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Food Chemistry 67 (1999) 101–102

Food Chemistry

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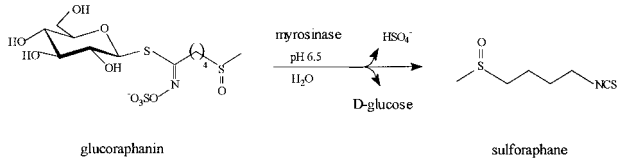
Letter to the editor

**To: Davide Bertelli, Maria Plessi, Daniela Braghiroli and Agar Monzani (1998). Separation by solid phase extraction and quantification by reverse phase HPLC of sulforaphane in broccoli. *Food Chemistry*, *63*(3), 417–421.**

Sir: In a recent issue of *Food Chemistry*, Bertelli, Plessi, Braghiroli, and Monzani (1998) claim that the method they proposed offers reliable and quantitative analysis of sulforaphane in broccoli. The main points of their method are: (i) acid hydrolysis of glucosinolates (GLs), which are present in florets, stalks and leaves of broccoli; (ii) solid phase extraction of sulforaphane (1-isothiocyanate-4-(methylsulfinyl)butane) (SFN) from crude extracts after acid hydrolysis of the corresponding GL; (iii) SFN determination by reverse-phase HPLC as compared with GC/MS analysis and spectrophotometric assay of isothiocyanates (ITCs) after reaction with 1,2-benzenedithiol.

Over the last decade, many authors have reported that some natural organic isothiocyanates such as SFN may prevent the formation of tumour in humans. SFN can be produced by myrosinase-catalysed hydrolysis at neutral pH (see the Reaction Scheme) of the precursor glucoraphanin (GRA), a GL contained in broccoli. In this vegetable, GRA is associated with other GLs, viz. glucoiberin, glucoerucin, 4-hydroxy-glucobrassicin, progoitrin, glucoibervirin and glucobrassicin. Together they represent the 40–50% of the total GLs content. Due to the growing interest in the potential role of SFN in protecting cells and complex organisms against malignancy, the availability of an analytical method for a rapid quantification of this compound is necessary, especially for routine assays. Unfortunately, in our opinion the method proposed by Bertelli et al. (1998) contains some unsuitable procedures and unconvincing statements.




glucoraphanin sulforaphane

Reaction Scheme. Myrosinase-catalysed hydrolysis of glucoraphanin into sulforaphane.

Recently, a GC/MS method for the analysis of SFN has been proposed (Chiang, Pusateri, & Leitz, 1998) and the authors described the thermal degradation of SFN to 3-butenyl isothiocyanate, that occurs during the injection of the sample in the GC. This decomposition was avoided using an appropriate injector liner and a precise control of the carrier gas flow rates, making the method very useful.

Bertelli et al. (1998) did not examine the above aspect and were unable to compare the GC/MS technique with their method for SFN analysis. In fact they asserted that the poor quality of the sulforaphane peak resulting from degradation on the column makes the GC/MS method unsuitable for the quantitative analysis of extracts.

Moreover, Bertelli et al. (1998) reported that acid hydrolysis performed before extraction consistently enhances the yield of SFN. Thus, stalks and leaves were treated only at pH 3.

Insofar as is known, the conversion of GLs into ITCs and, in particular, of GRA into the SFN is affected by temperature, pH, endogenous levels of myrosinase and reactant concentration.

Because the endogenous myrosinase is still active when pH drops to 3, the medium becomes more favourable for nitrile formation rather than for ITC (Fenwick, Heaney, & Mullin, 1983; Gil & MacLeod, 1980). Furthermore, in a recent review it was reported that myrosinase-induced hydrolysis of aliphatic GLs yields ITCs at pH 5–7, while under more acid conditions (ca. pH 3), even during autolysis, an increasing amount of nitriles are produced (Rosa, Heaney, Fenwick, & Portas, 1997). The analysis of the GRA solution, which was produced according to a recent method (Iori, 1998), shows the high stability of this compound when it is left at room temperature and at pH 3 for 5 days (unpublished result). Thus, the chemical degradation of GRA at pH 3 does not occur at all.

Thereby, it is surprising that Bertelli et al. (1998) only considered the recovery of synthetic SFN in their method, without verifying the complete transformation of GRA into SFN in broccoli samples. According to the reaction reported in our Reaction Scheme, the release of total ITCs from intact GLs in juice extracted from vegetables can be easily obtained, at neutral pH, adding a suitable amount of commercial myrosinase (Sigma Chemical Co., St Louis, MO) or that isolated from mustard seeds (Appelqvist & Josefsson, 1967; Palmieri, Iori, & Leoni, 1986). Because the sample preparation using myrosinase is quite simple, it was recently used by Chiang et al. (1998) and Jiao, Yu, Hankin, Low, and Chung (1998) to analyse ITCs in broccoli and in cooked cruciferous vegetables.

0308-8146/99/$ - see front matter 
PII: S0308-8146(99)00118-1

In conclusion, the method described by Bertelli et al. for the quantitative analysis of SFN in broccoli may not be suitable due to the favourable conditions for the formation of nitriles instead of ITCs.

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