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Analytical Methods Section

Quantitative determination and profiling of total sulphur compounds in garlic health products using a simple GC procedure

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A simple GLC procedure has been developed to examine the profile of sulphur compounds in garlic-based health products. The procedure can determine the content of three main sulphur compounds present in garlic, namely alliin, allicin and sulphides or estimate the total sulphur compounds present in a health product. A nearly 100% recovery was achieved using this procedure when pure alliin and garlic-oil sulphides were used to study the rate of conversion and recovery of the procedure. The procedure provides a simple and reliable method to determine total sulphur compounds and also to compare the composition of different product formulations.

INTRODUCTION

Garlic has been used world-wide since ancient times for its beneficial medicinal properties and as a flavouring agent. A variety of garlic-based health products are now readily available on the market. Various sulphur compounds have been suggested to be the active ingredient for its medicinal properties. Evaluation of the effectiveness of these health products requires a simple but reliable analytical procedure to monitor their compositions.

The prime active component in garlic and its health products is suggested to be allicin but also possibly involved are its degradation products, a range of sulphides. The intact garlic bulb is odourless, but when it is cut and/or homogenised, the active enzyme system alliinase converts alliin (*S*-allyl-L-cysteine sulphoxide) into allicin (diallyl thiosulphinate) (Stoll & Seebeck, 1948). Allicin is not a stable compound and readily degrades via several pathways to form the secondary products of sulphur compounds such as sulphides and vinyl dithins (Brodnitz *et al.*, 1971; Block, 1985). Under aqueous condition, allicin degrades to produce a range of sulphides, contributing the characteristic flavour and odour of garlic.

In the garlic health-product market, there are two main types of formulation, a garlic powder formulation (mainly containing alliin and possibly small levels of allicin) and a garlic-oil formulation (mainly containing sulphides).

The analysis of the sulphur compounds of garlic are currently performed using either GLC or HPLC methods. Several GLC and HPLC methods have been reported for the analysis of alliin, allicin, and related sulphides (Jansen *et al.*, 1987; Ziegler & Sticher, 1989; Iberl *et al.*, 1990*a,b*). The difficulty of using these proposed procedures to evaluate or compare the effectiveness of products lies in the contracting properties of those potentially active sulphur compounds. Each analytical method is primarily developed for one particular sulphur compound and the sample preparation procedure is therefore designed to create a suitable environment to preserve/produce the compound for analysis. These procedures very often make no distinction between the compounds originally present and those artificially produced during the sample preparation procedure. In order to assess the quality of a garlic product accurately, the profile of sulphur compounds need to be analysed. This task is not easy to accomplish since an individual method can only be chosen to analyse a particular sulphur compound or type of compound, and also any further degradation during analysis needs to be prevented. If this is not carefully done, the analyses might overlap each other or miss some of the actual sulphur compound present. Therefore, the sulphur compound profile may not accurately reflect the compounds present.

This paper reports the development of a simple GC procedure for the total sulphur compounds and their

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profile, mainly alliin, allicin and sulphides, in one health product.

MATERIAL AND METHODS

Materials

Dipropyl disulphide standard (purity 99%) was purchased from Fluka Chemicals (Gillingham, UK) Ltd, (Hull, UK). Garlic oil and powder were supplied by Seven Seas Health Care Ltd (UK). Alliin standard (purity 98%) was purchased from Wakunaga Pharmaceutical Ltd (Osaka, Japan).

Methods

GLC conditions

A Perkin-Elmer 8420 GC with a DBwax column (30 m × 0·32 mm, film thickness 0·25 μm) was used for the analysis. The carrier gas was helium at 22·5 psig. An on-column injection mode was used, which utilised an injection volume of 0·2 μl. The following temperature program was used:

$$55°C\ (10\ min) \xrightarrow{2°C\ min^{-1}} 150°C \xrightarrow{15°C\ min^{-1}} 220°C\ (10\ min)$$

Sample preparation for allicin and sulphides

About 0·3 g of garlic powder or tablet products was accurately weighed into a culture tube and vortexed with 5 ml of iso-octane for 2 min. The mixture was heated in a heating block at 100°C for 30 min. 0·1 ml dipropyl disulphide (10 mg/ml) was added to the tube after it was cooled to room temperature. The sample was vortexed for another minute and left to settle. The iso-octane extract was directly injected for GC analysis.

Sample preparation for total sulphur compounds

One gram garlic powder was homogenised with 30 ml water. Five ml of homogenate was transferred into a culture tube (15 ml). Iso-octane (2 ml) was carefully placed on top of the garlic homogenate. The contents were heated at 100°C for 30 min (or specified period as in the experiment on the effect of heating time) using a heating block. 0·1 ml dipropyl disulphide (10 mg/ml) was added to the tube after it was cooled to room temperature. The sample was vortexed for 1 min and centrifuged at 10 000 rpm for 10 min at 4°C. The upper iso-octane layer was directly injected into the GC for analysis.

GC quantitative determination of allicin

One gram garlic powder or 15–20 g fresh garlic cloves were homogenised with 30 ml distilled water. The homogenate was left at room temperature for 30 min before centrifuging for 15 min at 10 000 rpm. The supernatant (2 ml) was applied to a C_{18} cartridge and allicin was eluted with 15 ml iso-octane. 0·2 ml dipropyl disulphide (10 mg ml^{-1}) was added to the eluate. The iso-octane eluate was heated for 10 min using a heating block before GC analysis.

RESULTS AND DISCUSSION

Measurement of total sulphur compounds

Garlic sulphur compounds have different solubilities in water and organic solvents. Alliin and allicin are basically water-soluble, while sulphides dissolve in most organic solvents. During the preparation of garlic oil, a steam distillation procedure is normally used, and sulphur compounds change from water-soluble (alliin) to organic-solvent-soluble (sulphides). Currently, these sulphur compounds are determined using different methods and it is not any easy task to profile total composition of sulphur compounds in one product. If a conversion procedure is developed, the effectiveness of various products can be compared on the basis of their total converted sulphides. The procedure can therefore measure the sum of alliin, allicin and sulphides present in health products.

Garlic powder was homogenised with water and left to stand for 30 min, sufficient time for alliinase to convert alliin to allicin (Jansen *et al.*, 1987). The homogenate was heated with iso-octane from 10 min to 90 min to investigate the effect of heating time on the production of sulphides from allicin and to optimise the heating time for the conversion procedure. It was found that total sulphide content reached a maximum at 30 min. Following this time of heating, the total sulphides content started to decline slightly (Fig. 1). It has been reported that the polar GLC column cannot analyse tetra and higher sulphur-containing sulphides (Lawson *et al.*, 1990). It seems that long periods of heating garlic juice may lead to the production of those

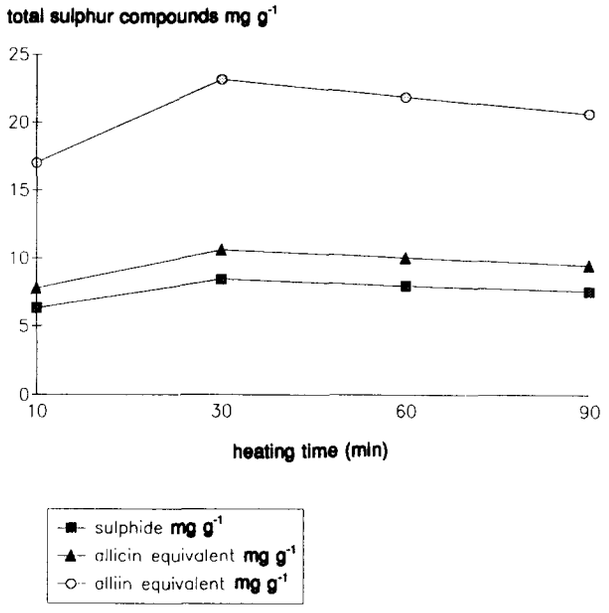

Fig. 1. Effect of heating time on the production of total sulphur compounds. Results were means of three experiments. Garlic powder was analysed for total sulphur compounds using the standard procedure except for being heated for the specified periods of time. Total sulphur compounds were determined as sulphides and allicin and alliin contents were calculated on sulphur balance.

Table 1. Total sulphur compounds in garlic powder

	Sulphides (mg g^{-1})	Allicin equivalent (mg g^{-1})	Alliin equivalent (mg g^{-1})
Mean	7·87 (0·39)	9·88 (0·56)	21·68 (1·07)

NB. Samples were prepared using the procedure for the total sulphur compound preparation. Sulphide contents were calculated using dipropyl disulphide as internal standard and relative GC FID response factor was incorporated into the calculation (Yan *et al.*, 1992). Results were means of six determinations and figures in the parenthesis were standard deviations. Alliin and Allicin contents were calculated from sulphide content based sulphur balance, i.e.

$$\text{Allicin content} = \Sigma[(\text{sulphide content} \times 162 \times N_s)/(M_s \times 2)]$$

$$\text{Alliin content} = [(\text{allicin content} \times 177 \times 2)/(162)]$$

where

162 = molecular weight of allicin,
177 = molecular weight of alliin,
M_S = molecular weight sulphide,
N_S = number of sulphur atoms in the sulphide.

tetra and higher sulphur containing sulphides, resulting in the apparent lower level of total GLC-determined sulphides. Therefore, the heating degradation process should avoid unnecessarily long heating times in order to achieve a high recovery. It was found that the sulphide recovery was very reproducible so long as the heating time was not altered.

The method was tested using garlic powder to study the reproducibility of the analysis. The garlic powder was analysed independently five times (Table 1). Alliin content was found to range from 20·5 to 23·4 mg g^{-1} with an average of 21·7 mg g^{-1} and an RSD of less than 5%. The resultant sulphide profile was very reproducible among the five replicates, but different from that of garlic oil (Fig. 2). The garlic powder preparation contained considerable amounts of vinyl-dithiins, decomposition products of allicin in non-polar solvent. During the heating conversion process, some allicin may enter into the solvent phase and lead to the production of two vinyl dithiins. There were two unidentified sulphides in the sample preparation, which may also be associated with the decomposition of allicin under non-polar conditions. However, the dominating sulphides were diallyl disulphide, allyl methyl trisulphide, and diallyl trisulphide. Recovery of sulphides from garlic oil and alliin using the procedure were studied (see the following section). Garlic oil (15 mg) was added to 5 ml water and alliin (10 mg) was added to garlic powder before homogenisation. Both preparations were heated with iso-octane for 30 min. The sulphide profile of the heating garlic oil was very similar to that of the original garlic oil, and addition of alliin did not change the resultant profile of garlic powder (Fig. 2). The difference in sulphur compound profiles in the sample preparation can give an indication of product formulation, i.e. the presence of two vinyl-dithiins indicates a garlic powder formulation.

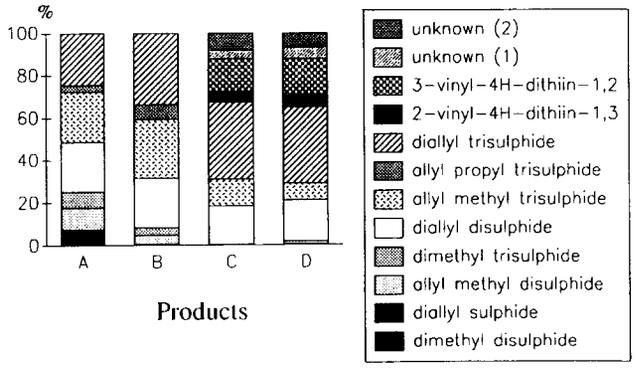

Fig. 2. Profile of sulphur compounds. A: Garlic oil; B: garlic oil analysed using the standard procedure; C: garlic powder analysed using the standard procedure; D: garlic powder with added alliin analysed using the standard procedure.

The procedure was very reproducible, providing a reliable estimation of total sulphur compounds in garlic products. The effectiveness of various health products can thus be truly evaluated and compared using this simple GC procedure. Without such a method, several analyses would need to be undertaken to measure alliin, allicin, and sulphide separately.

Studies on the recovery of the total-sulphur-compound procedure

Recovery of garlic-oil sulphides

The recovery of sulphides following the heating conversion analysis was investigated by either adding garlic oil to the homogenate before heating to determine the recovery of garlic sulphides or adding alliin to garlic powder to investigate the overall recovery. The garlic-oil recovery experiment was also performed to examine whether the composition of garlic oil changed during the heating process and if any sulphides might be lost during the process.

Garlic oil (15 mg) was added to water (5 ml) and the mixture was analysed for total sulphur compounds using the standard procedure. Neither sulphide composition nor total content of garlic sulphides had changed during the heating process (Fig. 2). The main three sulphides remained as diallyl disulphide (28·4%), allyl methyl trisulphide (21·8%), and diallyl trisulphide (33·4%). The eight major sulphides accounted for about 823 mg g^{-1} of garlic oil added (average of three analyses with an RSD of less than 2%). Garlic oil was found to have a similar content of sulphides (797 mg g^{-1}) without a heating process (Yan *et al.*, 1992). Therefore a recovery of 103% was achieved by calculating the total sulphide contents and it can be concluded that no significant change occurred in the sulphur-compound profile during the heating conversion process.

Recovery of added alliin

The second experiment was designed to estimate overall recovery. Since alliin is the original sulphur compound and it is available as a reasonably pure standard, it was chosen to determine the overall recovery including the conversion to sulphides during the heating process and the recovery of sulphides during extraction. The conversion from alliin to allicin needs the presence of

alliinase, and hence the recovery experiment was carried out by adding alliin (about 10 mg) to garlic powder before homogenisation. The increase in sulphide was taken to be the amount due to the added alliin. The recovery was found to be 79·5% on average of three analyses, with an RSD of less than 4%. During the analysis of garlic oil (Yan *et al.*, 1992), it was also found that the major sulphides made up about 79·7% of the garlic oil. Other minor sulphides may be present in trace quantities but below the detection limit. There might also be tetra- and higher sulphur-containing sulphides present, which are difficult to detect using a polar column GLC.

Degradation of allicin in non-polar solvent and GC analysis of allicin

It is commonly reported that allicin under the high temperatures of GC analysis, decomposes to two cyclic compounds, namely 3-vinyl-4*H*-dithiin-1,2 and 2-vinyl-4*H*-dithiin-1,3. The estimation of these two compounds can be related to the original content of allicin (Block, 1985). A GC procedure was proposed for this analysis (Saito *et al.*, 1989). However, the conversion from allicin to the two vinyl-dithiins is temperature-dependent and the ratio of conversion will be a function of the temperature programme used in the GC analysis. In some cases, less than 25% recovery has been reported (Iberl *et al.*, 1990*b*). Polar capillary GLC can recover 95% of vinyl-dithiiins (Lawson *et al.*, 1990), and the low recovery of allicin is the result of incomplete conversion under the GLC conditions. Allicin

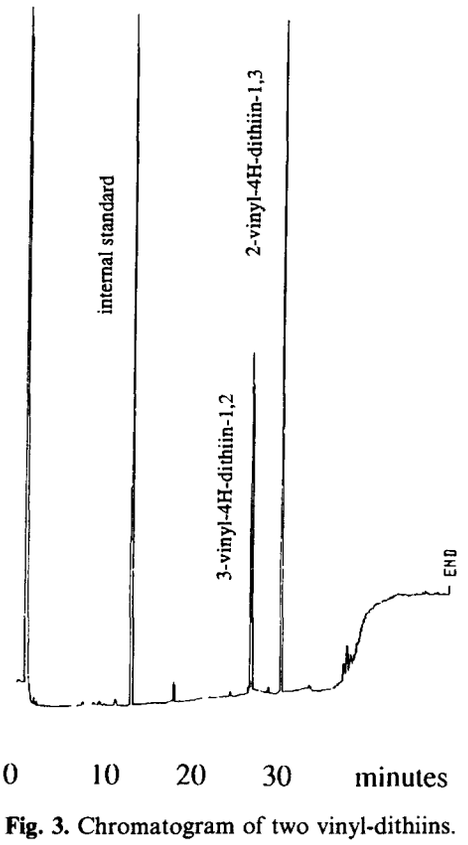

Fig. 3. Chromatogram of two vinyl-dithiins.

Table 2. Allicin content of garlic powder using direct GLC allicin procedure

Vinyl-dithins (mg g^{-1})	Allicin equivalent (mg g^{-1})	Alliin equivalent (mg g^{-1})
7·10 (0·59)	7·99	17·46

NB. Results were means of three determinations and figures in parentheses are standard deviations of the mean. Allicin and alliin were calculated based on the molecular balance.

decomposes to various secondary sulphur compounds under different environmental conditions. It mainly converts to dithiins in non-polar solvent. Therefore, if allicin is prepared in or transferred to an organic solvent and heated under controlled conditions before GC analysis, the rate of conversion to the two vinyl dithiins is likely to be consistent and improved. Therefore, a modified GC analysis may provide a reliable estimate of allicin contents.

The garlic powder used in the above study was homogenized with water and the homogenate was left to stand for 30 min at room temperature (in order for alliin to be converted to allicin by alliin lysase). The homogenate was centrifuged and 2 ml of supernatant was applied to a C_{18} cartridge. Allicin was eluted with several 3-ml portions of iso-octane and it was found that 12–15 ml of iso-octane can recover most of the allicin added. Dipropyl disulphide was added to the iso-octane preparation as internal standard. The preparation was then injected into the GC with or without being preheated for 10 min in a heating block. It was found that the samples without pre-GC heating gave an irregular recovery of the two dithiins though the chromatograms only showed two compounds but with broad peaks. A cold on-column injection mode was used in this study and the initial oven temperature was set to 55°C and gradually increased to 150°C. This temperature programme does not result in a complete and reproducible conversion from allicin to vinyl-dithiins. In contrast, the samples heated for 10 min before GC analysis gave a very consistent recovery of the two vinyl-dithiins (Table 2). The chromatogram showed only two sharp dithiin peaks (Fig. 3).

Allicin can be calculated based upon molar conversion. The garlic powder mainly contained alliin, and the allicin analysed in this experiment was produced from the alliin during the sample preparation. When alliin content was calculated, based upon molecular conversion, a slightly lower level of alliin (18·40 mg g^{-1}) was detected using the GLC allicin procedure in comparison to the total-sulphur-compounds analysis (21·34 mg g^{-1} (Table 2). This difference may be the result of incomplete recovery during the C_{18} cartridge elution.

The above analysis was designed to prepare allicin for the study of its conversion under non-polar solvent conditions and the analysis demonstrated that heating in iso-octane for 10 min can achieve a consistent conversion and recovery . This procedure can be used to

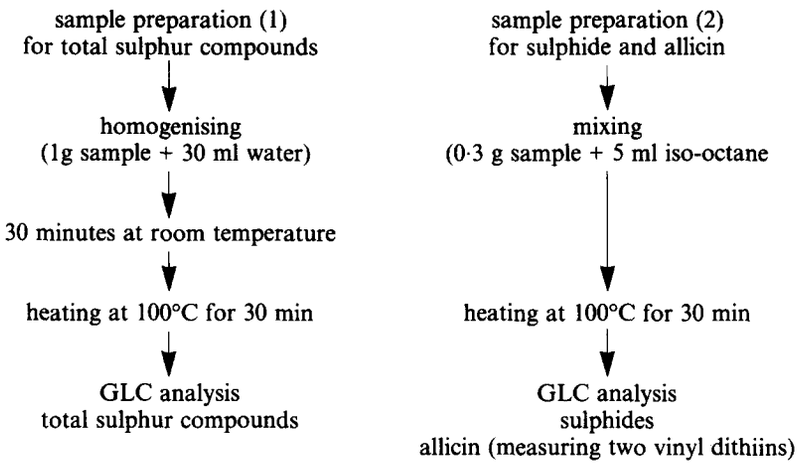

Fig. 4. Sample preparation scheme.

determine allicin content but care is needed in interpreting the original or converted allicin. The original level of alliin, allicin and sulphides can be determined using different sample preparation methods. The following section will discuss the development of a single GC procedure to profile the sulphur compounds in health products.

Development of a simple GC sulphur-profile procedure

In order to profile the composition of sulphur compounds using the GLC procedure, two sample preparations are required (Fig. 4). First the sample was prepared for total sulphur compounds, i.e homogenising with water and standing for 30 min followed by heating the aqueous preparation for 30 min at 100°C. This enables all the sulphur compounds to convert to sulphides and the total sulphur compounds can thus be estimated. A second sample was prepared by mixing the sample with iso-octane and heating for 30 min. This can recover sulphides upon heating and convert allicin to two vinyl-dithiins. The analysis can estimate both total sulphide and allicin contents. The garlic powder used for the above experiment and two tablet products, one formulated with garlic oil and the other with garlic powder, were tested using the proposed procedure. Table 3 illustrates the results of analysis. Direct iso-octane preparation and homogenising with water preparation estimates a similar level of sulphides for the tablet product of the garlic-oil formulation. No trace of vinyl dithiin was found in the analysis (Table 3). This indicates that the product is formulated with garlic oil with its main sulphur compounds being sulphides. Very low levels of sulphides and no vinyl–dithiins were found in the iso-octane preparation of garlic powder and the tablet product formulated with garlic powder, while homogenising with water indicated considerable amount of both sulphides and vinyl-dithiins (Table 3). This suggests that the main sulphur compound in the powder and the tablets was alliin and no allicin and very small amount of sulphides were present.

The above study demonstrates that the profile of sulphur compounds can be readily analysed using this simple GC procedure. Further studies on the comparison between this procedure and other well-known methods are to be undertaken and will form the basis of a subsequent paper.

Table 3. Sulphur compounds profiling of garlic powder and its health products

	Garlic powder	Tablet 1	Tablet 2
Sample prepared for sulphide and allicin (Prepared iso-octane)			
Sulphide determined (mg g^{-1})			
Dimethyl disulphide	0·046	0·000	0·000
Diallyl sulphide	0·060	0·028	0·000
Allyl methyl disulphide	0·020	0·009	0·000
Dimethyl trisulphide	0·000	0·085	0·000
Diallyl disulphide	0·008	0·212	0·018
Allyl methyl trisulphide	0·019	0·036	0·019
Diallyl trisulphide	0·022	0·143	0·067
2-Vinyl-dithiin-1,3	0·000	0·000	0·000
3-Vinyl-dithiin-1,2	0·000	0·000	0·000
Unknown (1)	0·000	0·000	0·000
Unknown (2)	0·000	0·000	0·000
Sample prepared for total sulphur compounds (prepared in distilled water)			
Sulphide determined (mg g^{-1})			
Dimethyl disulphide	0·000	0·000	0·000
Diallyl sulphide	0·019	0·027	0·022
Allyl methyl disulphide	0·138	0·008	0·037
Dimethyl trisulphide	0·000	0·066	0·000
Diallyl disulphide	1·359	0·215	0·368
Allyl methyl trisulphide	0·673	0·048	0·056
Diallyl trisulphide	2·457	0·152	0·299
2-Vinyl-dithiin-1,3	0·391	0·000	0·040
3-Vinyl-dithiin-1,2	1·211	0·000	0·115
Unknown (1)	0·285	0·000	0·000
Unknown (2)	0·606	0·000	0·000
Total sulphur compounds (mg g^{-1}) expressed as equivalent to alliin	21·850	1·481	2·447
Sulphides (mg g^{-1})	0·175	0·513	0·104
Allicin (mg g^{-1})	0·000	0·000	0·000
Alliin (mg g^{-1})	21·500	0·000	2·172

CONCLUSIONS

The described GC procedure provides a reliable estimate of total sulphur compounds in garlic and its products. The procedure was validated using both alliin and garlic oil sulphides as external standards. The procedure can recover nearly 100% of the garlic oil sulphides and achieved 80% recovery of alliin. Since garlic oil contains similar amounts of these sulphides, the procedure can secure a 100% conversion and recovery when alliin is the main compound of analysis. It can also provide a basis for the comparison of other analytical procedures. Since the procedure can analyse different types of product, the main advantage of the method is that it can be used to compare the quality of products of various formulations.

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