

Allium Thiosulfinates: Chemistry, Biological Properties and their Potential Utilization in Food Preservation

Noureddine Benkeblia^{1,2*} • Virginia Lanzotti³

¹ Department of Food and Nutrition Sciences, Graduate School of Dairy Science Research, Rakuno Gakuen University, Ebetsu, Hokkaido, 060-8501, Japan

² Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

³ Department of STAAM, University of Molise, 86100 Campobasso, Italy

Corresponding author: * ben-nour@rakuno.ac.jp, benkeblia@hotmail.com

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 thiosulfinates, volatile sulfur compounds which are responsible for the pungency of these vegetables. The thiosulfinates 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, thiosulfinates 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 thiosulfinates 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* thiosulfinates 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

CONTENTS

INTRODUCTION.....	193
CHEMICAL STRUCTURE AND BIOSYNTHESIS OF THIOSULFINATES	194
CHEMICAL STRUCTURE OF OTHER ORGANOSULFUR COMPOUNDS	195
ANTIBACTERIAL PROPERTIES	197
ANTIFUNGAL PROPERTIES	198
ANTIOXIDANT PROPERTIES	199
CONCLUDING REMARKS	199
REFERENCES.....	199

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 thiosulfinates and other organosulfur compounds, such as the well known lachrimatory factor. The thiosulfinates 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, thiosulfinates 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 thiosulfinates 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* thiosulfinates 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**, **3i–3m**) because of a sigmatropic [2,3] rearrangement. It has been found that garlic contains 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

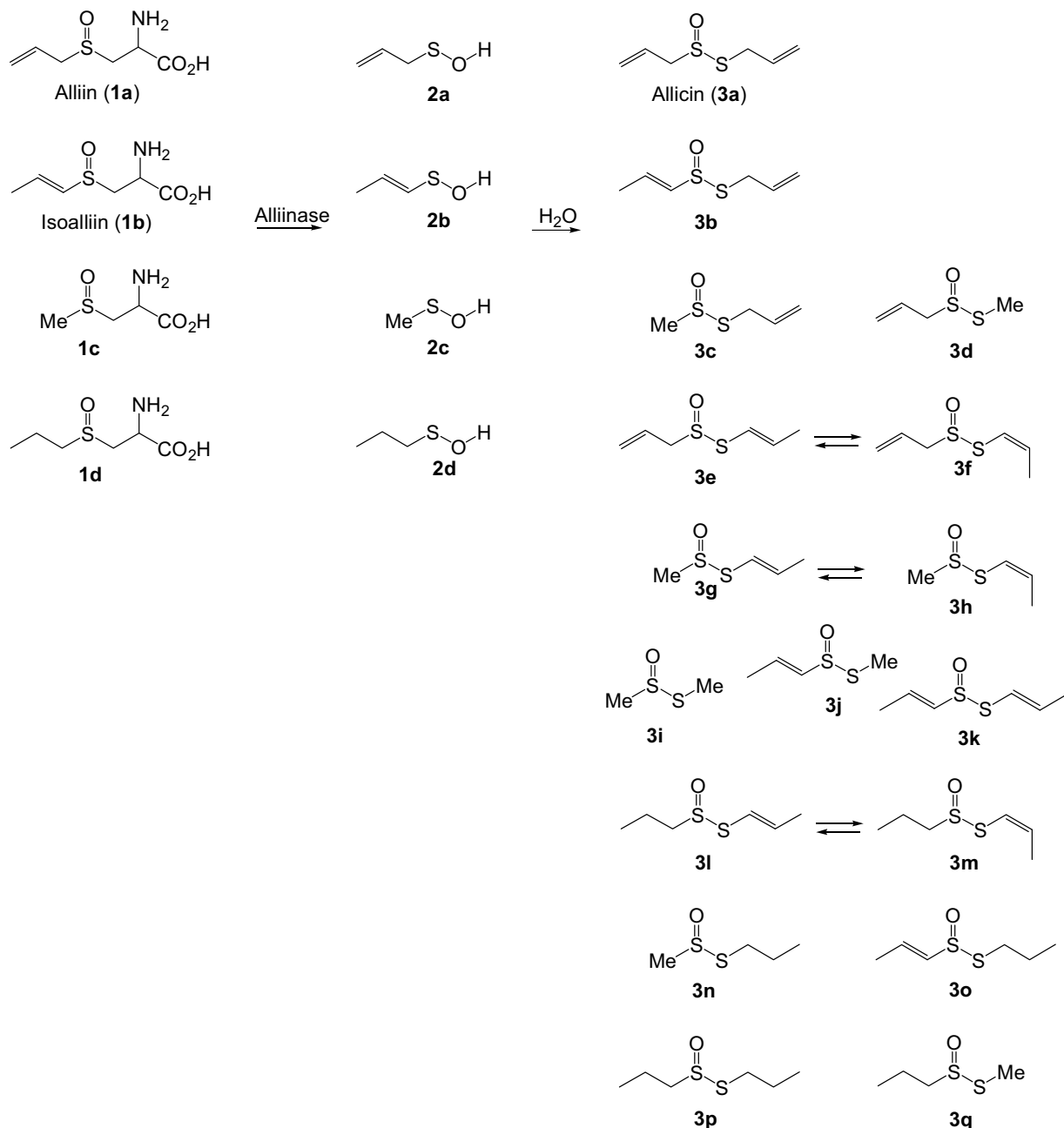


Fig. 1 Proposed biosynthetic pathway of thiosulfinates starting from their precursor, non protein sulfur amino acids, S-alk(en)yl-L-cysteine-S-oxide.

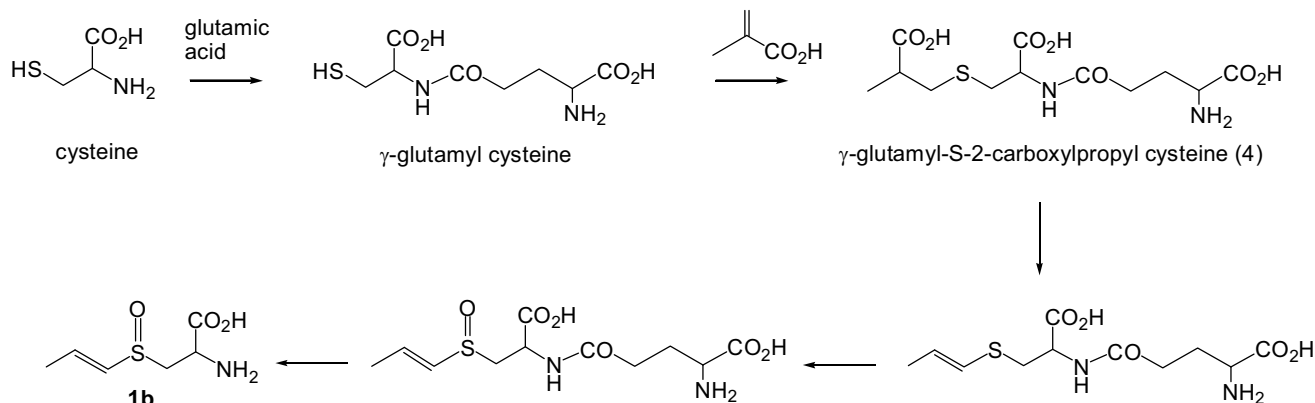


Fig. 2 Proposed biosynthetic pathway of isoalliin (**1b**) from cysteine, the amino acid precursor.

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 γ -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 γ -glutamylcysteine (Anderson 1980). Michael addition of this last compound with methacrylic acid, originating from valine, affords γ -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 hydro alcoholic 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 γ -glutamyl-S-(*E*)-1-propenylcysteine (**1b**) and γ -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

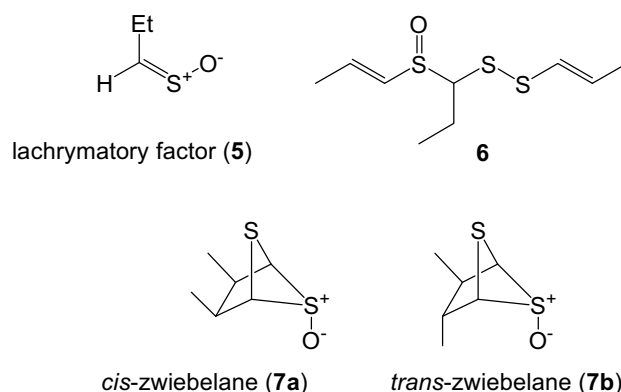


Fig. 3 Other headspace volatiles in onion formed when bulbs are cut or homogenated at room temperature.

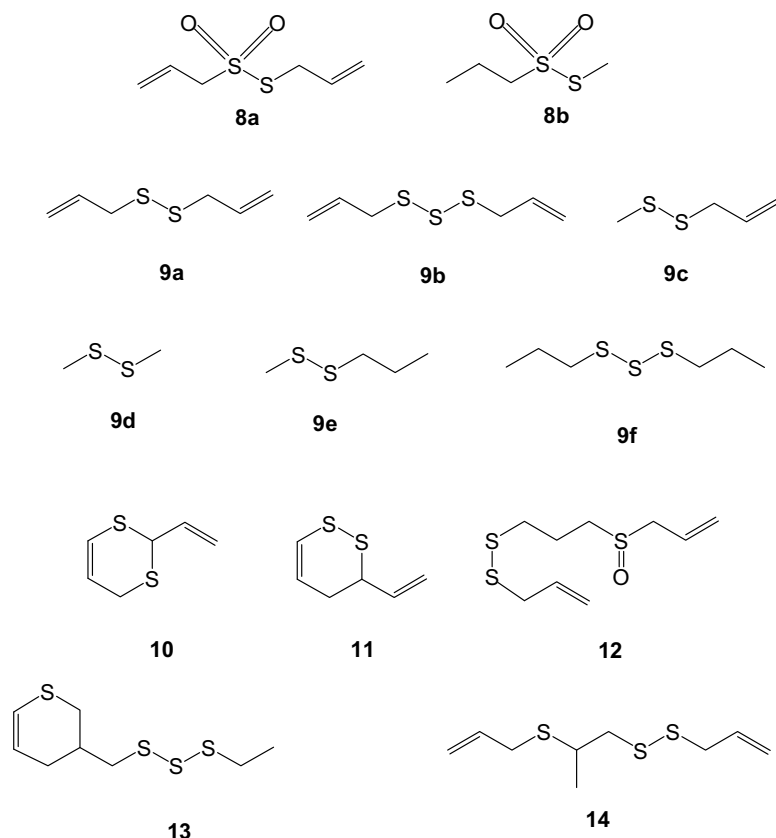


Fig. 4 Other organosulfur compounds formed when thiosulfonates stand in solution from room temperature up to 100°C.

(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 5-oxides, named *cis* and *trans*-zwibelane (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 β -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 ¹H 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–CO₂) 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–CO₂ onion extract. The compounds Me Pr trisulfide (**9c**; **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 methyl-allyl 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 (Cañazares *et al.*

Table 1 Antibacterial activity of garlic extract against *Staphylococcus aureus* and *Salmonella enteritidis*.

	Concentration (ml/l)				
	50	100	200	300	500
<i>Staphylococcus aureus</i>	6.3 ± 0.4	7.6 ± 0.8	7.9 ± 0.9	8.5 ± 0.7	9.3 ± 0.2
<i>Salmonella enteritidis</i>	8.3 ± 0.5	9.3 ± 0.1	9.9 ± 0.5	11.3 ± 1.0	13.1 ± 0.2

Zone of inhibition is expressed in mm.

2004). Similarly, this inhibiting effect was also noted on *Staphylococcus aureus* and results showed a complete inhibition of all strains tested at a concentration of 6.5 mg/ml (Gnan and Demello 1999). Furthermore, three thiosulfinates were isolated from oil-macerated garlic extract by Yoshida *et al.* (1998), and their antimicrobial activities against Gram-positive and Gram-negative bacteria were compared with allicin. The results have shown that antimicrobial activity of two isolated thiosulfinates were comparable but lower than that of allicin. They suggested that the antimicrobial activity were affected by alk-(en)-yl groups, and the order for antimicrobial activity was: allyl ≥ methyl > propenyl.

Among these numerous and abundant naturally occurring compounds, *Allium* extract has been considered a natural preservative or food additive, and can be used as additional methods of controlling pathogens (Whitemore and Naidu 2000).

Yin and Cheng (2003) reported the antimicrobial activity of four different thiosulfinates in ground beef. The presence of two thiosulfinates significantly reduced total aerobes and inhibited the growth of the inoculated pathogenic bacteria, *Salmonella typhimurium*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Campylobacter jejuni*. Wong and Kitts (2002) also reported that a further reduction in psychrotroph bacteria was observed with the pre-seasoning of garlic, in both non-irradiated and irradiated steaks. On the other hand, the initial aerobic flora was significantly reduced by a different form of added fresh and powder of garlic, and the shelf-life of dry chicken sausage was significantly extended to 21 days (Sallam *et al.* 2004).

ANTIFUNGAL PROPERTIES

Comparatively to the extensive literature on the effect of *Allium* extracts on bacteria, little has been done on their effect on fungi. The first investigation was reported by Raghunandana *et al.* (1946). Later, Yamada and Azuma (1977) reported that allicin was effective *in vitro* against *Candida*, *Cryptococcus*, *Trichophyton*, *Epidermophyton*, and *Microsporum*, however, decreased activity was demonstrated against *Aspergillus*. Nevertheless, these authors noted that the minimum inhibitory concentration (MIC) of allicin was considerably affected by incubation time, inoculum size, type of medium, and medium pH. For example, the MIC (minimum inhibitory concentration) of allicin against *Candida*, *Cryptococcus*, and *Aspergillus* remained constant after more than 3 days, while against *Dermatophytes* remained constant over 10 days of incubation. Decreasing the inoculum size increased the susceptibility to allicin. They also noted that antifungal activity of allicin was stronger on SG (Sabouraud glucose) agar medium with a pH of 5.6 than on the same medium with a pH of 6.0 or higher. These authors also reported that allicin induced morphological abnormalities in hyphae of *Trichophyton mentagrophytes* Morita, and the percent germination of spores of the Morita strain greatly decreased.

Another investigation confirmed the antifungal activity of thiosulfinates. Nagawa *et al.* (1996) reported that ajoene also inhibited yeast growth at concentrations below 20 mg/ml, and that *Saccharomyces cerevisiae* was killed by 30 mg/ml of ajoene after 24 h of cultivation. On the other hand, the antifungal activity and the minimal fungicidal concentration (MFC) of different *Allium* plants, namely garlic (*Allium sativum* L.), bakeri garlic (*Allium bakeri* L.), Chinese leek

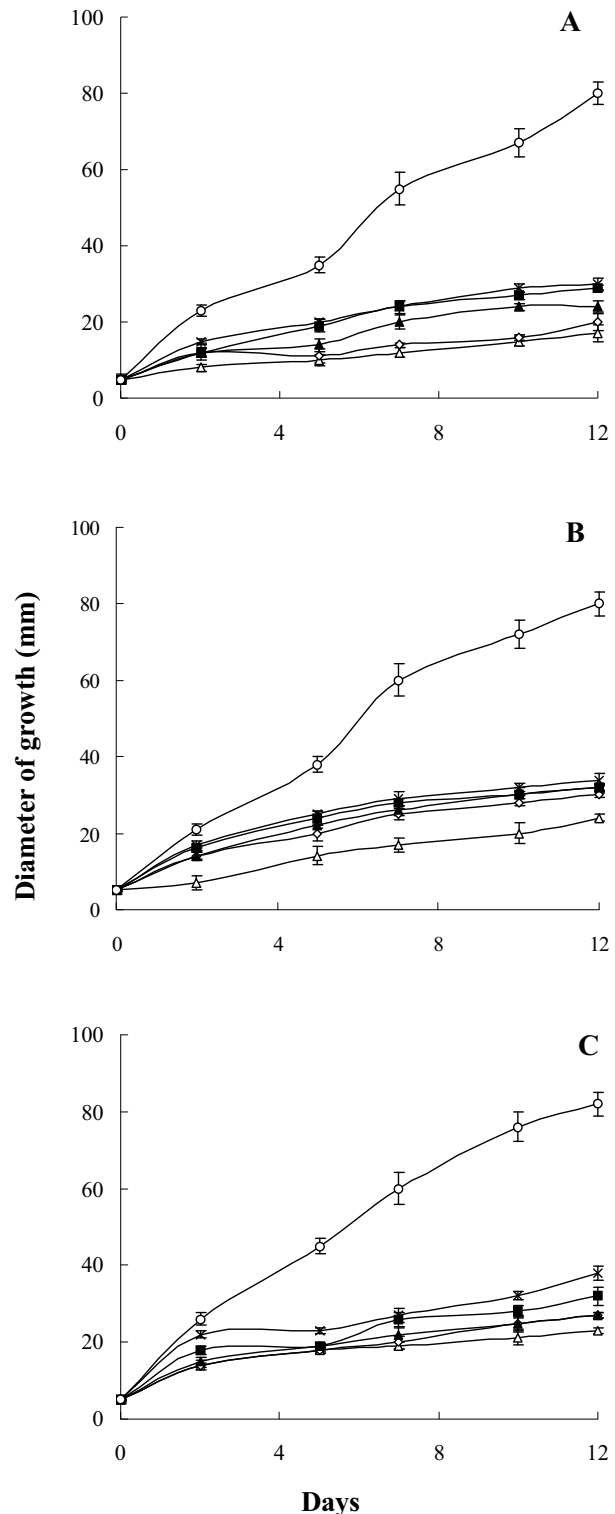


Fig. 5 Effect of garlic extract on growth of *Aspergillus niger* (A), *Fusarium oxysporum* (B) and *Penicillium cyclopium* (C). Concentrations of the extract: ○ Control, × 50, ■ 100, ▲ 200, ◇ 300 and △ 500 ml/l.

(*Allium odorum* L.), Chinese chive (*Allium tuberosum* Rotler), scallion (*Allium fistulosum* Auct.), onion bulb (*Allium cepa* L.) and shallot bulb (*Allium ascalonicum* L.), against *Aspergillus niger*, *A. flavus* and *A. fumigatus* were investigated and results have shown that all these *Allium* possessed antifungal activity, with garlic showing the lowest MFC

(Yin and Tsao 1999). Benkeblia (2004) investigated the effect of five different concentrations of essential oil extracts of three types of onion and garlic against three fungi, *Aspergillus niger*, *Penicillium cyclopium* and *Fusarium oxysporum*. Although *F. oxysporum* was less inhibited, *A. niger* and *P. cyclopium* were significantly inhibited (Fig. 5). Similarly, Shams-Ghahfarokhi *et al.* (2006) also studied the antifungal activity of aqueous extracts prepared from onion and garlic against *Malassezia furfur*, *Candida albicans*, and other *Candida* spp. These authors noted that all onion and garlic inhibited growth of all fungi tested and the inhibition was dose-dependent. The most observed inhibitory dose of onion was 2 mg/ml, while for garlic this dose ranged from 65 to 125 µg/ml. In contrast, López-Díaz *et al.* (2002) reported that garlic extract tested had no or a very little effect on the growth of *Penicillium olsonii* and *Penicillium nalgiovense* isolated from the surface of Spanish fermented meat sausage.

ANTIOXIDANT PROPERTIES

The antioxidant activities in *Allium* tissue extracts have been of particular interest because of the relationship between oxidative stress and pathologies such as atherosclerosis, cancer, and aging, in which free radicals and reactive oxygen species are implicated as having a role (Cavaliere and Rogan 1992; Halliwell *et al.* 1992; Salvemini and Botting 1993). Recent investigations on antioxidant properties of *Allium* components have used crude extracts or other derivatives. However, due to the complexity of such preparations, some investigators have reached unequivocal conclusions (Kourounakis and Rekkas 1991; Rekkas and Kourounakis 1994; Prasad *et al.* 1995) or have insinuated that the thiosulfonates or related organosulfur compounds are primarily responsible for the observed antioxidant effects (Augusti 1990; Kim *et al.* 1997; Siegers *et al.* 1999), although other endogenous components, such as phenolics, may have antioxidant properties (Yin and Cheng 1998; Benkeblia 2005). Nevertheless, pure thiosulfonates and related organosulfur compounds have exhibited real antioxidant properties under specific conditions (Imai *et al.* 1994; Rabinkov *et al.* 1998). However, it is equivocal to attribute a host of antioxidant properties to the thiosulfonates in crude *Allium* preparations, simply because they are present. Earlier it was claimed that S-2-propenyl-L-cysteine sulfoxide is an effective antioxidant (Hirata and Matsushita 1996), however, the case in point was the finding that pure alliin (S-allyl-L-cysteine sulfoxide) has no anti-oxidant activity in a linoleic acid emulsion and has only weak reducing power (Yin and Cheng 1998). Thus, the antioxidant functionality of thiosulfonates – which are not present in intact tissues – and other related organosulfur compounds in *Allium* plants will remain ambiguous until pure compounds are assessed.

Xiao and Parkin (2002) investigated the antioxidant properties of some pure thiosulfonates. Among them, some were not capable of scavenging hydrogen peroxide or superoxide anion. Relative to standard antioxidants (ascorbic acid, *n*-propyl gallate, butylated hydroxytoluene, Trolox, and reduced glutathione), these thiosulfonates were 1- to 3-fold less efficient at reducing DPPH radical, 0.5-2-fold less efficient at quenching singlet oxygen, and about equally effective at scavenging hydroxyl radical, while allicin was the most effective thiosulfonate in these assays. On the other hand, *in vitro* antioxidant activities of five *Allium* species' extracts, namely of *Allium neveshirense*, *A. sivasicum*, *A. dictyoprosom*, *A. scrodoprosom* subsp. *rotundum* and *A. atroviolaceum* were investigated by Tepe *et al.* (2005). The extracts were screened for their potential antioxidant activities. They reported that non-polar sub-fractions of the extracts did not show any antioxidant potential, while the polar sub-fractions exhibited marked activity. Among the polar ones, the most active one was *A. atroviolaceum*.

In food technology and preservation, meat and meat products are the products generating most concern by the addition of plant extracts for their preservation. Thus, a

major cause of meat product deterioration is oxidative rancidity, and this oxidation of lipids is responsible for changes in its nutritional quality: loss of vitamins and essential amino acids, color, flavor, odor and texture (Gray and Pearson 1987; Gray *et al.* 1996). Various synthetic antioxidants have been utilized to retard the development of rancidity in meat products, and thus extend their shelf life, such as BHT, BHA, and propyl gallate. However, questions regarding the safety of synthetic antioxidants together with consumer's preference have led to increased interest and research on natural antioxidants, and it has shown that spices (sage, red pepper, black pepper, garlic and marjoram) inhibit rancidity, often showing synergism (Madsen and Bertelsen 1995; Karpińska *et al.* 2001).

Aguirrezábal *et al.* (2000) compared the antioxidant effect of garlic with a mixture of nitrate, nitrite and ascorbic acid in dry sausage, and they noted that garlic was as effective as the mixture of additives in inhibiting lipid oxidation. The antioxidant protection of diallyl sulfide (DAS), diallyl disulfide (DADS), S-ethyl cysteine (SEC), N-acetyl cysteine (NAC) in ground beef against lipid oxidation was studied by Yin and Cheng (2003). These authors found that the exogenous addition of these garlic-derived organosulfur compounds significantly delayed both oxymyoglobin and lipid oxidations.

Moreover, pre-seasoning with garlic followed by electron beam irradiation treatment was shown to enhance the quality of beef sirloin steaks (Wong and Kitts 2002). Similarly, the antioxidant effects of different forms of garlic were investigated against lipid oxidation in raw chicken sausage during storage, and the antioxidant activity was also compared to BHA (butylated hydroxyanisole) (Sallam *et al.* 2004). The authors found that the addition of either garlic or BHA delayed lipid oxidation, and fresh garlic showed the highest antioxidant activity followed by garlic powder, BHA and garlic oil.

CONCLUDING REMARKS

The last decade has seen an enormous trend towards plant extracts such as essential oils, volatiles, and other molecules released by the secondary metabolism of plants. However, investigation on the biological activities of *Allium* compounds, as well as other phyto-compounds, and their mechanisms of action is still a major challenge for biochemistry, microbiology and food industries.

The *in vivo* and *in vitro* data reported by the extensive literature showed that thiosulfonates have different degrees of antibacterial and antifungal inhibition, therefore, the findings to date are very important in order to try and compare the sensitivity of species and the different medium where these activities are assessed. Presently, it is difficult to explain the small discrepancy concerning the effects and stability of thiosulfonates, but it may have occurred because they are chemically complex or because their adsorption on living organisms is more stable than free thiosulfonates in a medium. On the other hand, relative to some common antioxidants, thiosulfonates are found to be effective antioxidants in terms of scavenging, and this activity is only for pure allicin. Although thiosulfonates have shown other scavenging ability, this activity was very weak comparatively, and many claims suggest that the antioxidant properties in crude extracts of *Allium* are more likely due to components other than thiosulfonates and related organosulfur compounds, and these compounds remain to be identified. Fortunately, studies on the mechanism of these different actions are now in progress and research is progressing faster than initially expected.

REFERENCES

- Aguirrezábal MM, Mateo J, Domínguez MC, Zumalacárregu JM (2000) The effect of paprika, garlic and salt on rancidity in dry sausages. *Meat Science* 54, 77-81
- Anderson JW (1980) Assimilation of inorganic sulfate into cysteine. In: Mifflin

- BJ (Ed) *The Biochemistry of Plants* (Vol 5), Academic Press, New York, USA, pp 203-223
- Augusti KT** (1990) Therapeutic and medicinal values of onions and garlic. In: Brewster JL, Rabinowitch HD (Eds) *Onions and Allied Crops (Vol III) Biochemistry, Food Science and Minor Crops*, CRC Press, Boca Raton, FL, USA, pp 93-108
- Bayer T, Wagner H, Block E, Grisoni S, Zhao SH, Neszmelyi A** (1989) Zwiebelanaloge: Novel biologically active 2,3-dimethyl-5,6-dithiabicyclo[2.1]hexane 5-oxides from onion. *Journal of the American Chemical Society* **111**, 3085-3086
- Benkeblia N** (2005) Free-radical scavenging capacity and antioxidant properties of some selected onions (*Allium cepa* L.) and garlic (*Allium sativum* L.) extracts. *Brazilian Archives of Biology and Technology* **48**, 753-759
- Benkeblia N** (2004) Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). *Lebensmittel-Wissenschaft und-Technologie* **37**, 263-268
- Block E** (1992) The organosulfur chemistry of the genus *Allium*: Implications for the organic chemistry of sulfur. *Angewandte Chemie* **31**, 1135-1178
- Block E** (1993) Flavor artifacts. *Journal of Agricultural and Food Chemistry* **41**, 692-692
- Block E, Putman D, Zhao SH** (1992a) Allium chemistry: GC-MS analysis of thiosulfinates and related compounds from onion, leek, scallion, shallot, chive, and Chinese chive. *Journal of Agricultural and Food Chemistry* **40**, 243-2438
- Block E, Naganathan S, Putman D, Zhao SH** (1992b) Allium chemistry: HPLC analysis of thiosulfinates from onion, garlic, wild garlic (ramson), leek, scallion, shallot, elephant (great-headed) garlic, chive, and Chinese chive. Uniquely high allyl to methyl ratios in some garlic samples. *Journal of Agricultural and Food Chemistry* **40**, 2418-2430
- Breu W** (1996) *Allium cepa* (onion). Part 1. Chemistry and analysis. *Phyto-medicine* **3**, 293-306
- Cañizares P, Gracia I, Gómez LA, Martín de Argila C, Boixeda D, García A, de Rafael L** (2004) Allyl-thiosulfinates, the bacteriostatic compounds of garlic against *Helicobacter pylori*. *Biotechnology Progress* **20**, 397-401
- Cavaliere EL, Rogan EG** (1992) The approach to understanding aromatic hydrocarbon carcinogenesis. The central role of radical cations in metabolic action. *Pharmacological Therapy* **55**, 183-199
- Cavallito CJ, Bailey JH** (1944a) Allicin, the antibacterial principle of *Allium sativum*. I. Isolation, physical properties and antibacterial action. *Journal of the American Chemical Society* **66**, 1950-1951
- Cavallito C, Bailey JH, Buck JS** (1944b) Allicin, the antibacterial principle of *Allium sativum* II. Its precursor and "essential oil" of garlic. *Journal of American Chemical Society* **67**, 1032-1033
- Cavallito CJ, Buck JS, Suter CM** (1944) Allicin, the antibacterial principle of *Allium sativum*. II. Determination of chemical structure. *Journal of the American Chemical Society* **66**, 1952-1954
- Cruz-Villalon G** (2001) Synthesis of allicin and purification by solid-phase extraction. *Analytical Biochemistry* **290**, 376-378
- Feldberg RS, Chang SC, Kotik AN, Nadler M, Neuwirth Z, Sundstrom DC, Thompson NH** (1988) *In vitro* mechanism of inhibition of bacterial cell growth by allicin. *Antimicrobial Agents and Chemotherapy* **32**, 1763-1768
- Fenwick GR, Hanley AB** (1990) Chemical composition. In: Brewster JL, Rabinowitch HD (Eds) *Onion and Allied Crops*, CRC Press, Boca Raton, Florida, USA, pp 17-31
- Garlic & Health Group** (2007) Special section. *Medicinal and Aromatic Plant Science and Biotechnology* **1**, 1-36
- Gnan SO, Demello MT** (1999) Inhibition of *Staphylococcus aureus* by aqueous goiaba extracts. *Journal of Ethnopharmacology* **68**, 103-108
- Gray JI, Pearson AM** (1987) Rancidity and warmed-over flavor. In: Pearson AM, Dutson TR (Eds) *Advances in Meat Research* (Vol 3), Van Nostrand Co., New York, USA, pp 221-269
- Gray JJ, Goma EA, Buckley DJ** (1996) Oxidative quality and shelf life of meats. *Meat Science* **43** (Suppl), S111-S123
- Halliwel B, Gutteridge JMC, Cross EEJ** (1992) Free radicals, antioxidants, and human disease: where are we now? *Journal of Laboratory and Clinical Medicine* **119**, 598-620
- Han J, Lawson L, Han G, Han P** (1995) A spectrophotometric method for quantitative determination of allicin and total garlic thiosulfinates. *Annals of Biochemistry* **225**, 157-160
- Hirata R, Matsushita S** (1996) Reducing activity level of alliin. *Bioscience, Biotechnology and Biochemistry* **60**, 484-485
- Hsieh PC, Mau JL, Huang SH** (2001) Antimicrobial effect of various combination of plant extracts. *Food Microbiology* **18**, 35-43
- Iida H, Hashimoto S, Miyazawa M, Kameoka H** (1983) Volatile flavor components of nira (*Allium tuberosum* Rottl.). *Journal of Food Science* **48**, 660-661
- Imai J, Ide N, Nagae S, Moriguchi T, Matsuura H, Itakura Y** (1994) Antioxidant and radical scavenging effects of aged garlic extract and its constituents. *Planta Medica* **60**, 417-420
- Karpińska M, Borowski J, Danowska-Oziewicz M** (2001) The use of natural antioxidants in ready-to-serve food. *Food Chemistry* **72**, 5-9
- Kim SM, Kubota K, Kobayashi A** (1997) Antioxidative activity of sulfur-containing flavor compounds in garlic. *Bioscience, Biotechnology and Biochemistry* **61**, 1482-1485
- Krest I, Glodek J, Keusgen M** (2000) Cysteine sulfoxides and alliinase activity of some *Allium* species. *Journal of Agricultural and Food Chemistry* **48**, 3753-3760
- Kourounakis PN, Rekká EA** (1991) Effect on active oxygen species of alliin and *Allium sativum* (garlic) powder. *Research Communication in Chemistry Pathology and Pharmacology* **74**, 249-252
- Kubec R, Hrbacova M, Musah RA, Velisek J** (2004) Allium discoloration: Precursors involved in onion pinkening and garlic greening. *Journal of Agricultural and Food Chemistry* **52**, 5089-5094
- Kubec R, Velisek J** (2007) Allium discoloration: The color-forming potential of individual thiosulfinates and amino acids: Structural requirements for the color-developing precursors. *Journal of Agricultural and Food Chemistry* **55**, 3491-3497
- Lancaster JE, Collin HA** (1981) Presence of alliinase in isolated vacuoles and of alkyl cysteine sulfoxides in the cytoplasm of bulbs of onion (*Allium cepa*). *Plant Science Letters* **22**, 169-176
- Lanzotti V** (2006) The analysis of onion and garlic. *Journal of Chromatography A* **1112**, 3-22
- Lawson LD** (1996) The composition and chemistry of garlic cloves and processed garlic. In: Koch HP, Lawson LD (Eds) *Garlic: The Science and Therapeutic Application of Allium sativum and Related Species*, Williams and Wilkins, Baltimore, USA, pp 37-107
- Lawson LD, Hughes BG** (1992) Characterization of the formation of allicin and other thiosulfinates from garlic. *Planta Medica* **58**, 345-350
- Lawson LD, Wang Z-Y, Hughes BG** (1991a) γ -Glutamyl-S-alkylcysteines in garlic and other *Allium* species: precursors of age-dependent *trans*-1-propenyl thiosulfinates. *Journal of Natural Products* **54**, 436-444
- Lawson LD, Wang Z-Y, Hughes BG** (1991b) Identification and HPLC quantitation of the sulfides and dialk(en)yl thiosulfinates in commercial garlic products. *Planta Medica* **57**, 363-370
- López-Díaz TM, González CJ, Moreno B, Otero A** (2002) Effect of temperature water activity pH and some antimicrobials on the growth of *Penicillium olsonii* isolated from the surface of Spanish fermented meat sausage. *Food Microbiology* **19**, 1-7
- Madsen HL, Bertelsen G** (1995) Spices as antioxidants. *Trends in Food Science and Technology* **6**, 271-277
- Mondy N, Duplat D, Christides JP, Arnault I, Auger J** (2002) Aroma analysis of fresh and preserved onions and leek by dual solid-phase microextraction-liquid extraction and gas chromatography-mass spectrometry. *Journal of Chromatography A* **963**, 89-93
- Mütsch-Eckner M, Meier B, Wright AD, Sticher O** (1992) Gamma-glytamyl peptides from *Allium sativum* bulbs. *Phytochemistry* **31**, 2389-2391
- Nagawa R, Iwate N, Ishikawa K, Fukuda H, Fujimi T, Suzuki A** (1996) Inhibition of microbial growth by ajoene, a sulfur-containing compound derived from garlic. *Applied and Environmental Microbiology* **62**, 4238-4242
- Phay N, Higashiyama T, Tsuji M, Matsuura H, Fukushi Y, Yokota A, Tomita F** (1999) An antifungal compound from roots of welsh onion. *Phytochemistry* **52**, 271-274
- Prasad K, Laxdal VA, Yu M, Raney BL** (1995) Antioxidant activity of allicin, an active principle in garlic. *Molecular and Cellular Biochemistry* **148**, 183-189
- Rabinkov A, Miron T, Konstantinovski, L, Wilchek, M, Mirelman D, Weiner L** (1998) The mode of action of allicin: trapping of radicals and interaction with thiol containing proteins. *Biochimica et Biophysica Acta* **1379**, 233-244
- Raghuandana RR, Srinivasa RS, Venkatarman PR** (1946) Investigations on plant antibiotics. I. Studies on allicin, the antibacterial principle of *Allium sativum* (garlic). *Journal of Scientific and Industrial Research* **1B**, 31-35
- Randle WM** (1997) Onion flavor chemistry and factors influencing flavor intensity. *ACS Symposium Series* **660**, 41-52
- Randle WM, Block E, Littlejohn MH, Putman D, Bussard ML** (1994) Onion (*Allium cepa* L.) thiosulfinates respond to increasing sulfur fertility. *Journal of Agricultural and Food Chemistry* **42**, 2085-2088
- Rekká EA, Kourounakis PN** (1994) Investigation of the molecular mechanism of the antioxidant activity of some *Allium sativum* ingredients. *Pharmazie* **49**, 539-540
- Renzenberg H** (1982) Glutathione metabolism and possible biological roles in higher plants. *Phytochemistry* **21**, 2771-2781
- Rose P, Whiteman M, Moore PK, Zhu YZ** (2005) Bioactive S-alk(en)yl cysteine sulfonamide metabolites in the genus *Allium*: The chemistry of potential therapeutic agents. *Natural Products Reports* **22**, 351-368
- Sallam KM, Ishioroshi M, Samejima K** (2004) Antioxidant and antimicrobial effects of garlic in chicken sausage. *Lebensmittel-Wissenschaft und-Technologie* **37**, 849-855
- Salvemini D, Botting R** (1993) Modulation of platelet function by free radicals and free-radical scavengers. *Trends in Pharmacological Science* **14**, 36-42
- Semmler FW** (1892) The essential oil of garlic. *Archiv der Pharmazie* **230**, 434-448
- Shams-Ghahfarokhia M, Shokoohamiria MR, Amirrajaba N, Moghadasia B, Ghajarib A, Zeinic F, Sadeghid G, Razzaghi-Abyanehd M** (2006) *In vitro* antifungal activities of *Allium cepa*, *Allium sativum* and ketoconazole against some pathogenic yeasts and dermatophytes. *Fitoterapia* **77**, 321-323

- Siegers C-P, Röbbke A, Pentz R** (1999) Effects of garlic preparations on superoxide production by phorbol ester activated granulocytes. *Phytomedicine* **6**, 13-16
- Sinha NK, Guyer DE, Gage DA, Lira CT** (1992) Supercritical carbon dioxide extraction of onion flavors and their analysis by gas chromatography-mass spectrometry. *Journal of Agricultural and Food Chemistry*, **40**, 842-845
- Sivam GP, Lampe JW, Ulness B, Swanzy SR, Potter JD** (1997) *Helicobacter pylori* in vitro susceptibility to garlic (*Allium sativum*) extract. *Nutrition and Cancer* **27**, 118-121
- Stoll A, Seebeck E** (1948) Über alliin, die genuine mettersubstanz des knoblauchols. 1. Mitteilung über *Allium* substanzen. *Helvetica Chimica Acta* **31**, 189-210
- Tepe B, Sokmen B, Akpulata AH, Sokmena A** (2005) *In vitro* antioxidant activities of the methanol extracts of five *Allium* species from Turkey. *Food Chemistry* **92**, 89-92
- Virtanen AI** (1965) Studies on organic sulphur compounds and other labile substance in plants. *Phytochemistry* **4**, 207-228
- Ward PM, Fasitsas S, Katz SE** (2002) Inhibition, resistance development, and increased antibiotic and antimicrobial resistance caused by neutraceuticals. *Journal of Food Protection* **65**, 528-533
- Wertheim T** (1844) *Protozoology*, Tindall & Cassel, Baillière, London, UK, 251 pp
- Whitemore BB, Naidu AS** (2000) Thiosulfinates. In: Naidu AS (Ed) *Natural Food Antimicrobial Systems*, CRC Press, Boca Raton, Florida, USA, pp 265-380
- Wong PYY, Kitts DD** (2002) The effects of herbal pre-seasoning on microbial and oxidative changes in irradiated beef steaks. *Food Chemistry* **76**, 197-205
- Xiao H, Parkin KL** (2002) Antioxidant functions of selected *Allium* thiosulfinates and S-alk(en)yl-L-cysteine sulfoxides. *Journal of Agricultural and Food Chemistry* **50**, 2488-2493
- Yamada Y, Azuma K** (1977) Evaluation of the *in vitro* antifungal activity of allicin. *Antimicrobial Agents and Chemotherapy* **11**, 743-749
- Yin M-C, Cheng W-S** (2003) Antioxidant and antimicrobial effects of four garlic-derived organosulfur compounds in ground beef. *Meat Science* **63**, 23-28
- Yin M-C, Cheng W-S** (1998) Antioxidant activity of several *Allium* members. *Journal of Agricultural and Food Chemistry* **46**, 4097-4101
- Yin M-C, Tsao M-S** (1999) Inhibitory effect of seven *Allium* plants upon three *Aspergillus* species. *International Journal of Food Microbiology* **49**, 49-56
- Yoshida H, Iwata N, Katsuzaki H, Naganawa R, Ishikawa K, Fukuda H, Fujino T, Suzuki A** (1998) Antimicrobial activity of a compound isolated from an oil-macerated garlic extract. *Bioscience, Biotechnology and Biochemistry* **62**, 1014-1017
- Ziegler SJ, Sticher O** (1989) HPLC of S-alk(en)yl-L-cysteine derivatives in garlic. *Planta Medica* **55**, 373-378