Int. J. Vitam. Nutr. Res., 72 (1), 2002, 26–31

Glucosinolates: Bioavailability and Importance to Health

Ian.T. Johnson

Institute of Food Research, Norwich Research Park, Colney, Norwich, NR4 7UA, UK

Received for publication: September 10, 2001

Abstract: Epidemiological studies suggest that brassica vegetables are protective against cancers of the lungs and alimentary tract. Cruciferous vegetables are the dietary source of glucosinolates, a large group of sulfur-containing glucosides. These compounds remain intact unless brought into contact with the enzyme myrosinase by pests, food processing, or chewing. Myrosinase releases glucose and breakdown products, including isothiocyanates. These highly reactive compounds are potent inducers of Phase II enzymes *in vitro*. Isothiocyanates also inhibit mitosis and stimulate apoptosis in human tumor cells, *in vitro* and *in vivo*. To understand and exploit such effects it is important to determine the routes of absorption of glucosinolate breakdown products, their metabolism, and delivery to systemic tissues. Glucosinolates can be gained or lost by vegetables during storage. They may be degraded or leached during processing, or preserved by thermal inactivation of myrosinase. Glucosinolates are broken down by plant myrosinase in the small intestine or by bacterial myrosinase in the colon. Isothiocyanates are absorbed from the small bowel and colon, and metabolites are detectable in human urine two to three hours after consumption of brassica vegetables. Interpretation of epidemiological data and exploitation of brassica vegetables for human health requires an understanding of glucosinolate chemistry and metabolism, across the whole food chain, from production and processing to the consumer.

Key words: Brassica, glucosinolate, isothiocyanate, health, cancer, bioavailability

Introduction

Doll and Peto [1] estimated from epidemiological data that diet was responsible for approximately 35% of all cancers in the USA and other industrialized countries, although the uncertainty attached to this estimate was very high. The reasons for the relationship between diet and cancer remain poorly defined. The World Cancer Research Fund has recently confirmed the central importance of diet as a major determinant of many forms of cancer across the globe, and has emphasised that the consumption of diets rich in fruits and vegetables reduces the risk of many types of cancer [2]. This discovery has led government bodies to encourage consumption of fruits and vegetables as a public health measure. It has also aroused the interest of the relevant agricultural industries and focused the attention of research workers on the biological mechanisms underlying this protective effect [3].

Plant materials are usually rich sources of micronutrients and dietary fiber, but they also contain an immense variety of biologically active secondary metabolites, many of which provide plants with color, flavor, and other properties. The glucosinolates are a large family of sulfur-containing compounds found in plant families of the order Capparales, which includes the Brassicas; many of these are economically important vegetables [4]. All the glucosinolates possess a common fundamental structure comprised of a β-D-thioglucose group, a sulfonated oxime moiety, and a variable side-chain derived from methionine, tryptophan, phenylalanine, and some branched chain amino acids [5]. The glucosinolates are themselves chemically stable and biologically inactive. They are sequestered within subcellular compartments throughout the plant, but following tissue damage caused by pests, harvesting, food processing, or chewing, they are brought into contact with the endogenous enzyme "myrosinase," (thioglu-

coside glycohydrolase EC 3.2.3.1). The immediate reaction is hydrolysis of the glucosidic bond, releasing glucose and an unstable intermediate that degrades spontaneously, forming a complex variety of breakdown products the nature of which depends upon the ambient conditions (Fig. 1). The isothiocyanates, a group of hot and bitter compounds commonly termed "mustard oils," are probably the most important and most thoroughly investigated of these products.

There is a significant body of data indicating that brassica vegetables protect against cancer in humans. Van Poppel *et al* [6] conducted a meta-analysis of cohort and case-control studies and concluded that a high consumption of brassica vegetables is associated with a decreased risk of carcinomas of the lung, stomach, colon, and rectum. The protective effects of cabbages, broccoli, cauliflower, and Brussels sprouts were thought most probably to be a consequence of their glucosinolate content. In this review I will consider briefly the biological effects of glucosinolate breakdown products and their likely mechanisms of action as anticarcinogens, and summarize the present state of knowledge about their absorption from foods and delivery to target tissues.

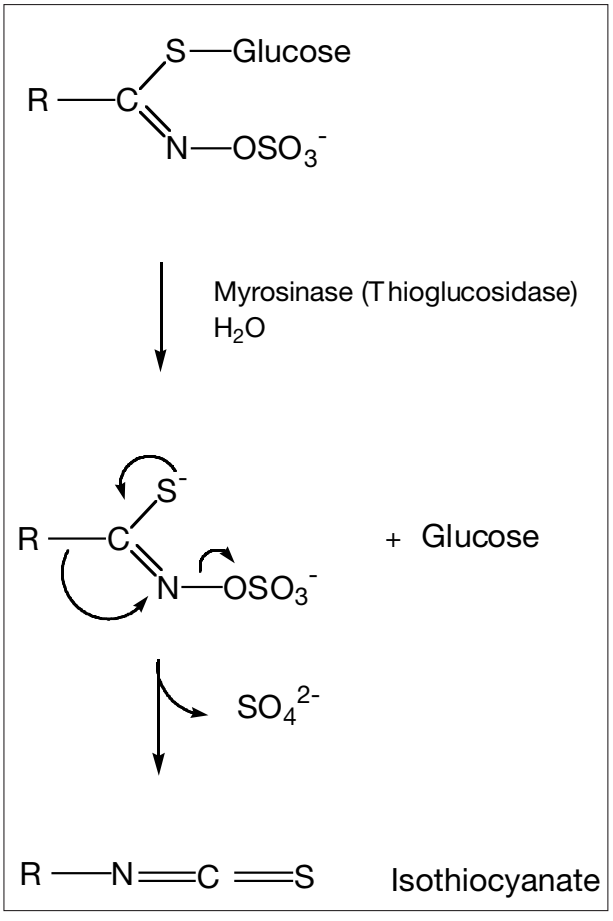

Figure 1: The enzyme myrosinase is localized in specialized myrosin cells, and probably within the cytoplasm of other cells. Tissue disruption liberates myrosinase, which cleaves the glucose from glucosinolates, leaving an unstable intermediate aglycone. This product then spontaneously rearranges by one of several possible routes. The Lossen rearrangement, leading to formation of an isothiocyanate, is the most common.

Biological effects of glucosinolate breakdown products in humans

About 90% of cancers in the UK are carcinomas arising from epithelial cells. Of these, around 80% are associated with the surface epithelia of organs such as the bladder, lungs, and alimentary tract. Nevertheless there are large geographical variations in the incidence of carcinomas of these tissues, indicating that in principle they are preventable. Even amongst smokers, fruits and vegetables appear to play an important role as determinants of lung cancer risk. Glucosinolates are of particular interest in this context because of the impressive convergence of different types of evidence, indicating that they may function as natural chemopreventive components of the human diet.

From a meta-analysis of both cohort and case-control studies, Verhoeven *et al* [7] concluded that consumption of brassica vegetables provided protection against cancers of the lung, stomach, colon, and rectum. Some of the most impressive evidence for a plausible mechanism to explain this relationship comes from the work of Hecht and coworkers [8]. Isothiocyanates such as phenethyl isothiocyanate (PEITC), benzyl isothiocyanate (BITC), and sulforaphane modify the balance of Phase I and II xenobiotic metabolizing enzymes that are expressed in liver, and in epithelial cells including those of the colon. Phase I enzymes such as the cytochrome p450 family are monooxygenases, which metabolize lipophilic procarcinogens, often converting them to highly carcinogenic epoxides in the process [3]. Phase II enzymes such as those in the glutathione transferase (GST) family metabolize these products to form inactive, water-soluble conjugates that are readily excreted in urine. Hecht and colleagues have studied the effects of PEITC on the induction of lung tumors in a rat model by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), in considerable detail. In a chronic study with simultaneous administration of NNK and PEITC, lung tumors were induced in 70% of the control rats given only NNK, however only 5% of rats cotreated with PEITC developed lung tumors. In addition, there was a marked reduction in a biomarker of NNK activation, 4-hydroxy-1-(3-pyridyl)-1-butanone-releasing hemoglobin adducts in rats given PEITC, and a significant increase in

excretion of two NNK metabolites, (4-(methylnitrosamino)-1-(3- pyridyl)-1-butanol and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol glucuronide). Taken together, these findings provide good evidence that PEITC, for which watercress is the major source in the human diet, has the potential to block the carcinogenic effects of tobacco smoke.

One frequently voiced criticism of such animal models is that the concentrations of both carcinogens and anticarcinogens upon which they depend are often unrealistically high. In this case, however, there is evidence from epidemiological studies implying that the models have real implications for human health. In a recent paper, London *et al* [9] described the relationship between the concentration of isothiocyanate metabolites in urine and subsequent risk of lung cancer in a large cohort of Chinese men. The presence of deletion polymorphisms of the *GSTM1* and *GSTT1* genes, which code for different members of the GST enzyme family, were determined at the time of urine sampling. During 10 years of follow-up there were 232 cases of lung cancer that were compared with 710 matched controls. Individuals with detectable levels of isothiocyanate metabolites in urine were at a reduced risk of cancer compared to controls (RR = 0.65). However, further analysis showed that the effect was observable only in subjects with homozygous deletion of *GSTM1* or *GSTT1*, and strongest in those with deletion of both *GSTM1* and *GSTT1* (RR = 0.28; $p < 0.01$). The results were sustained when the analysis was restricted to present or past smokers. These observations suggest a very complex interplay between environmental factors, genotype, and risk of cancer. The GST enzymes play a major role in the detoxification of environmental mutagens, but they also metabolize anticarcinogenic phytochemicals, including the isothiocyanates. Thus loss of *GSTT1* and *GSTM1* might be expected to compromise an individual's ability to metabolize and dispose of tobacco smoke carcinogens. This assumption was borne out by the fact that individuals with null genotype for both *GSTM1* and *GSTT1* were at considerably greater risk of lung cancer if they lacked isothiocyanates in their urine, and therefore presumably consumed few brassica vegetables (RR = 2.79). However in the case of a high consumer of brassica vegetables, the adverse effect of the reduced metabolic capacity resulting from GST-null status was supposedly outweighed by delayed excretion of isothiocyanates, and prolonged exposure of target cells to the anticarcinogenic effects.

The dual role of GST enzymes in the metabolism of both carcinogens and anticarcinogens thus poses something of a paradox, and the precise nature of the anticarcinogenic effects of glucosinolate breakdown products remains to be established. If the blocking effect of enzyme modulation is indeed the principal anticarcinogenic effect of glucosinolate breakdown products in *GSTM1*- and *GSTT1*-null individuals, then other members of the GST family, or other xenobiotic metabolizing enzymes, must be involved. Alternatively, entirely different mechanisms of protection may be at work. Destruction by apoptosis of cells carrying potentially carcinogenic mutations is an important mechanism for the arrest of carcinogenesis [10]. Glucosinolate breakdown products block the cell cycle and induce apoptosis in cancer cells *in vitro* [11], and in the colon after treatment with the carcinogen 1,2 dimethylhydrazine (DMH) *in vivo* [12]. The mechanisms involved are not entirely clear, but are not directly related to Phase II enzyme activity. It is interesting to note that a protective effect of broccoli consumption against precancerous adenomatous polyps of the colon has been observed in individuals with the *GSTM1*-null genotype [13]. It is not clear yet whether isothiocyanates can induce apoptosis of precancerous cells in other target tissues such as the lung, but this possibility merits further investigation.

Bioavailability of glucosinolates and their breakdown products

The delivery of glucosinolate breakdown products to the intestinal mucosa and other systemic tissues depends crucially upon the timing and site of myrosinase activity during consumption and digestion of brassica vegetables. The activation of myrosinase occurs after physical disruption of the plant tissue, which can occur at any stage during harvest, processing, or food preparation. In practice, limited chopping induces only small amounts of glucosinolate loss at the cut surfaces of vegetables, so that large broccoli florets for example undergo only minimal losses up to the point of cooking. If the vegetables are eaten raw, then both active myrosinase and its substrates are ingested and the reaction can continue within the alimentary tract. This has been confirmed in both experimental animals and humans. When rats were fed benzyl glucosinolate in the presence of active myrosinase derived from Brussels sprouts, a substantial proportion of the administered dose appeared as isothiocyanate excretion products in the urine [14]. Colonic bacteria were able to metabolize some of the ingested glucosinolate but plant myrosinase appeared to be the dominant factor. Getahun and Chung [15] described a somewhat similar experiment carried out in human subjects using watercress as a source of PEITC. Volunteers consumed 350 g (475 μmol glucosinolates) of watercress in which the myrosinase had been completely inactivated by cooking, or 150 g (972 μmol glucosinolates) of raw watercress, which retained its myrosinase activity. In the case of cooked watercress, the rate of conversion of glu-

cosinolates to isothiocyanates ranged from 1.2 to 7.3%, compared with 17.2 to 77.7% for the raw plant material. Given the considerable loss of glucosinolates that resulted from cooking, it is clear that the method of preparation can make a very large difference both to the intake of glucosinolates and to the bioavailability of their breakdown products. Cooking influences both the activity of myrosinase through denaturation of the enzyme, and the level of glucosinolates in the plant tissue through thermal breakdown and leaching. Dekker *et al* [16] have recently described a predictive mathematical approach to this complex issue.

The bacterial microflora of the human colon are known to express myrosinase activity, but their importance in the delivery of glucosinolate breakdown products to the colon and to systemic tissues remains unresolved. Significant quantities of isothiocyanate metabolites are excreted in the urine of healthy human volunteers after eating brassica vegetables, even when myrosinase has been completely inactivated by cooking [15, 17]. However this route of assimilation falls to negligible levels when the numbers of colonic bacteria are reduced by antibiotics [17]. Rabot *et al* [18] isolated a strain of *Bacteroides thetaiotaomicron* (II8) from human feces that was capable of degrading glucosinolates. In germ-free rats inoculated either with this bacterium alone or with an intact human microflora, consumption of sinigrin led to a considerably higher excretion of allyl isothiocyanate compared to germ-free animals, and a correspondingly lower fecal excretion of intact sinigrin [19, 20]. This is clear confirmation that bacterial myrosinase activity can deliver glucosinolate metabolites to the gut, and accounts for the absorption and metabolism of isothiocyanates from thoroughly cooked brassica vegetables observed in human studies [15]. One might assume that bacterial breakdown of sinigrin in the rat colon would be the optimal mechanism for delivery of allyl isothiocyanate to the crypts of the colonic mucosa, where it appears to induce increased rates of apoptosis in cells damaged by DMH [12]. However this is not necessarily the case. Cooked Brussels sprouts are less effective at inducing apoptosis in this model than either a raw juice [11] or uncooked whole sprouts (Smith and Johnson, unpublished). This suggests that delivery of glucosinolate breakdown products to the basal zones of the colonic crypts via the mucosal vascular bed, after absorption from the small intestine and passage through the liver, may be more effective than direct luminal delivery in the colon.

Summary and conclusions

As a result of recent research by several different groups in both Europe and North America, an outline of the main features of glucosinolate absorption and metabolism in humans is beginning to emerge (Fig. 2). Brassica vegetables prepared for consumption contain glucosinolates or their breakdown products, together with myrosinase. The levels and proportions of these components vary in a complex way that depends on the food production chain from farm to the cooked food [16]. Once ingested, the plant material is disrupted by mastication and gastric motility so that any remaining myrosinase can act on whatever intact glucosinolates may be present. The site of absorption within the upper gastrointestinal tract is not known with any precision, but this intraluminal "digestion" of glucosinolates by plant myrosinase appears to be the major route for the delivery of isothiocyanates, and possibly other breakdown products such as nitriles, to the circulation. In the absence of myrosinase activity, some intact glucosinolates are thought to be absorbed from the human alimentary tract but the biological significance of this, if any, is unknown (S Rabot, personal communication). Most of the glucosinolates will be delivered to the colonic microflora and a sizable fraction will be broken down to isothiocyanates, some of which are absorbed and metabolized, so that they become available to exert effects on target tissues. The colonic bacteria probably also metabolize isothiocyanates further before absorption can occur, but the breakdown products, their fate, and their biological significance are poorly understood at present. There is now strong evidence that both the pharmacokinetics of isothiocyanates in individuals, and their effectiveness as anticarcinogens, depends upon the patterns of expression of glutathione transferases and perhaps other Phase II enzymes. These are determined by common genetic polymorphisms.

Unlike some other phytochemicals, glucosinolates are present at relatively high concentrations in the diet. An individual consuming three to four portions of broccoli per week, which is the level at which a protective effect against adenomatous polyps has been reported [13], might easily be consuming 300–400 mg of glucosinolates. However the level of exposure depends greatly upon the commercial variety of vegetable eaten. The levels of glucosinolates in brassica vegetables can be manipulated by selective breeding, and their presence influences both the palatability and the nutritional properties of these crops [21]. Both issues need to be considered when breeding new varieties of vegetable and it must not be forgotten that, like synthetic drugs, biologically active secondary plant metabolites may have adverse side effects [5]. Further research is needed to define the biological activities of

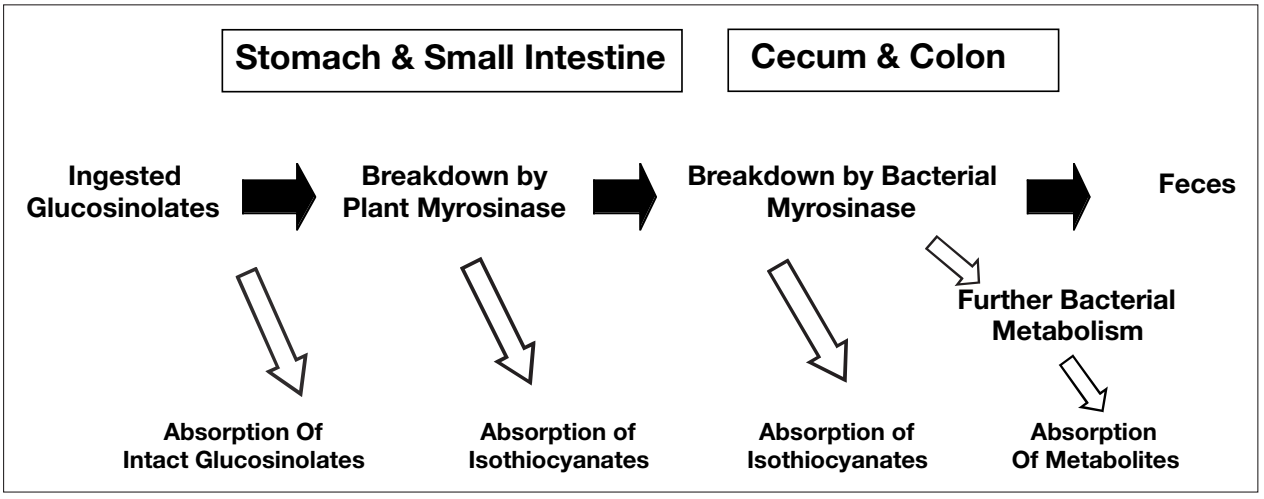

Figure 2: The figure summarizes the fate of glucosinolates in the human gut. The level of intact glucosinolates ingested depends upon the physical disruption, and hence activation of myrosinase, that the vegetable tissue has undergone during food preparation. Cooking can destroy the activity of myrosinase, but also causes leaching into the cooking water and some thermal degradation of glucosinolates. If the vegetables are lightly cooked or raw, significant levels of myrosinase activity remain, causing glucosinolate degradation during digestion. The isothiocyanates released can be absorbed in the upper alimentary tract. Bacterial myrosinase also releases isothiocyanates, and some are absorbed via this route in humans.

individual glucosinolates in greater detail, so that the balance of benefit, risk, and consumer preference can be properly defined.

Acknowledgements

The author is grateful to the BBSRC, and to the European Commission (FAIR CT97 3029; *Effects of Food-Borne Glucosinolates on Human Health*) for financial support.

References

1. Doll, R. and Peto, R. (1981) The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J. Natl. Cancer Inst. 66 (6), 1191–1308.
2. World Cancer Research Fund Food (1997) Nutrition and the Prevention of Cancer: a Global Perspective. American Institute for Cancer Research, Washington, DC, 216–251.
3. Johnson, I., Williamson, G. and Musk, S. (1994) Anticarcinogenic factors in plant foods: a new class of nutrients? Nutrition Research Reviews 7, 175–204.
4. Fenwick, G. R., Heaney, R. K. and Mullin, W. J. (1983) Glucosinolates and their breakdown products in food and food plants. Crit. Rev. Food Sci. Nutr. 18, 123–201.
5. Mithen, R. F., Dekker, M., Verkerk, R., Rabot, S. and Johnson, I. T. (2000) The nutritional significance, biosynthesis and bioavailability of glucosinolates in human foods. J. Sci. Food Agric. 80, 967–984.
6. van Poppel, G., Verhoeven, D.T., Verhagen, H. and Goldbohm, R. A. (1999) Brassica vegetables and cancer prevention. Epidemiology and mechanisms. Adv. Exp. Med. Biol. 472, 159–168.
7. Verhoeven, D.T., Goldbohm, R. A., van Poppel, G., Verhagen, H., van den Brandt, P. A. (1996) Epidemiological studies on brassica vegetables and cancer risk. Cancer Epidemiol. Biomarkers Prev. 5 (9), 733–748.
8. Hecht, S. S. (1999) Chemoprevention of cancer by isothiocyanates, modifiers of carcinogen metabolism. J. Nutr. 129 (3), 768S–774S.
9. London, S. J., Yuan, J. M., Chung, F. L., Gao, Y.T., Coetzee, G. A., Ross, R. K. and Yu, M. C. (2000) Isothiocyanates, glutathione S-transferase M1 and T1 polymorphisms, and lung-cancer risk: a prospective study of men in Shanghai, China. Lancet 356 (9231), 724–729.
10. Evan, G. I., Vousden, K. H. (2001) Proliferation, cell cycle and apoptosis in cancer. Nature 411, 342–348.
11. Smith, T. K., Clarke, R., Scott, J. and Johnson, I.T. (2000) Raw Brussels sprouts block mitosis in colorectal cancer cells (HT29) and induce apoptosis in rat colonic mucosal crypts in vivo. In: Dietary Anticarcinogens and Antimutagens: Chemical and Biological Aspects (Johnson, I.T. and Fenwick, G. R. eds.), pp. 338–344, Royal Society of Chemistry, Cambridge.
12. Smith, T. K., Lund, E. K. and Johnson, I.T. (1998) Inhibition of dimethylhydrazine-induced aberrant crypt foci and induction of apoptosis in rat colon following oral administration of the glucosinolate sinigrin. Carcinogenesis 19, 267–273.
13. Lin, H. J., Probst-Hensch, N. M., Louie, A. D., Kau, I. H., Witte, J. S., Ingles, S. A., Frankl, H. D., Lee, E. R. and Haile, R.W. (1998) Glutathione transferase null genotype, broccoli,

and lower prevalence of colorectal adenomas. Cancer Epidemiol. Biomarkers Prev. 7 (8), 647–652.
14. Rouzard, G., Duncan, A. J., Rabot, S., Ratcliffe, B., Durao, S., Garrido, S. and Young, S. (2000) Factors influencing the release of cancer-protective isothiocyanates in the digestive tract of rats following consumption of glucosinolate-rich brassica vegetables. In: Dietary Anticarcinogens and Antimutagens: Chemical and Biological Aspects (Johnson, I.T. and Fenwick, G. R. eds.), pp. 92–95, Royal Society of Chemistry, Cambridge.
15. Getahun, S. M. and Chung, F.-L. (1999) Conversion of glucosinolates to isothiocyanates in humans after ingestion of cooked watercress. Cancer Epidemiol. Biomarkers Prev. 8, 447–451.
16. Dekker, M., Verkerk, R. R. and Jongen, W. M. F. (2000) Predictive modelling of health aspects in the food production chain: A case study on glucosinolates in cabbage. Trends Food Sci. Technol. 11, 174–181.
17. Shapiro, T. A., Fahey, J.W., Wade, K. L., Stephenson, K. K. and Talalay, P. (1998) Human metabolism and excretion of cancer-chemoprotective glucosinolates and isothiocyanates of cruciferous vegetables. Cancer Epidemiol. Biomarkers Prev. 7 (12), 1091–1100.
18. Rabot, S., Guerin, C., Nugon-Baudon, L. and Szylit, O. (1995) Glucosinolate degradation by bacterial strains isolated from a human intestinal microflora. Proc. 9th Intl-Rapeseed Congr. 1, 212–214.
19. Elfoul, L., Rabot, S., Khelifa, N., Garrido, S. and Durao, J. (2000) Production of allyl isothiocyanate from sinigrin in the distal gut of gnotobiotic rats harbouring a human colonic bacterial strain of Bacteroides thetaiotaomicron. In: Dietary Anticarcinogens and Antimutagens: Chemical and Biological aspects (Johnson, I.T. and Fenwick, G. R. eds.), pp. 88–91, Royal Society of Chemistry, Cambridge.
20. Elfoul, L., Rabot, S., Khelifa, N., Quinsac, A., Duguay, A. and Rimbault, A. (2001) Formation of allyl isothiocyanate from sinigrin in the digestive tract of rats monoassociated with a human colonic strain of Bacteroides thetaiotaomicron. FEMS Microbiol. Lett. 197, 99–103.
21. van Doorn, H. E., van der Kruk, G. C., van Holst. G.-J., Raaijmakers-Ruijs, N. C. M. E., Postma, E., Groeneweg, B. and Jongen, W. H. F. (1998) The glucosinolates sinigrin and progoitrin are important determinants for taste preference and bitterness of Brussels sprouts. J. Sci. Food Agric. 78 (1), 30–38.

Ian. T. Johnson

Institute of Food Research
Norwich Research Park
Colney, Norwich, NR4 7UA, UK
Tel. +44 (0)1603 255330
ian.johnson@bbsrc.ac.uk