Food Chemistry 30 (1988) 157–162

Research Note

Benzyl Isothiocyanate in Onion (*Allium cepa* L.) and Mushroom (*Agaricus bisporus*)—A Re-examination

ABSTRACT

*Modern chemical and physico-chemical techniques have been applied to the analysis of benzyl isothiocyanate and glucosinolate in onion (*Allium cepa *L.) and mushroom (*Agaricus bisporus*). No such compounds could be detected above the limits of the analytical methods used and earlier claims for the presence of these compounds in these foods must be regarded as tentative.*

INTRODUCTION

Glucosinolates, also known as mustard oil glycosides, co-occur with myrosinase (thioglucoside glucohydrolase EC3.2.3.1) in all of the species of the order *Capparales* (including the agriculturally important brassicas) so far investigated (Ettlinger & Kjaer, 1968; Heaney & Fenwick, 1987). Glucosinolate degradation, catalysed by myrosinase, yields characteristic products, the most ubiquitous of which are isothiocyanates (Fenwick *et al.*, 1983; Tookey & VanEtten, 1983). There have been occasional reports of the occurrence of glucosinolates and derived isothiocyanates in species which are systematically remote from the Capparales. Thus, whilst Gill *et al.* (1984) suggested that glucosinolates were present in cocoa (*Theobroma cacao* L., Sterculiaceae) this was not confirmed by later, more extensive, work (Bjerg *et al.*, 1987).

In 1983, MacLeod and Panchasara reported traces of benzyl isothiocyanate and benzyl nitrile (another product of the hydrolysis of glucosinolates) in mushroom (*Agaricus bisporus*) and claimed this to be 'virtual positive proof' of the existence of the precursor glucosinolate. Later, Lorsch *et al.*

Food Chemistry 0308-8146/88/$03.50

(1985) proposed that benzyl isothiocyanate was amongst antiasthmatic components isolated from the onion (*Allium cepa* L.). Since this laboratory has had an extensive interest in biologically active principles of brassicas (Fenwick *et al.*, 1983; Heaney & Fenwick, 1987) and, latterly, onions and their allies (Fenwick & Hanley, 1985*a*, *b*, *c*), we have re-examined the above claims using a variety of chemical and physico-chemical techniques. The results of these investigations, which do not support the claims of previous authors, are described herein.

MATERIALS AND METHODS

Materials

Benzyl glucosinolate was isolated from *Lepidium sativum* seed meal (Hanley *et al.*, 1983). Benzyl isothiocyanate and benzyl nitrile were obtained from the Aldrich Chemical Co., Gillingham, Dorset, UK. Myrosinase was supplied by Biocatalysts Ltd. Two samples of fresh mushrooms were obtained from local retail outlets. Two samples of onions were obtained from the same sources. Samples of onion extracts, obtained in the course of work on the antiasthmatic principles of onion, were supplied by Professor W. Dorsch, Munich.

Preparation of Samples

(i) Volatile fractions

Onions (500 g) were chopped into boiling water, cooled and blended. The mixture was steam distilled and the distillate extracted twice with chloroform. The organic phase was dried, concentrated in vacuo and treated as described by Dorsch *et al.* (1985). Mushrooms (500 g) were chopped into water and extracted with a Likens and Nickerson apparatus as used by MacLeod & Panchasara (1983). The extract was worked up as described by these workers. Small samples of the mushrooms and onions (50 g) were macerated in water for 30 min with myrosinase (100 mg) and ascorbate (100 mg), before being extracted with chloroform as described above.

(ii) Involatile fractions

Onions and mushroom samples were chopped into boiling 70% methanol, extracted for 15 min, filtered and the process repeated twice more. Combined extracts were concentrated *in vacuo* and applied to Sephadex A-25 columns

for glucosinolate analysis (see below) either before or after clean up by flash chromatography (Peterka & Fenwick, 1988).

Analysis

Benzyl isothiocyanate was analysed by gas chromatography as described by MacLeod & Panchasara (1983) and by combined gc–ms. Intact glucosinolates were determined by glucose release (Heaney & Fenwick, 1981) and desulphoglucosinolates were analysed by gc (Heaney & Fenwick, 1980) and hplc (Spinks *et al.*, 1984), by combined gc–ms (Eagles *et al.*, 1981) and hplc–ms (Mellon *et al.*, 1987) and by fast atom bombardment mass spectrometry (probe sample) as described by Fenwick *et al.* (1982). The procedures of Wilkinson *et al.* (1984*a*, *b*) were employed for the analysis of myrosinase.

RESULTS AND DISCUSSION

Care was taken to reproduce as far as possible the isolation procedures described by the original workers. Whilst no attempt was made to identify the components separated in the mushroom extract, it did appear to be quantitatively similar to that reported by MacLeod & Panchasara (1983). Investigations using gc and gc–ms (single ion monitoring of the molecular ion, m/z 91) failed to confirm the presence of benzyl isothiocyanate in any of the extracts, irrespective of whether myrosinase had been added before the extraction. Spiking experiments using both onion and mushroom extracts revealed that the presence of benzyl isothiocyanate could be confirmed by gc–ms if present at less than one-tenth of the figure ($<0{\cdot}1\ \mu g\ g^{-1}$) reported by the earlier workers.

It was thought possible that the glucosinolate precursor might have been present in the original material, but that maceration and addition of myrosinase was ineffective in causing hydrolysis due to the presence of an inhibitor. Consequently, procedures were employed to isolate any glucosinolates present in the original plant/fungal material. Quantitative analysis, in line with earlier work (Bjerg *et al.*, 1987), revealed the gc and hplc methods to have a detection limit for benzyl glucosinolate of *ca* $0{\cdot}5\ \mu g\ g^{-1}$. Whilst small peaks were observed in the gas chromatogram of the mushroom extracts in the approximate position of benzyl desulphoglucosinolate, no ions corresponding to this assignment were found upon examination of the gc–ms data (Eagles *et al.*, 1981).

The isolation procedures employed for the analysis and/or isolation of intact glucosinolates in brassicas (Hanley *et al.*, 1983; McGregor *et al.*, 1983)

require that cellular myrosinase be inactivated by extracting the chopped tissue with boiling solvent. The same procedure was employed in this study. Onions, and other alliums, contains a C—S lyase (allinase, alliin alkyl sulphenate lyase, EC 4.4.1.4) which is responsible for conversion of involatile S-substituted cysteine sulphoxides into the flavour-active components. This compound may also cause the breakdown of glucosinolates. However, examination of onions extracted with boiling 70% methanol revealed the presence of alliinase substrates (Lancaster & Kelly, 1983) thus indicating the enzyme to have been effectively inactivated during processing.

Preliminary examination of mushroom extracts revealed these to possess glucosinolate-degrading activity. The activity was lost upon storage at 0°C overnight, readily lost upon boiling in 70% methanol, increased with addition of 1 mM ascorbate and unaffected by addition of pyridoxal phosphate. The activity, determined using sinigrin as substrate, was found to be 0·07 μmol min^{-1} g^{-1} powdered tissue, this being less than 10% of the value of turnip myrosinase and less than 25% that of watercress (Wilkinson *et al.*, 1984*b*). Thus, it would seem unlikely that the absence of glucosinolates in either mushroom or onion is a result of their rapid enzymatic breakdown during isolation.

Unequivocal proof of the absence of glucosinolates in plant material is difficult to obtain. On the basis of the analysis conducted here the levels of benzyl glucosinolate in the onion and mushroom samples analysed must be less than 0·5 $\mu g\, g^{-1}$ and that of benzyl isothiocyanate less than 0·01 $\mu g\, g^{-1}$. Where glucosinolates have been found in the plant kingdom their levels are usually very much higher (> 10 mg g^{-1}). The antiasthmatic effects of onions have been ascribed by other workers to compounds other than isothiocyanates (see Fenwick & Hanley, 1985*c*) and according to W. Dorsch (private communication) guinea pigs pretreated intraperitoneally with benzyl glucosinolate (20 and 50 mg kg^{-1}), with or without myrosinase, showed the same degree of bronchial obstruction, when later inhaling an allergen, as control groups. Thus, present chemical and biological evidence is such as to call into question the claims for the presence of benzyl glucosinolate and benzyl isothiocyanate in mushroom and onions.

ACKNOWLEDGEMENTS

The authors are grateful to Professor W. Dorsch, Department of Pediatrics, Poliklinik, Ludwig Maximillian University, Munich, for supplying onion extracts examined in this study. Mass spectra were obtained by the Mass Spectrometry Group at this Laboratory and the work was funded by MAFF.

REFERENCES

Bjerg, B., Fenwick, G. R., Spinks, A. & Sørensen, H. (1987). Failure to detect glucosinolates in cocoa, *Phytochemistry*, **26**, 567–8.

Dorsch, W., Adam, O., Weber, J. & Ziegeltraum, T. (1985). Antiasthmatic effects of onion extracts—Detection of benzyl—and other isothiocyanates (mustard oils) as antiasthmatic compounds of plant origin, *Europ. J. Pharmacol.*, **107**, 17–24.

Eagles, J., Fenwick, G. R. & Heaney, R. K., (1981). Gas chromatography–chemical ionisation mass spectrometry of glucosinolate derivatives. *Biomed. Mass Spectrom.*, **8**, 278–82.

Ettlinger, M. G. & Kjaer, A. (1968). Sulphur compounds in plants. In *Recent Advances in Phytochemistry*. Vol. 1. ed. T. J. Mabry, R. E. Alston & V. C. Runeckles, Appleton-Century-Crofts, New York, pp. 60–144.

Fenwick, G. R. & Hanley, A. B. (1985*a*). The genus *Allium*—Part 1, *CRC Crit. Rev. Fd Sci. Nutr.*, **22**, 199–271.

Fenwick, G. R. & Hanley, A. B. (1985*b*). The genus *Allium*—Part 2, *CRC Crit. Rev. Fd Sci. Nutr.*, **22**, 273–377.

Fenwick, G. R. & Hanley, A. B. (1985*c*). The genus *Allium*—Part 3, *CRC Crit. Rev. Fd Sci. Nutr.*, **23**, 1–73.

Fenwick, G. R., Eagles, J. & Self, R. (1982). The fast atom bombardment mass spectra of glucosinolates and glucosinolate mixtures, *Org. Mass Spectrum*, **17**, 544–6.

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

Gill, M. S., MacLeod, A. J. & Moreau, M. (1984). Volatile components of cocoa with particular reference to glucosinolate products, *Phytochemistry*, **23**, 1937–42.

Hanley, A. B., Heaney, R. K. & Fenwick, G. R. (1983). Improved isolation of glucobrassicin and other glucosinolates. *J. Sci. Fd Agric.*, **34**, 869–73.

Heaney, R. K. & Fenwick, G. R. (1980). The analysis of glucosinolates in *Brassica* species using gas chromatography. Direct determination of the thiocyanate-ion precursors, glucobrassicin and neoglucobrassicin *J. Sci. Fd Agric.*, **31**, 593–9.

Heaney, R. K. & Fenwick, G. R. (1981). A micro-column method for the rapid determination of total glucosinolate content of cruciferous materal, *Z. Pflanzenzuchtg.*, **87**, 89–95.

Heaney, R. K. & Fenwick, G. R. (1987). Identifying toxins and their effects. In *Natural Toxicants in Food, Progress and Prospects*, ed. D. H. Watson, Ellis Horwood, Chichester, pp. 76–109

Lancaster, J. E. & Kelly, K. E. (1983). Quantitative analysis of the S-alk(en)yl-L-cysteine sulphoxides in onion (*Allium cepa*), *J. Sci. Fd Agric.*, **34**, 1229–35.

MacLeod, A. J. & Panchasara, S. D. (1983). Volatile aroma compounds, particularly glucosinolate products of cooked edible mushroom (*Agaricus bisporus*) and cooked dried mushroom, Phytochemistry, **22**, 705–9.

McGregor, D. I., Mullin, W. J. & Fenwick, G. R. (1983). Analytical methodology for determining glucosinolate composition and content, *J. Assoc. Off. Analyt. Chem.*, **66**, 825–49.

Mellon, F. A., Chapman, J. R. & Pratt, J. A. E. (1987). Thermospray liquid chromatography–mass spectrometry in food and agricultural research, *J. of Chromatography*, **394**, 209–22.

Peterka, S. & Fenwick, G. R. (1988). The use of flash chromatography for the isolation and purification of glucosinolates (mustard oil glucosides). *Fat Science Technol.*, **90**, 61–4.

Spinks, E. A., Sones, K. & Fenwick, G. R. (1984). The quantitative analysis of glucosinolates in cruciferous vegetables, oilseeds and forages using high performance liquid chromatography, *Fette, Seifen, Anstrichmittel*, **86**, 228–31.

Tookey, H. L. & VanEtten, C. H. (1983). Glucosinolates. In *CRC Handbook of Naturally Occurring Food Toxicants*, ed. M. Rechcigl, Jr. CRC Press, Boca Raton, Florida, 15–30.

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. Fd Agric.*, **35**, 543–52.

A. B. Hanley, R. K. Heaney, J. Lewis, K. Sones, E. A. Spinks, A. P. Wilkinson & G. R. Fenwick,
Molecular Science Division, AFRC Institute of Food Research,
Norwich Laboratory, Colney Lane,
Norwich, UK.

(Received 13 January 1988; revised version received and accepted 15 January 1988)