

# *Allium* Thiosulfates: Chemistry, Biological Properties and their Potential Utilization in Food Preservation

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## ABSTRACT

Onion (*Allium cepa* L.), garlic (*Allium sativum* L.) and other edible *Allium* are among the oldest cultivated plants, and are used for multiple purposes. They are a rich source of several phytonutrients, and recognized to have significant and wide biological activities. These biological activities are related to the thiosulfates, volatile sulfur compounds which are responsible for the pungency of these vegetables. The thiosulfates or alkane(ene) thial-S-oxide are formed by the action of the enzyme alliinase (E.C. 4.4.1.4) from their respective S-alk(en)yl cysteine sulfoxides. However, depending on the *Allium* species, and under differing conditions, thiosulfates can decompose to form additional sulfur constituents, including diallyl, methyl allyl, and diethyl mono-, di-, tri-, tetra-, penta-, and hexasulfides, vinylidithiins, and (*E*)- and (*Z*)-ajoene. With increasing interest into the utilization of natural biologically active compounds and the development of specific packaging, mainly active packaging, the thiosulfates aroused much interest for the improvement of shelf-life and safety of perishable foods, and their potency as food preservatives and substitutes for chemicals. This review examines the nature and the biological activities of *Allium* thiosulfates and their potential values as food preservatives in food preservation and shelf-life extension.

**Keywords:** antimicrobial, antioxidant, biosynthesis, chemistry, food preservation

**Abbreviations:** BHT, butylated hydroxytoluene; BHA, butylated hydroxyanisole; DPPH, 1,1-diphenyl- 2-picrylhydrazyl

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## INTRODUCTION

*Allium* species are supposed to be ones of the world's oldest cultivated vegetables and they have been large reported. It is presumed that our predecessors discovered and consumed wild *Allium* species long before farming or writing was invented. Because *Allium* plants are small and leave no archaeological evidence, the exact origin remains still mysterious. Onion and garlic could probably be the first cultivated crops due to their growing versatility, long storage time, and portability. They could be dried and preserved for times when food was scarce.

At the present time, the *Allium* family has over 600 members, distributed all over Europe, North America, Northern Africa and Asia, each differing in taste, form and color, but close in biochemical, phytochemical and nutraceutical content. The plants can be used as ornamentals, vegetables, spices, or as medicine. There are over 120 different documented uses of the *Allium* plants, and besides their remarkable medicinal powers, *Allium* plants are generally consumed for their flavors, while their nutritive values have been appreciated only recently (Fenwick and Hanley 1999; Garlic & Health Group 2007).

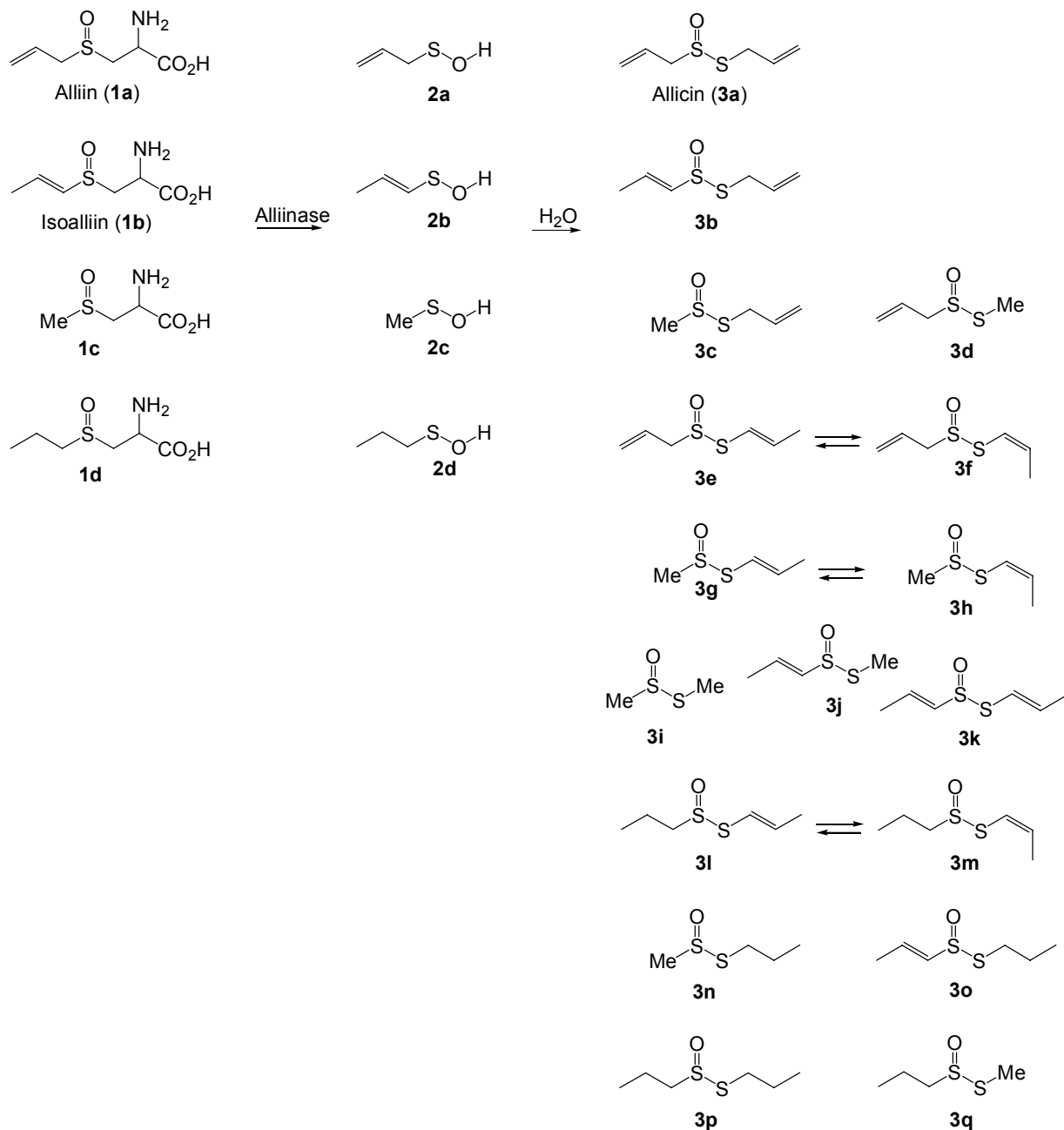
*Allium* species, especially *Allium* vegetables, are characterized by their rich content of thiosulfates and other organosulfur compounds, such as the well known lachrimatory factor. The thiosulfates or alkane(ene) thial-S-oxide are formed by the action of the enzyme alliinase (E.C. 4.4.1.4) from their respective S-alk(en)yl cysteine sulfoxides which are the main responsible of onion flavor and produce the eye-irritating compounds that induce lachrimation. However, depending on the *Allium* species, and under differing conditions, thiosulfates can decompose to form additional sulfur constituents, including diallyl, methyl allyl, and diethyl mono-, di-, tri-, tetra-, penta-, and hexasulfides, vinylidithiins, and (*E*)- and (*Z*)-ajoene.

With the increasing interest into the utilization of natural biological active compounds and the development of specific packaging, mainly active packaging, the thiosulfates arouse much interest for the improvement the shelf-life and safety of perishable foods, and their potency as food preservatives and substitutes for chemicals. This review examines the nature and the biological activities of *Allium* thiosulfates and their potential value as preservatives in food preservation and shelf-life extension.

## CHEMICAL STRUCTURE AND BIOSYNTHESIS OF THIOSULFINATES

Thiosulfinates are the best studied compounds arising from *Allium* species. Their finding was first reported by Wurtzheim (1844) and later by Semmler (1892) who identified the correct disulfide structure as the main component of distilled oil of garlic and onion. Fifty years later, it became clear that these compounds are not present in the intact bulbs but are formed by enzymatic reaction of precursor (Cavallito *et al.* 1944a; Cavallito *et al.* 1944b; Stoll and Seebeck 1948). In fact, they demonstrated that disulfides are absent when the bulbs are frozen in dry ice, pulverized, and extracted with acetone. The white powder obtained by this procedure does not show the classic pungent smell while it appears only after addition of water. On the contrary, when the powder is treated with hydro alcoholic solution the enzyme is inactivated and the disulfide compound is not found. So it was also clear that the pungency of garlic *Allium* plants is associated with the thiosulfinate compounds. **Fig. 1** shows the proposed biosynthesis of such compounds starting from their precursor, non protein sulfur amino acids, S-alk(en)yl-L-cysteine-S-oxide (**1a–1d**; **Fig.**

**1**). They are all present in all *Allium* spp. analyzed so far constituting from 1 to 5% of the dry weight of the plant. As shown in **Fig. 1**, two molecules of precursor are needed to form the volatile thiosulfinates. Four sulfoxides are commonly present in *Allium* plants: S-2-propenyl- (**1a**), S-(*E*)-1-propenyl- (**1b**), S-methyl- (**1c**), S-propyl-L-cysteine-S-oxide (**1d**) and their homo and hetero coupling give rise to a number of resulting thiosulfinates (Rose *et al.* 2005). Due to the fact that most species also contain the S-ethyl- and S-butyl-L-cysteine-S-oxide (Krest *et al.* 2000), the number of resulting thiosulfinates is even higher. The cysteine sulfoxide precursors (**1a–1d**; **Fig. 1**), located in the cytoplasm, through an enzymatic reaction catalyzed by alliinase, a C-S lyase present in the vacuoles (Lancaster and Collin 1981), initially give sulfenic acid intermediates (**2a–2d**; **Fig. 1**). These highly reactive compounds immediately produce thiosulfinates by a condensation reaction (**3a–3q**; **Fig. 1**). Compounds possessing a 1-propenyl residue at the thiolic site exist as a mixture of the *E*, *Z* isomers (**3e–3f**, **3g–3h**, **3l–3m**) because of a sigmatropic [2,3] rearrangement. It has been found that garlic contain compounds **1a–1c**, alliin (**1a**) being the major compound. This last compound is the precursor of alliin (**3a**) (Cavallito *et al.* 1944a). In contrast, in



**Fig. 1** Proposed biosynthetic pathway of thiosulfinates starting from their precursor, non protein sulfur amino acids, S-alk(en)yl-L-cysteine-S-oxide.

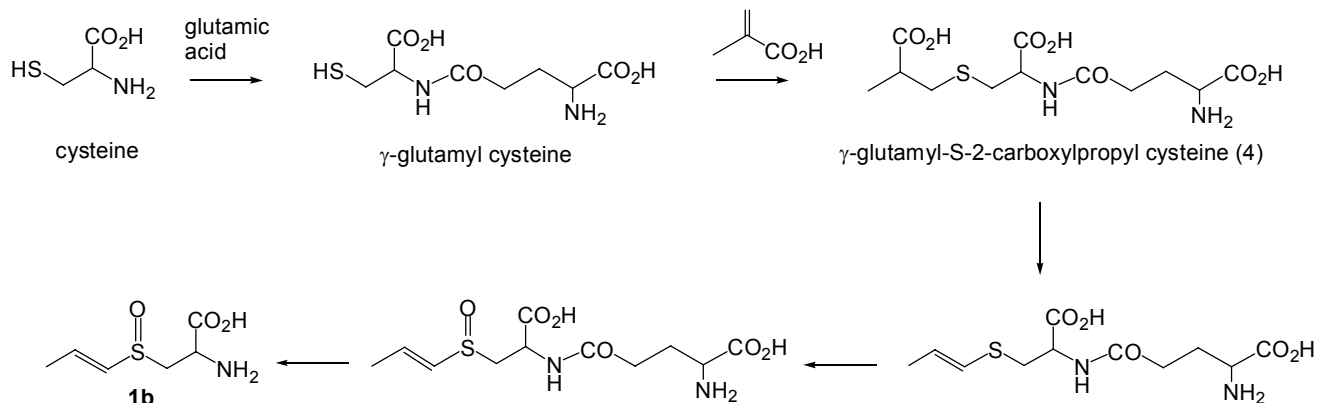


Fig. 2 Proposed biosynthetic pathway of isoalliin (**1b**) from cysteine, the amino acid precursor.

onion **1a** is the only compound absent while **1b**, the compound with the 1-propenyl residue, named isoalliin, is the major metabolite (Virtanen 1965; Lawson *et al.* 1991a).

Also present in *Allium* are  $\gamma$ -glutamyl peptides of sulfur amino acids that are the biosynthetic precursor of organic sulfur compounds. Fig. 2 shows the proposed biosynthetic pathway of isoalliin (**1b**) from cysteine, the amino acid precursor. The sulfate after reduction is incorporated into cysteine in the chloroplast (Rennenberg 1982) and then is converted into  $\gamma$ -glutamylcysteine (Anderson 1980). Michael addition of this last compound with methacrylic acid, originating from valine, affords  $\gamma$ -glutamyl-S-2-carboxypropylcysteine (**4**; Fig. 2) which in onion undergoes sequential decarboxylation, oxidation, and cleavage by glutamyl transpeptidase [E.C. 2.3.2.1] to **1b**. By boiling the bulbs with a hydroalcoholic solution the allinase enzyme is inactivated thus allowing the analysis of peptide precursors (Ziegler and Sticher 1989; Mutsch-Eckner *et al.* 1992).

The total thiosulfonates of different *Allium* spp. have been quantified (Block *et al.* 1992b) by HPLC analysis. Garlic (*A. sativum*) showed higher amount of total thiosulfonates compared to the other spp. analyzed. Lower content (15  $\mu\text{mol/g}$  wet fresh weight average concentration) has been found in garlic grown in colder climate (21°C average temperature), while concentration increases (23  $\mu\text{mol/g}$  average concentration) for store-purchased garlic (25°C average temperature). Highest thiosulfonate amount (36  $\mu\text{mol/g}$  average concentration) was found for garlic grown in a warmer climate (31°C average temperature). All the other species analyzed possessed thiosulfonate contents ranging from 21  $\mu\text{mol/g}$  (wild garlic, *A. ursinum*) to 53  $\mu\text{mol/g}$  (elephant garlic, *A. ampeloprasum*) to 2  $\mu\text{mol/g}$  (Chinese chive, *A. tuberosum*) to 0.35, 0.20, and 0.14  $\mu\text{mol/g}$  (respectively yellow, red, and white onion, *A. cepa*) to 0.25  $\mu\text{mol/g}$  (shallot, *A. ascalonicum*) to 0.19  $\mu\text{mol/g}$  (chive, *A. schoenoprasum*) to 0.15  $\mu\text{mol/g}$  (leek, *A. porrum*) to 0.08 (scallion, *A. fistulosum*).

It has been found (Lawson *et al.* 1991a) that the concentration of  $\gamma$ -glutamyl-S-(*E*)-1-propenylcysteine (**1b**) and  $\gamma$ -glutamyl-S-2-propenylcysteine (**1a**), the major metabolites of fresh garlic extract, decreased markedly with storage at temperature above 0°C. When the storage temperature is colder, the concentration of **1b** increases and therefore those of the related thiosulfonates. Thus, climate has been demonstrated to affect not only the total content of thiosulfonates but also their relative amounts. In fact, in garlic grown in colder climate (21°C average temperature) the ratio **3a**/methyl thiosulfonate is higher than garlic grown in temperate climates 10°C warmer (Block 1992). It was suggested that *Allium* spp., particularly garlic (*A. sativum*) but also elephant garlic (*A. ampeloprasum*) grown in colder climates are subjected to stress and that this stress manifest itself in reduced synthesis of S-methyl-L-cysteine sulfoxide, the immediate precursor of methyl thiosulfonates. Storage at temperature above 0°C can also result in diminishes formation of methyl thiosulfonates, possibly by selective destruction of

a methyl-specific alliinase (Lawson and Hughes 1992).

The composition of thiosulfonates depends also on the *Allium* spp. and this variation has been used in chemotaxonomic studies. In particular, only Chinese chive (*A. tuberosum*) showed a preponderance of methyl groups, although all *Allium* plants examined contain methyl groups (Iida *et al.* 1983). Garlic (*A. sativum*), elephant garlic (*A. ampeloprasum*), and wild garlic (*A. ursinum*) show a preponderance of the allyl group that is also present in detectable amounts in Chinese chive. The allyl/methyl ratio ranged from 94:2 (garlic grown at 22–23°C) to 80:16 (store bought garlic) to 74:24 (garlic grown at 32°C) to 62:35 (elephant garlic) to 50:49 (wild garlic) to 11:86 (Chinese chive). Allyl groups are absent in onion (*A. cepa*), scallion (*A. fistulosum*), shallot (*A. ascalonicum*), leek (*A. porrum*), and chive (*A. schoenoprasum*). In these last species the propyl group is a major alkyl group with methyl/propyl ratio varying from 1:5.8 (chive), to 1:1.52.0 in scallion, shallot, and leek. In onion the methyl/propyl ratio varies from 1.7–1.5:1 (yellow and red) to 1:1 (white). Thus, the propyl group is absent in garlic, elephant garlic, wild garlic, and Chinese chive, while it is present in onion taxa. Finally, the 1-propenyl group is present in all species, but is dominant only in onion.

## CHEMICAL STRUCTURE OF OTHER ORGANOSULFUR COMPOUNDS

The main fate of the sulfenic acid intermediate is condensation to produce thiosulfonates, however these compounds can participate in a variety of reactions (dehydration, rearrangement, condensation, Diels-Alder reaction, hydrolysis, pyrolysis) depending on the conditions (Lanzotti 2006) which afford other classes of organosulfur compounds (Figs. 3, 4). The last compounds can be classified as headspace volatiles (formed when bulbs are cut or homogenated at room temperature) (**5–7a/7b**; Fig. 3), compounds formed when thiosulfonates stand in solution at room temperature

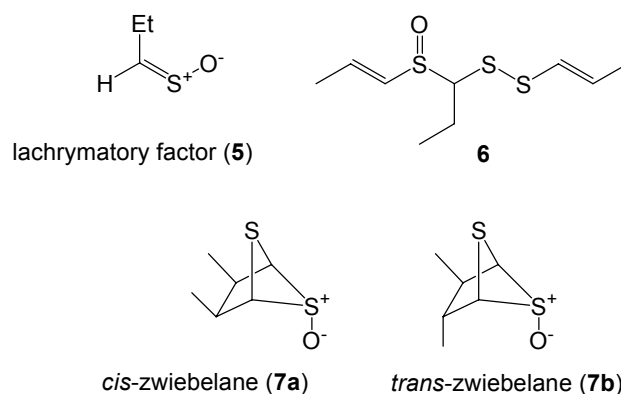
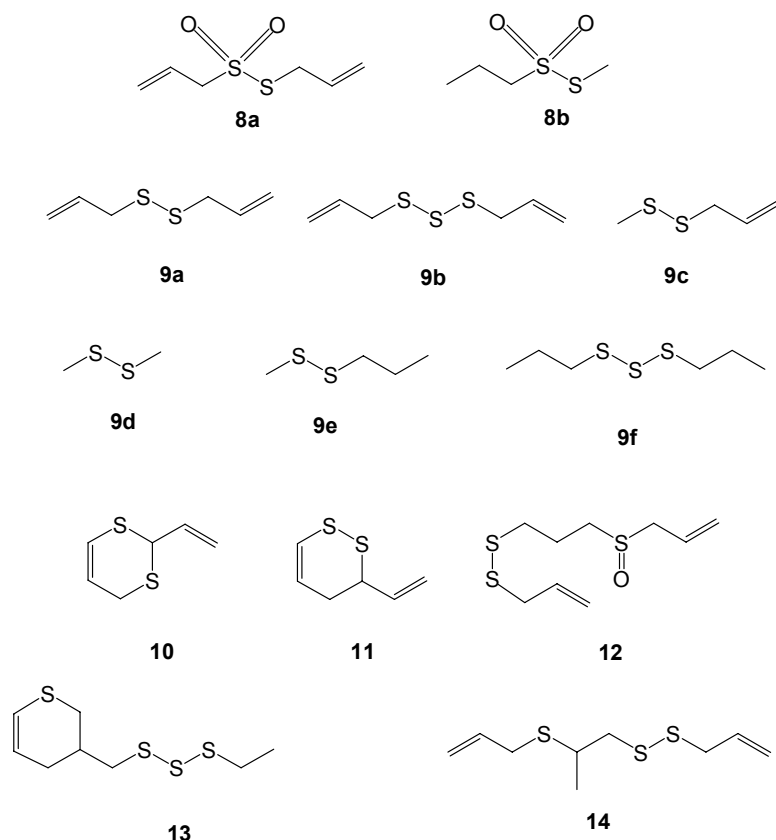


Fig. 3 Other headspace volatiles in onion formed when bulbs are cut or homogenated at room temperature.



**Fig. 4** Other organosulfur compounds formed when thiosulfonates stand in solution from room temperature up to 100°C.

(8a/8b–12; Fig. 4) and compounds formed from thiosulfonates when the temperature is increased from room temperature up to 100°C (13, 14, Fig. 4) (Block 1993).

The main headspace volatiles (Fig. 3) formed in onion bulbs from sulfenic 1-propenyl acid intermediate (2b) by internal transfer of hydrogen are (*Z,E*)-propanethial *S*-oxide, named lachrymatory factor (LF) (5, *Z*-isomer) (Block 1992; Breu 1996). Their names derive from “cry” because they are the compounds that make people cry when they slice an onion. Other onion volatiles are cepaenes (e.g. 6) (Block 1992; Breu 1996), whose name derives from *cepa*, and 2,3-dimethyl-5,6-dithiabicyclo[2.1.1] hexane *S*-oxides, named *cis* and *trans*-zwiebelane (7a and 7b, respectively) (Bayer *et al.* 1989), from “zwiebel”, onion in German.

From thiosulfinate solution at room temperature could originate (Fig. 4) thiosulfonates (e.g. 8a and 8b), di- and trisulfur compounds (e.g. 9a–9f), 2-vinyl-2,4-dihydro-1,3-dithiin (10), 3-vinyl-3,4-dihydro-1,2-dithiin (11), and ajoene (12, from the Spanish word “ajo” that means garlic). At higher temperatures (about 100°C) polysulfur compounds (Fig. 4) are formed (e.g. 13 and 14) containing up to 5 sulfur atoms.

Kubec *et al.* (2004) studied the precursors involved in the formation of pink and green-blue pigments generated during onion and garlic processing. It has been confirmed that the pigment formations are similar, with (*E*)-*S*-(1-propenyl) cysteine sulfoxide (1b, Fig. 1), isoalliin, serves as the primary precursor. Upon disruption of the tissue, isoalliin and other *S*-alk(en)ylcysteine sulfoxides are enzymically cleaved, yielding 1-propenyl-containing thiosulfonates (3j, 3b, 3o, with a Me, allyl or propyl groups at the thiolic side, respectively). A thiosulfinate possessing 1-propenyl residues on both sides (3k; Fig. 1) of the molecule has also been found. These compounds have been shown to subsequently react with amino acids to produce the pigments. Whereas the Pr, 1-propenyl, and Me derivatives (3o, 3k, and 3j, respectively) form pink, pink-red, and magenta compounds, those containing the allyl group (3b, Fig. 1) give rise to blue products after reacting with glycine at acid condition (pH 5.0). This reaction does not take place in the cells where the cellular pH is basic.

Very recently, the abilities of all 20 protein amino acids and six naturally occurring 1-propenyl-containing thiosulfonates to form these pigments have been studied. Furthermore, several analogs of these thiosulfonates were synthetically prepared, and their color-forming abilities were evaluated, together with those of various amino compounds by reaction with glycine. It has been found that an unsubstituted primary amino group and a free carboxyl group are essential structural features required for amino compounds to be able to generate the pigments. Out of the thiosulfinate analogs tested, only those containing at least a three-carbon chain with the  $\beta$ -carbon bearing a hydrogen atom yielded the pigments. Thiosulfonates, sulfoxides, sulfides, and disulfides did not form any colored products. The pH optimum for pigment formation has been found to be between 5.0 and 6.0 for all thiosulfonates tested (Kubec *et al.* 2007).

The variation in thiosulfonates and related compounds between fresh bulbs and cloves, and commercial garlic products was investigated (Lawson *et al.* 1991b). The analysis indicated that thiosulfonates were released only from garlic cloves and garlic powder products. The vinylthiins (10, 11; Fig. 4) and ajoene (12; Fig. 4) were found only in products containing garlic macerated in vegetable oil. The disulfide compounds containing two allyl (9a; Fig. 4), or methyl allyl (9c; Fig. 4), or two methyl (9d; Fig. 4) residues were found only in products containing the oil of steam-distilled garlic. Typical steam-distilled garlic oil products contained about the same amount of total sulfur compounds as the total thiosulfonates released from freshly homogenized garlic cloves. However, oil-macerated products contained only 20% of that amount, while for garlic powder products the amount varied from 0 to 100%. Products containing garlic powder suspended in a gel or garlic aged in aqueous alcohol did not contain detectable amounts of these non-ionic sulfur compounds. A comparison of several brands of each type of garlic product revealed a large range in content (4-fold for oil-macerates and 33-fold for steam-distilled garlic oils), indicating the importance of analysis before using garlic products for clinical investigations or commercial distribution (Lawson 1996).

Mondy *et al.* (2002) analyzed the organosulphur com-

pounds of fresh onion and transformation products, such as frozen, freeze-dried powders and sterilized products. In particular, LF (**5**; **Fig. 3**) was directly analysed on fresh onion juice which showed to contain thiosulfonates and zwiebelanes (**7a** and **7b**; **Fig. 3**) also after 80 minutes maceration. With the exception of LF, frozen onion compounds were similar to those of fresh onion sample. Conversely, the other transformed samples have lost most of the initially formed compounds and produced mainly di- and trisulfides (e.g. **9–12**; **Fig. 4**) corresponding to the degradation of thiosulfonates and zwiebelanes. These dramatic changes explained the very different flavours of these manufactured products compared to fresh material.

Randle (1997) reviewed the literature about the onion flavour chemistry and reported as main components LF (**5**; **Fig. 3**) and various thiosulfonates (**3g–3q**; **Fig. 1**) which are also responsible for the characteristic onion flavours. As shown in **Fig. 2**, sulfate through cysteine can proceed through several peptide pathways and terminate in the synthesis of one of three flavour precursors. Flavour intensity is governed by genetic factors within the onion and environmental conditions under which the onions grow (Randle *et al.* 1994). Onion cultivars differ in the ability to absorb sulfate and in the efficiency of synthesizing flavour precursors. Increased sulfate fertility, higher growing temperatures and dry growing conditions all contribute to increased flavour intensity in onion (Randle *et al.* 1994).

The thiosulfonates are best analyzed by milder chromatographic methods than gas chromatography and mass spectrometry (GC–MS), usually used for stable volatile compounds. Then, HPLC and supercritical fluid chromatography (SC) have been showed to yield the best analytical results (Lanzotti 2006).

Block and his group published several papers on this matter. They first performed a quantitative GC–MS analysis (Block *et al.* 1992a) of thiosulfonates and related compounds from onion and other common related *Allium* species, such as leek (*A. porrum*), scallion (*A. fistulosum*), shallot (*A. ascalonicum*), and chive (*A. schoenoprasum*). A wide-bore capillary column was used with cryogenic (0°C) on-column injection and initial column temperature conditions, slow column heating rates (2–5°C/min), and GC–MS transfer line temperature of 80–100°C. Authentic samples of suspected components were used to verify identities and quantify the compounds, using benzyl alcohol as internal standard. Under these conditions, the thiosulfonates **3g–3j**, **3l–3n**, **3p**, **3q** (**Fig. 1**), and *cis*- and *trans*-zwiebelanes (**7a** and **7b**, **Fig. 3**, respectively) were all identified in onion and in leek, scallion, shallot, and chive. No satisfactory peaks were obtained for the (*E*)-isomer **3o** (**Fig. 1**), or for thiosulfonates containing a 2-propenyl (allyl) group (**3a–3f**; **Fig. 1**), which were best identified by HPLC.

The data regarding quantitative HPLC analysis were reported in an accompanying paper (Block *et al.* 1992b) in which room-temperature vacuum distillates and extracts of onion, garlic, and other related *Allium* species such as leek, scallion, shallot, chive, wild garlic, and elephant (or great-headed) garlic were analysed by HPLC and <sup>1</sup>H NMR by using authentic samples of suspected thiosulfonate components to evaluate the methods. It was concluded that GC, as typically performed with high injector and column temperatures, creates a wrong picture of the volatile extracts of *Allium* species, and that HPLC provides instead a reliable measure, both qualitative and quantitative of the compounds present.

In the same year, Sinha *et al.* (1992) published a method based on the supercritical carbon dioxide extraction (SC–CO<sub>2</sub>) of onions and their quantitative analysis by GC–MS. This analysis evidenced the presence of 28 sulfur compounds, including allicin (**3a**; **Fig. 1**) (or its isomer, di-1-propenyl thiosulfonate, **3k**; **Fig. 1**), Pr methanethiosulfonate (**8b**; **Fig. 4**), dithiin derivatives (**10–12**; **Fig. 4**), diallyl sulfide (**9a**; **Fig. 4**), and diallyl trisulfide (**9b**; **Fig. 4**). A common steam-distilled onion oil, analysed under similar conditions, did not contain detectable amounts of these listed

compounds while other compounds were in common with the SC–CO<sub>2</sub> onion extract. The compounds Me Pr trisulfide (**9e**; **Fig. 4**), di-Pr trisulfide (**9f**; **Fig. 4**), and di-Pr tetrasulfide were detected only in the commonly steam-distilled onion oil and were present in high concentration.

Finally, Cruz-Villalon (2001) developed a method to synthesize allicin (**3a**; **Fig. 1**), based on the oxidation of diallyldisulfide (**9a**; **Fig. 4**) with an aqueous solution of magnesium monoperoxyphthalate (MMPO) and tetrabutylammonium hydrogen sulfate, as a phase transfer catalyst. The allicin obtained was isolated by solid-phase extraction and its concentration was detected spectrophotometrically, allowing the allicin solution to be used also as a standard for HPLC calibration.

## ANTIBACTERIAL PROPERTIES

While investigating plant extracts for antibacterial activity, it was observed that a freshly prepared infusion of ground garlic cloves possessed high antibacterial activity. A literature investigation showed that *A. sativum*, the common garlic, has been endowed with therapeutic virtues both in legend and in the scientific literature, and most of the claims have been poorly substantiated, until recently (Garlic & Health Group 2007). Several investigators have observed antibacterial activity of garlic extracts and have attributed this activity to diallyl sulfide, unstable sulfur in alkyl polysulfides, a bacteriophage, acrolein or some similar unsaturated aldehyde, and recently to a chemically undefined group of substances designated as phytoncides, however, the antibacterial principle of *A. sativum*, allicin, was first isolated, characterized, and its physical properties and antibacterial action assessed by Cavallito and Bailey (1944) and Cavallito *et al.* (1944b). Later, Feldberg *et al.* (1988) reported an *in vitro* mechanism of inhibition of bacterial cell growth by allicin. They noted that allicin delayed and inhibited partially DNA and protein syntheses, while inhibition of RNA synthesis was immediate and total, suggesting that this is the primary target of allicin action.

The antibacterial and antifungal activities against a variety of Gram-negative and Gram-positive bacteria were, and continue to be extensively investigated (Whitemore and Naidu 2000). Han *et al.* (1995) reported that the antibiotic activity of 1 mg of allicin has been equated to that of 15 IU of penicillin. Recent investigations have also demonstrated an inhibitory effect by aqueous extracts on numerous bacterial species such as *Helicobacter pylori*, *Bacillus subtilis*, *Escherichia coli*, *Flavobacterium* sp., *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Vibrio parahaemolyticus* (Sivam *et al.* 1997; Hsieh *et al.* 2001; Ward *et al.* 2002; Benkeblia 2004), and fungi such as *Aspergillus flavus* and *Aspergillus niger* (Phay *et al.* 1999; Hsieh *et al.* 2001; Benkeblia 2004). However, these authors reported different minimum level of inhibition depending on the extraction method, the final content of thiosulfonates in the extracts and also the applied method for the assessment of the inhibitory effect.

Onion extract is effective *in vitro* against many bacteria species including *Bacillus subtilis*, *Salmonella*, and *E. coli* (Cavallito and Bailey 1944; Yoshida *et al.* 1998; Yin and Cheng 2003; Benkeblia 2004) (**Table 1**). From a fundamental point of view, the antibacterial activity of *Allium* thiosulfonate-extracts has been extensively investigated, however, these investigations focused mainly on onion and garlic. However, onion is not as potent as garlic since the sulfur compounds in onion are only about one-quarter the level found in garlic. Allicin and allyl-methyl plus methylallyl thiosulfonate extracted from garlic have shown inhibition of the *in vitro* growth of *Helicobacter pylori* (Hp). The capacity and effectiveness of isolated natural thiosulfonates have been tested, and this has enabled the identification of the main compounds responsible for the bacteriostatic activity. Additionally, microbiological analyses have also shown that these compounds have a synergic effect on the inhibition of the *in vitro* growth of Hp (Cañizares *et al.*









