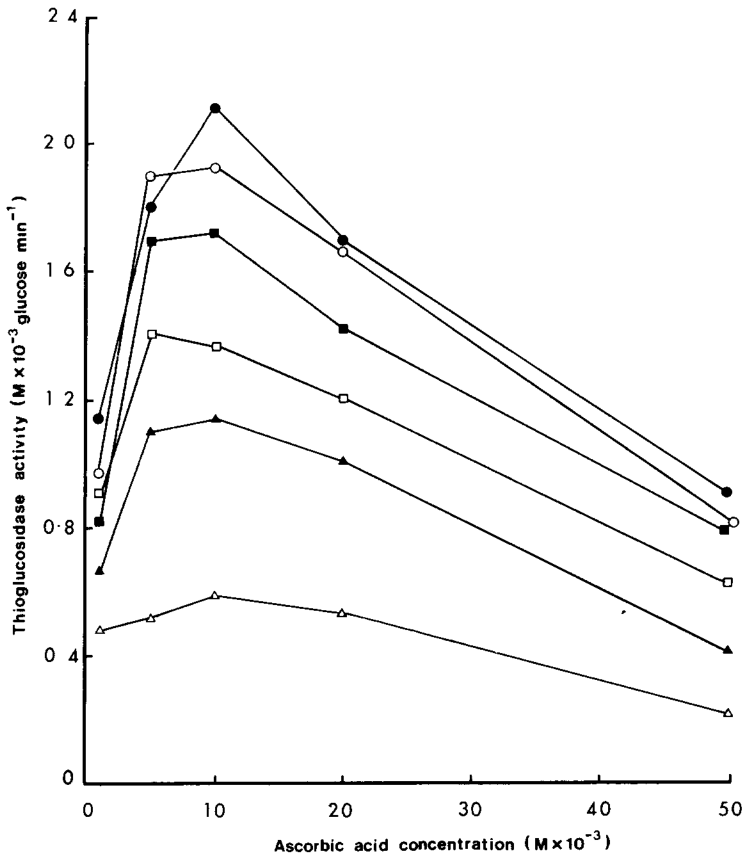

Fig. 1. Dependence of thioglucosidase activity on sinigrin and ascorbic acid concentrations. △ = 1×10^{-3}M, ▲ = $2{\cdot}5 \times 10^{-3}$M, □ = 5×10^{-3}M, ■ = 10×10^{-3}M, ○ = 15×10^{-3}M, ● = 20×10^{-3}M sinigrin.

concentration, an optimum activity was observed at 10^{-2}M ascorbic acid with activities increasing as sinigrin concentration was increased up to $2{\cdot}0 \times 10^{-2}$M (Fig. 1). Activation by ascorbic acid can also be seen from the V_{max} data whilst the increasing K_m values suggest that ascorbic acid is acting as an inhibitor (Table 2). In the absence of ascorbic acid, no thioglucosidase activity was detected under the present conditions. Wilkinson *et al.* (1984*b*) have reported that the ascorbic acid concentration for optimum thioglucosidase activity lay between 0·7 and 5×10^{-3}M depending on the species of cruciferous vegetable. For Brussels sprouts, they obtained an optimum activity between 0·7 and 1×10^{-3}M ascorbic acid. However, their peak was very broad with enzyme activity between 0·1 and 55×10^{-3}M ascorbic acid and with plateaux at approximately 2·5 and $7{\cdot}5 \times 10^{-3}$M. The latter behaviour suggests the presence of isoenzymes with different ascorbic acid

TABLE 2
Effect of Ascorbic Acid Concentration on the Michaelis Constant (K_m) and Maximal Thioglucosidase Rate (V_{max})

Ascorbic acid concentration (M × 10^{-3})	K_m[a] (M × 10^{-3} *sinigrin*)	V_{max}[a] (M × 10^{-3} *glucose min*$^{-1}$)
1	1·63 (0·29)	1·17 (0·049)
5	2·38 (0·31)	2·07 (0·070)
10	2·98 (0·44)	2·31 (0·099)
20	2·69 (0·31)	1·90 (0·057)
50	3·77 (0·64)	1·07 (0·055)

[a] Standard errors in parentheses, 2 determinations.

activation and inhibition properties. The relative activity of such isoenzymes could vary with Brussels sprouts cultivar and this may offer an explanation for the difference in ascorbic acid concentration for optimal activity compared with the present work. In support of this suggestion, Henderson & McEwen (1972) and Björkman & Lönnerdal (1973) have shown a variation in response to ascorbic acid among several thioglucosidase isoenzymes extracted from cruciferous oilseeds.

At the concentration of sinigrin present in Brussels sprouts (0·27–3·76 × 10^{-3}M; Fenwick *et al.* 1983), thioglucosidase activity should be between 11·4% and 64·3% of the V_{max} at 5 × 10^{-3}M ascorbic acid. As ascorbic acid is naturally present in sprouts at about 6 × 10^{-3}M (Paul & Southgate, 1979), this would be the approximate range of activities expected in practice. The sinigrin concentration depends primarily on cultivar and this could greatly affect the observed rate of hydrolysis of the glucosinolate and the final concentration of degradation products which influence the perceived flavour. Also the cultivar dependence of thioglucosidase concentration would be expected to be important as this would directly affect V_{max}. However, this latter relationship has not yet been explored.

Ascorbic acid appears to activate mustard thioglucosidase by binding to an aglycone effector site (Tsuruo & Hata, 1967) and inhibit it either by binding at the substrate site (Tsuruo & Hata, 1968) or, possibly, by destabilising the enzyme/substrate complex (Björkman & Lönnerdal, 1973). At the pH used in the present work (pH 7·0), the ascorbic acid (pK_a 4·04 and 11·34, Dawson *et al.* (1986)) would be present mainly as a singly charged anion and although the interaction with thioglucosidase is unknown, it is feasible that the negative charge on the ascorbate has an important role to play in activation. Inhibition may be caused by a keto form of the ascorbate as suggested by Tsuruo & Hata (1968).

Effect of pH on thioglucosidase activity

Using the crude enzyme extract, two activity maxima were observed at pH 6–6·5 and pH 8 (Fig. 2). As the pH of unfrozen Brussels sprouts lies between 6 and 7, the form of thioglucosidase with the pH optimum at 6–6·5 is probably most active in sprout tissue. Thioglucosidases from other sources also show variation of activity with pH value. West *et al.* (1977) have shown two thioglucosidase optima at pH 5 and pH 8 in crude extracts from cabbage, and isoenzymes have been isolated from mustard and rape seed with different pH optima (Björkman & Lönnerdal, 1973). The presence of different thioglucosidase isoenzymes in Brussels sprouts would appear to be the most probable cause of the two activity maxima.

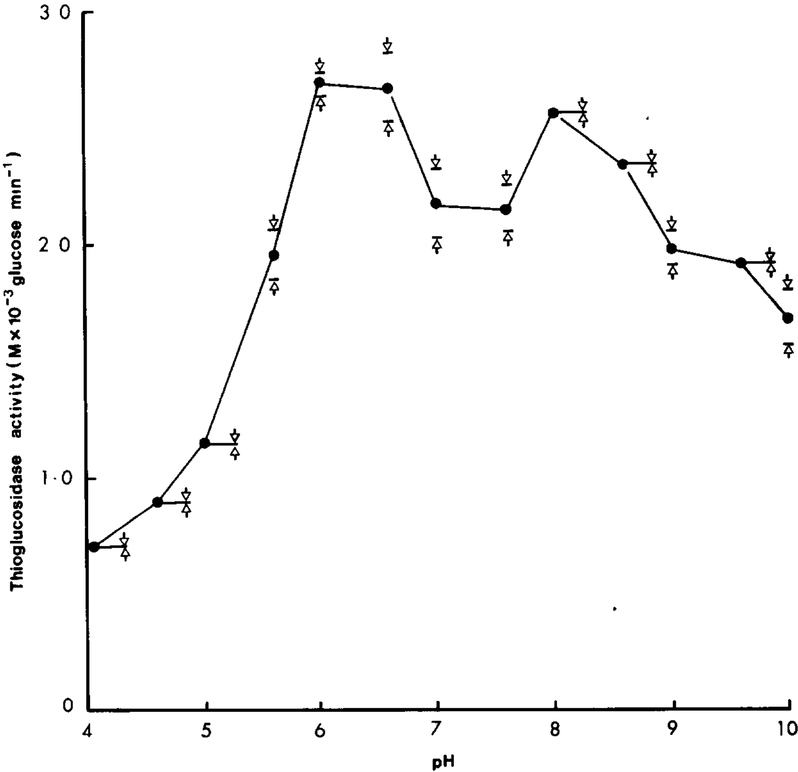

Fig. 2. Effect of pH on thioglucosidase activity. Arrows denote range.

Changes in thioglucosidase activity with temperature

The thioglucosidase activity in the crude enzyme extract varied with temperature in the range −12°C to +50°C as shown in Fig. 3 (Arrhenius

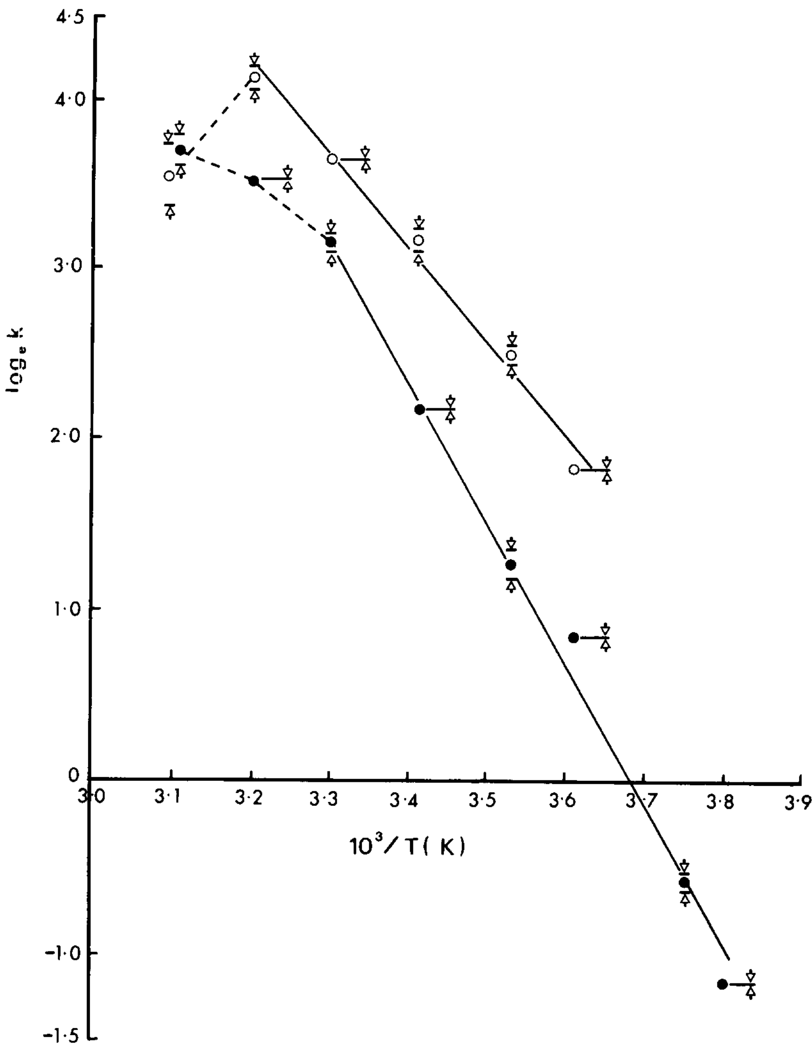

Fig. 3. Effect of temperature on thioglucosidase activity. ○ = No glycerol, ● = 35% (w/w) glycerol. Arrows denote range.

plots). Without glycerol, the activation energy over the range +4°C to +40°C was 44·5 kJ mol^{-1}. In the presence of 35% (w/w) glycerol, the enzyme activity was reduced with increasing effect as the temperature was lowered. The activation energy over the range −12°C to +30°C was then determined as 67·7 kJ mol^{-1}. Deviations from linearity at the higher temperatures are probably caused by the thermal inactivation of the thioglucosidase. The differences in the activation energies with and without

glycerol clearly indicate different reaction pathways although the same reaction product was found in both cases (glucose).

The lack of thioglucosidase activity at −6°C and −12°C in the absence of glycerol, even after 10 months' storage, is in agreement with observations on other non-cellular systems (Fennema, 1975). Possible reasons for this which have been considered in the present study are reduction of water activity on freezing, cold denaturation of the thioglucosidase and inactivation or inhibition of the enzyme as a result of freeze-concentrating the solutes. Using the freezing point depression formula of Chen (1987), the water activities of ice–water at −6°C and −12°C were calculated as 0·94 and 0·89, respectively. Whilst the lower water activity may have reduced thioglucosidase activity, it would not be expected to eliminate enzyme activity completely. The fact that significant thioglucosidase activity was found at −6°C and −12°C in 35% (w/w) glycerol which has approximately the same water activity as ice-water at these temperatures (0·89, calculated using the freezing point depression data of Wolf *et al.* (1972) and Chen's formula), is further evidence that the reduced water activity is not the sole cause of enzyme activity loss on freezing.

On thawing samples held at −12°C, rapid and complete hydrolysis of the sinigrin occurred, showing that a significant proportion of the enzyme activity remained after the freezing and thawing process. This suggests a reversible cold denaturation of the enzyme possibly due to weakening of hydrophobic bonds in an oligomeric complex as suggested for potato phosphofructokinase (Dixon *et al.*, 1981).

The increased concentration of solutes on freezing is known to give rise to a number of effects which could influence enzyme activity. Changes in pH value can be caused either by concentration effects as such or by the crystallisation of the buffer components under eutectic conditions. In the present case, Na_2HPO_4 is in excess and would crystallise in preference to NaH_2PO_4 causing the pH to fall. A pH of 3·6 has been observed by van den Berg & Rose (1959) at the eutectic point of mixed NaH_2PO_4 and Na_2HPO_4 (−9·9°C). From the results at 30°C, the thioglucosidase activity would be considerably reduced at this pH value. Freezing would have also increased the ascorbic acid concentration, perhaps to such an extent that it became inhibitory as described earlier. The re-appearance of enzyme activity on thawing could then be due to the return of the ascorbic acid concentration to its pre-freezing value. In practice, the complete loss of thioglucosidase activity on freezing may be caused by a number of factors acting simultaneously and synergistically.

Evidence for thioglucosidase activity in frozen Brussels sprouts has come from the observation of considerably higher levels of propenyl isothiocyanate (4-5 fold) in unblanched sprouts, stored for 4 months at −18°C,

than in similar material that had been blanched (Springett, 1987). In addition, the finding of increased levels of mustard flavour in sprouts blanched for short times and stored for 3–6 months at −23°C supports the isothiocyanate studies (Adams, 1983). Thus, the glycerol model system may represent the environment in frozen Brussels sprouts more nearly than the buffer system.

Thermal inactivation of thioglucosidase

Heat treatment of the thioglucosidase preparation was carried out with a view to determining the inactivation parameters (Arrhenius activation energy and rate constant at a reference temperature) which could be used to compute residual enzyme activities in Brussels sprouts with known temperature histories. Preliminary work had shown no reappearance of thioglucosidase activity after partial inactivation at 70°C (Springett, 1987), which made it possible to determine enzyme activity as soon as possible after the heat treatment, without it being necessary to allow regeneration of activity to a constant level. However, the first order plots of thioglucosidase activity against time were nonlinear (Fig. 4) and accurate rate constants could not be determined. Subsequent calculation of the inactivation parameters was therefore ruled out. Further work at longer heating times is needed to determine whether or not biphasic kinetics with respect to time can be assumed.

Two types of mechanism could have led to the nonlinear first order kinetics, a parallel-type which involves the concurrent inactivation of isoenzymes with different heat stabilities and a series-type involving the conversion of the native enzyme to intermediate forms with lower specific activities and enhanced heat stabilities. If the parallel mechanism applies, variation in isoenzyme activity from one Brussels sprouts cultivar to another could cause differences in the observed heat-stability of thioglucosidase, whereas if the series mechanism applies, similar heat-stabilities should be found in all cultivars. The presence of isoenzymes has been inferred from the pH studies and it is possible that these putative species have different stabilities. Further work with highly purified thioglucosidase isoenzymes from sprouts would be necessary to differentiate between the parallel and series mechanisms.

In conclusion, the results have shown a number of properties of Brussels sprouts' thioglucosidase of relevance to the processor. In particular, the location of the enzyme, the variation of activity with sinigrin and ascorbic acid concentrations, the variation of activity with temperature, and the behaviour of the enzyme on heating are important factors which have potential consequences for the flavour of the frozen product. The main product from sinigrin and residual thioglucosidase would be 2-propen-1-yl

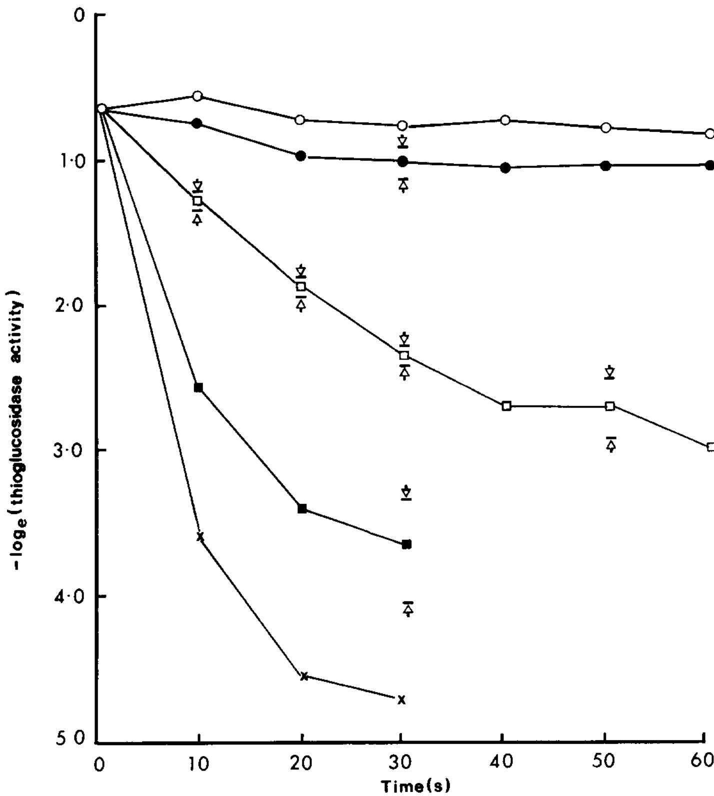

Fig. 4. Thermal inactivation of thioglucosidase. ○ = 50°C, ● = 60°C, □ = 70°C, ■ = 80°C, × = 90°C. Arrows denote range.

isothiocyanate at the normal pH value of Brussels sprouts and this would be expected to impart a 'mustard' note to the flavour. However, the presence of epithiospecifier protein may divert some of the aglycone intermediate of sinigrin hydrolysis to 1-cyano-2,3-epithiopropane (Springett & Adams, 1988). The contribution which this compound makes to overall flavour is unknown but its formation would cause a reduction in isothiocyanate and thereby a possible reduction in 'mustard' note. Other glucosinolates may also be affected by the presence of residual thioglucosidase. In particular, progoitrin can be converted to goitrin which imparts a bitter flavour (Fenwick & Griffiths, 1981) and glucobrassicin can give rise to various indole compounds which may have distinct flavour characteristics despite being only slightly volatile.

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REFERENCES

Adams, J. B. (1978). The inactivation and regeneration of peroxidase in relation to the high temperature—short time processing of vegetables. *J. Food Technol.*, **13**, 281–97.

Adams, J. B. (1983). The effect of blanching on enzyme activities and their relationship to the quality of frozen vegetables. Part 1C—Brussels sprouts. Campden Food and Drink Research Association, Technical Memorandum No. 316, Chipping Campden, UK.

Björkman, R. & Lönnerdal, B. (1973). Studies on myrosinases: III Enzymatic properties of myrosinases from *Sinapis alba* and *Brassica napus* seeds. *Biochim. et Biophys. Acta*, **327**, 121–31.

Bradford, M. N. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. *Analyt. Biochem.*, **72**, 248–54.

Chen. C. S. (1987). Relationship between water activity and freezing point depression of food systems. *J. Food Sci.*, **52**, 433–5.

Coaker, T. H. (1969). New approaches to cabbage root fly control. *Proc. 5th Br. Insectic. and Fungic. Conf., Brighton, UK*, **3**, 704–10.

David, W. A. L. & Gardiner, B. O. C. (1966). Mustard oil glucosides as feeding stimulants for *Pieris brassicae* larvae in a semi-synthetic diet. *Ent. Exp. and Appl.*, **9**, 247–55.

Dawson, R. M. C., Elliott, D. C., Elliott, W. H. & Jones, K. M. (1986). *Data for Biochemical Research.* (3rd edn). Clarendon Press, Oxford.

Dixon, W. L., Franks, F. & ap Rees, T. (1981). Cold lability of phosphofructokinase from potato tubers. *Phytochem.*, **20**, 969–72.

Duggleby, R. G. (1981). A nonlinear regression programme for small computers. *Analyt. Biochem.*, **110**, 9–18.

Fennema, O. (1975). Activity of enzymes in partially frozen aqueous systems. In *Water Relations of Foods.* ed. R. B. Duckworth. Academic Press, London, pp. 397–413.

Fenwick, G. R. & Griffiths, N. M. (1981). The identification of the goitrogen, (-)5-vinyloxazolidine-2-thione (goitrin), as a bitter principle of cooked Brussels sprouts (*Brassica oleracea* L. var. *gemmifera*). *Z. Lebensm. unters Forsch*, **172**, 90–2.

Fenwick, G. R., Heaney, R. K. & Mullin, W. J. (1983). Glucosinolates and their breakdown products in food and food plants. *CRC Crit. Rev. Food Sci. Nut.*, **18**, 123–201.

Henderson, H. M. & McEwen, T. J. (1972). Effect of ascorbic acid on thioglucosidases from different crucifers. *Phytochem.*, **11**, 3127–33.

MacLeod, A. J. (1976). Volatile flavour compounds of the *Cruciferae*. In *The Biology and Chemistry of the Cruciferae*, ed. J. G. Vaughan, A. J. MacLeod & B. M. G. Jones. Academic Press, London, pp. 307–30.

Paul, A. A. & Southgate, D. A. T. (1979). *McCance and Widdowson's The Composition of Foods.* Her Majesty's Stationery Office, London.

Springett, M. B. (1987). Flavour changes in Brussels sprouts in relation to preservation by freezing. PhD Thesis, University of Reading, UK.

Springett, M. B. & Adams, J. B. (1988). Identification of 1-cyano-2,3-epithiopropane in volatiles of a model system from Brussels sprouts (*Brassica oleracea* L. var. *bullata* subvar. *gemmifera* D.C.). *J. Sci. Food Agric.* **46**, 211–19.

Thorsteinson, A. J. (1953). The chemotactic responses that determine host specificity in an oligophagous insect (*Plutella maculipennis* (Curt.) *lepidoptera*). *Can. J. Zool.*, **31**, 52–72.

Tsuruo, I. & Hata, T. (1967). Studies on the myrosinase in mustard seed. Part II. On the activation mode of the myrosinase by L-ascorbic acid. *Agr. Biol. Chem.*, **31**, 27–32.

Tsuruo, I. & Hata, T. (1968). Studies on myrosinase in mustard seed. Part V. On the β-glucosidase activity of myrosinase and the interaction of ascorbate with myrosinase. *Agr. Biol. Chem.*, **32**, 1425–31.

van den Berg, L. & Rose, D. (1959). Effect of freezing on the pH and composition of sodium and potassium phosphate solutions: The reciprocal system KH_2PO_4–Na_2HPO_4–H_2O. *Arch. Biochem. Biophys.*, **81**, 319–29.

West, L. G., Badenhop, A. F. & McLaughlin, J. L. (1977). Allyl isothiocyanate and allyl cyanide production in cell-free cabbage leaf extracts, shredded cabbage and coleslaw. *J. Agric. Food Chem.*, **25**, 1234–8.

Wilkinson, A. P., Rhodes, M. J. C. & Fenwick, G. R. (1984*a*). Determination of myrosinase (thioglucoside glucohydrolase) activity by a spectrophotometric coupled assay. *Analyt. Biochem.*, **139**, 284–91.

Wilkinson, A. P., Rhodes, M. J. C. & Fenwick, G. R. (1984*b*). Myrosinase activity of cruciferous vegetables. *J. Sci. Food Agric.*, **35**, 543–52.

Wolf, A. V., Brown, M. G. & Prentiss, P. G. (1972). Concentrative properties of aqueous solutions: Conversion tables. In *Handbook of Chemistry and Physics.* (53rd edn) ed. R. C. Weast, The Chemical Rubber Co., Cleveland, OH, USA, pp. D181–235.