Chapter 4
Antimicrobial Properties of Organosulfur Compounds

Osman Sagdic and Fatih Tornuk

Abstract Organosulfur compounds are defined as organic molecules containing one or more carbon-sulfur bonds. These compounds are present particularly in *Allium* and *Brassica* vegetables and are converted to a variety of other sulfur containing compounds via hydrolysis by several herbal enzymes when the intact bulbs are damaged or cut. Sulfur containing hydrolysis products constitute very diverse chemical structures and exhibit several bioactive properties as well as antimicrobial. The antimicrobial activity of organosulfur compounds has been reported against a wide spectrum of bacteria, fungi and viruses. Despite the wide antimicrobial spectrum, their pungent flavor/odor is the most considerable factor restricting their common use in foods as antimicrobial additives. However, meat products might be considered as the most suitable food materials in this respect since *Allium* and *Brassica* vegetables especially garlic and onion have been used as flavoring and preservative agents in meat origin foods. In this chapter, the chemical diversity and *in vitro* and *in food* antimicrobial activity of the organosulfur compounds of *Allium* and *Brassica* plants are summarized.

Keywords Organosulfur compounds • Garlic • Onion • *Allium* • *Brassica* • Thiosulfinates • Glucosinolates

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4.1 Introduction

Phytochemicals are defined as non-nutrient bioactive plant origin compounds naturally present in fruits, vegetables and whole grains (Liu 2004). Dietary intake of phytochemicals has been strongly linked to reduced risk of major chronic diseases such as cancer, diabetes and cardiovascular diseases (Liu 2003). Use of fruit and vegetables as health remedies traditionally is very common in a considerable part of world population (Salama and Marraiki 2010).

One group of sulfur forming in plants is known as organosulfur compounds. Organosulfur compounds, defined as organic molecules containing one or more carbon-sulfur bonds, are one of the groups present in foods as natural preservatives (Cremlyn 1996). These compounds are valued not only due to their rich and varied chemistry, but also for their many important biological properties (Polshettiwar and Kaushik 2004).

The families *Alliaceae* and *Brassicaceae,* two members of the plant kingdom, contain sulfur containing materials which possess biological properties (Stoewsand 1995). Today, it is well established that these vegetables are natural sources of a group of phytochemicals known as organosulfur compounds. *Allium* and *Brassica* vegetables especially garlic and onion have been known to be used as health remedies in ancient civilizations. Therapeutic application of these vegetables for the prevention of various diseases such as cancer and cardiovascular diseases has been well-studied (Vazquez-Prieto and Miatello 2010).

Organosulfur compounds in *Allium* and *Brassica* plants are called thiosulfinates and glucosinolates, respectively. These phytochemicals are characterized by the properties that give the plants their specific flavors and odors. Thiosulfinates and glucosinolates are also converted to various new sulfur containing materials which exhibit a variety of bioactive properties such as antimicrobial, anticarcinogenic, antitumor and pesticidal etc. via a number of biosynthetic reactions. This chapter outlines the antimicrobial properties of the organosulfur compounds present in *Allium* and *Brassica* plants and their derivatives on diverse microorganisms including bacteria, fungi and viruses.

4.2 Thiosulfinates

The major sources of organosulfur compounds are *Allium* vegetables. The *Allium* genus includes approximately 600 species, the most widely consumed of which are onions (*Allium cepa*), garlic (*Allium sativum*), leeks (*Allium porrum*), chives (*Allium schoenoprasum*), and shallots (*Allium ascalonicum*) (Bianchini and Vainio 2001; Benkeblia and Lanzotti 2007). Among the *Allium* species, onion and garlic are the oldest and most commonly cultivated plants by different cultures (Lanzotti 2006).

The earliest records of onion and garlic date back to the sixteenth century BC when several medicinal formulas based on garlic and onions as a curative agent for a variety of diseases such as heart problems, headache and tumors were mentioned

Table 4.1 Approximate composition of garlic and onion (Lawson 1996; Rahman 2003; Dini et al. 2008)

Components	Garlic (%)	Onion
Moisture	62–68	88.6–92.8%
Carbohydrates	26–30	5.2–9.0%
Proteins	1.5–2.1	0.9–1.6%
Ash	–	0.6%
γ-Glutamylcysteines	0.5–1.6	–
Lipids	0.1–0.2	Trace–0.2%
Fiber	1.5	1.7%
Total sulfur compounds	1.1–3.5	–
Sulfur	0.23–0.37	50–51 mg g^{-1}
Calcium	–	190–540 mg g^{-1}
Nitrogen	0.6–1.3	–
Minerals	0.7	–
Vitamins	0.015	100 mg g^{-1}
Saponins	0.04–0.11	–

– no data

in the Codex Ebers, the Egyptian medical papyrus (Block 1985). The Sumerians, Indians, Romans, Ancient Egyptians and Greeks are also all known to have consumed garlic and onions for thousands of years (Ahmad 1996). In China, tea made from garlic and onion has been used as a remedy for fever, headache, cholera and dysentery; garlic was utilized as an antiseptic in the treatment of gangrene in the World Wars (Corzo-Martinez et al. 2007). Garlic and onion are still used in folk medicine in all over the world. At present, these vegetables are among the most cultivated plants in the world with production amounts of 9 and 55 million tons per year, respectively (Peter 2000; FAO 2004).

4.2.1 Occurrence and Chemistry of Thiosulfinates

Not only do people consider *Allium* vegetables important because of their characteristic flavor that contributes to the savoriness of various dishes in world cuisine, they are also aware of the nutritional potential of these vegetables since they are rich in nutrients such as high levels of phosphor, potassium, zinc, vitamins A, C and D, folic acid, calcium and selenium etc. is also considered by people (Peter 2000; Rahman 2003). They are also rich sources of phenolic compounds (Vinson et al. 1998). Table 4.1 shows the approximate composition of fresh garlic and onion bulbs.

As in most fresh foods, water is the main constituent of *Allium* vegetables (Table 4.1). Although garlic and onion contain similar compounds to a certain extent, the amounts of major components in onion are rather low compared to those of garlic. Fresh garlic contains a moisture level of 62–68% (Table 4.1), while it is

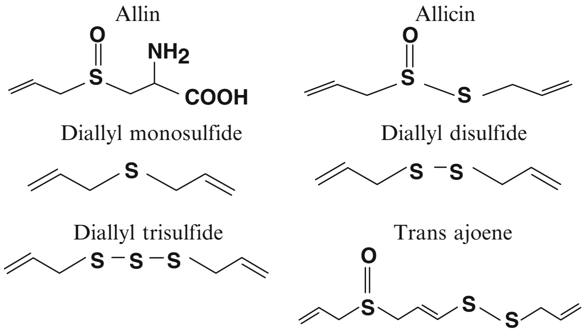

Fig. 4.1 Chemical structures of alliin and its main hydrolysis products

even higher (88.6–92.8% of fresh weight) in onion than that in garlic (Peter 2000). When considering the biological importance of these vegetables, sulfur containing compounds attract the attention. As seen Table 4.1, the proportion of total sulfur compounds in garlic is 1.1–3.5%, meanwhile its amount in onion is only one third of that found in garlic (Benkeblia 2004).

Thiosulfinates are the most studied compounds among the active constituents of *Allium* vegetables. Their main structures were first reported by Wertheim and later by Semmler in garlic and onion oils in the nineteenth century (Benkeblia and Lanzotti 2007). Block (1992) identified eight different thiosulfinates from nine *Allium* vegetables including garlic and onion by HPLC analysis. Garlic was found to be the richest source of thiosulfinates with amounts ranging from 15 μmol/g (garlic grown at low temperature, 21°C) to 53 μmol/g (elephant garlic). The thiosulfinate contents of the onion species were all <0.35 μmol/g.

Intact garlic bulbs possess certain sulfur containing compounds known as S-alk(en)yl-L-cysteine sulphoxides (ACSOs). Alliin (S-allylcysteine sulfoxide), the major ACSO of these vegetables is a colorless, odorless water soluble amino acid so long as the bulb is intact or undamaged (Corzo-Martinez et al. 2007). These are present in all *Allium* spp. constituting 1–5% of dry weight of the plant. However, when the cells are damaged, the enzyme alliinase (or alliin lyase) releases and converts alliin into certain volatile and reactive components called thiosulfinates (Block 1985). Allicin (allyl 2-propene thiosulfinate) is the most abundant thiosulfinate produced (70% of overall garlic thiosulfinates), whereas allyl methyl thiosulfinate (AMTS) is the second (18%) and additional thiosulfinates are also formed in lower concentrations (Mazza 2002). Since the thiosulfinates as well as allicin are very unstable, they readily undergo various transformations to form more stable compounds, to alkyl sulfides such as di- and trisulfides, allyl sulfides, vinyl dithiins, ajoenes and mercaptocysteines (Shi et al. 2005). These compounds can be yielded in a variety of reactions such as dehydration, rearrangement, condensation, Diels-Alder reaction, hydrolysis and pyrolysis depending on conditions and still exhibit biological activity (Lanzotti 2006). Figure 4.1 illustrates chemical structures of alliin and sulfur containing compounds forming from alliin in *Allium* vegetables. Onions also produce these sulfides but only at the level of 4 mg/100 g of fresh weight which means barely any amount compared to garlic (Shi et al. 2005).

4.2.2 Antimicrobial Properties of Thiosulfinates

Even though it is common knowledge that *Allium* vegetables contain a variety of functional components, the beneficial aspects of the *Allium* species have been mainly attributed to specific organosulfur compounds. In addition to the traditional use of *Allium* vegetables for thousands of years, scientific studies carried out on these vegetables, their extracts or specific organosulfur compounds have also proved their diverse pharmacological and biological benefits such as antimicrobial, antioxidative, antitumor and antiasthmatic (Lanzotti 2006), while effects of various *Allium* products such as garlic oil and methanol extract to inhibit rumen fermentation and methane production were also discussed (Patra and Saxena 2010). In general, cooked or waited vegetables are recommended for human consumption due to their prolonged protective effect resulting from the increased level of bioactive compounds in the vegetal tissues (Goncagul and Ayaz 2010).

Allium vegetables have been shown to have antibacterial, antifungal, antiviral and antiprotozoal activities. This activity has been attributed to thiosulfinates and to the other sulfur containing compounds present in these vegetables. It has been found that the main antimicrobial agents are breakdown products of allicin such as diallyl disulfide (DADS), diallyl trisulfide (DATS), diallyl sulfide (DAS) and ajoene, antimicrobial strength of which is higher than that of allicin (Corzo-Martinez et al. 2007). It is obvious that the enzyme alliinase plays a critical role in antimicrobial action since thiosulfinates are generated following the activation of this enzyme after the vegetable tissues are injured. Inactivation of alliinase as a result of prolonged heating causes the failure of antimicrobial activity (Lawson 1996). In general, each sulfur compound has individually been found to exhibit weaker antimicrobial activity as compared to the crude extracts of *Allium* spp. (Lawson 1998). It is also known that some proteins, saponins and phenolic compounds can contribute to antimicrobial activity (Griffiths et al. 2002).

As in other natural antimicrobial compounds of plant origin (spice essential oils, phenolics and other plant extracts etc.), these compounds may generally show their antimicrobial activity by altering the permeability of microbial cell walls and the replacing of intracellular and extracellular materials with each other. Moreover, the main mechanism involved in the antimicrobial effect is assumed to be the inhibition of thiol-containing enzymes in microorganisms by the rapid reaction of thiosulfinates with thiol groups (Ankri and Mirelman 1999).

4.2.2.1 Antibacterial Activity

The antibacterial activities of the *Allium* species have been well documented. Historically, Louis Pasteur first noted the antibacterial activity of garlic (Block et al. 1993). By the 1900s, substances showing antibacterial action were beginning to be identified. Allicin was the first organosulfur compound to be isolated and defined as an antibacterial agent in garlic (Cavallito and Bailey 1944). The antibacterial activity of allicin is remarkably more bacteriostatic than bactericidal. Allicin in concentrations of 1:85.000 in broth is bactericidal against a wide variety of Gram-negative and Gram-positive organisms (Cavallito and Bailey 1944). Feldberg et al. (1988) suggested that the antibacterial action of allicin caused a quick and complete inhibition of RNA biosynthesis and additionally a partial inhibition of DNA and protein synthesis. In addition to allicin, other thiosulfinates have also been found to exhibit antibacterial activity. A great number of *in vitro* and *in situ* studies have been made in order to reveal the antibacterial properties of individual organosulfur compounds or various extracts of *Allium* vegetables.

It has been proven that the *Allium* species can inhibit both Gram (+) and Gram (−) bacteria as well as toxin production. Table 4.2 summarizes the *in vitro* studies made to identify antibacterial activities of *Allium* vegetables or their components. Garlic, the most studied vegetable, has been reported to be effective against strains of *Pseudomonas, Proteus, Escherichia coli, Staphylococcus aureus, Klebsiella, Salmonella, Micrococcus, Bacillus subtilis, Mycobacterium* and *Clostridium*. It may also inhibit beneficial intestinal microflora, but the potentially harmful species of the *Enterobacteriaceae* family are more sensitive to garlic compounds especially allicin (Corzo-Martinez et al. 2007). The sensitivity of *Listeria monocytogenes,* a psychrotrophic pathogen which can be found in foods, was measured against various extracts of *Allium vinelae* (wild garlic) by the disc diffusion method and methanol extract was found to be more active than ethanol and *n*-hexane extracts (Sagun et al. 2006). The efficiency of garlic shoot juice on *L. monocytogenes* was also shown (Kim et al. 2007). General lysis of the cytoplasm, lysis of the cell wall and cellular swelling were observed in bacterial cells by scanning electron microscopy in the same study. The inhibitory effect of garlic against *S. aureus, Salmonella* Typhi*, E. coli* and *L. monocytogenes* was measured using the turbidity method in another study. 80% inhibition level for *E. coli, S.* Typhi*, S. aureus* and *L. monocytogenes* were provided in the presence of garlic with levels of 3.95%, 7.0%, 5.0% and 8.8% in *in vitro* conditions, respectively (Kumar and Berwal 1998). Allicin and other *Allium* products were also considered as potential control agents for oral pathogens, causes of the dental diseases, such as *Streptococcus mutans, S. sobrainus, Actinomyces oris, Act. viscosus* and *Porphyromonas gingivalis* (Bakri and Douglas 2005; Bachrach et al. 2011).

As can be seen in Table 4.2, the *Bacillus* species are the most sensitive bacteria against *Allium* compounds or extracts while ajoene has the highest antibacterial effect. The sensitivity of *E. coli* to allicin is higher compared to other sulfur containing compounds. It can also be seen that *P. aeruginosa* is the most resistant species against all organosulfur compounds in general. However, the different minimum inhibitory concentration (MIC) levels reported by researchers may arise from extraction method, final thiosulfinate concentration in the extracts and the method applied for the assessment of the inhibitory effect (Benkeblia and Lanzotti 2007).

Studies on the antibacterial activity of thiosulfinates have mainly focused on garlic and onion. These studies showed that garlic was more effective in bacterial inhibition than onion since the sulfur compound content of garlic is four times higher compared to the level found in onion. Furthermore, both onion and garlic are

Table 4.2 *In vitro* antibacterial activity of *Allium* vegetables or their components

Antibacterial organosulfur compounds	Bacteria	MIC value (µg/mL)	Reference
Ajoene	*Bacillus cereus*	4	Naganawa et al. (1996)
	Staphylococcus aureus	16	
	Escherichia coli	116	
Z-ajoene	*B. cereus*	5	Yoshida et al. (1998)
	B. subtilis	5	
	S. aureus	20	
	E. coli	100	
	P. aeruginosa	>500	
Allicin[a]	*S. aureus*	12	Ankri and Mirelman (1999)
	E. coli	15	
Garlic oil	*P. aeruginosa*[b]	16–20	Tsao and Yin (2001)
DAMS		80–88	
DADS		64–72	
DATS		32–36	
DATTS		40893	
Garlic oil	*K. pneumoniaee*[b]	24–48	Tsao and Yin (2001)
DAMS		96–104	
DADS		72–80	
DATS		40–48	
DATTS		20–24	
A. atroviolaceum[c]	*B. subtilis*	0.31	Chehregani et al. (2007)
A. eriophyllum var. *laceratum*[c]		0.31	
A. scabriscapum[c]		0.75	
Z-10-devinylajoene	*B. cereus*	60	Yoshida et al. (1999)
	B. subtilis	50	
	S. aureus	70	
	E. coli	>100	
	P. aeruginosa	>100	
DAMS[d]	*Helicobacter pylori*	2.07–4.15	O'gara et al. (2000)
DADS[d]		100	
DATS[d]		13–25	
DATTS[d]		3–6	
Allicin[d]		6–12	
Garlic oil[e]		8–32	
Garlic powder[e]		250–500	
Z-10-devinylajoene	*B. cereus*	5	Yoshida et al. (1998)
	B. subtilis	5	
	S. aureus	30	
	E. coli	125	
	P. aeruginosa	170	

(continued)

Table 4.2 (continued)

Antibacterial organosulfur compounds	Bacteria	MIC value (µg/mL)	Reference
Fresh Garlic	*Vibrio* ssp.[f]	0.16–0.31	Kasornchandra et al. (2005)
	E. coli	0.31	

MIC Minimum inhibitory concentration, *DAMS* Diallyl monosulfide, *DADS* Diallyl disulfide, *DATS* Diallyl trisulfide, *DATTS* Diallyl tetrasulfide

[a] The results are 50% lethal dose concentrations of allicin

[b] Total numbers of clinical isolates of *P. aeruginosa* and *K. pneumoniae* were 123 and 114, respectively

[c] Aqueous extracts of the plant bulbs and flowers were used for testing the antibacterial activities of *A. atroviolaceum* – *A. eriophyllum* var. *laceratum* and *A. scabriscapum*, respectively

[d] Three strains of *H. pylori* were tested

[e] Eight strains of *H. pylori* were tested

[f] Seven strains of *Vibrio* ssp. were tested

the most commonly used food ingredients for flavor enhancement purposes. Meat and dairy products are especially favorable for flavoring by these materials (Sagun et al. 2006; Salem et al. 2010). In addition to food flavoring, a number of biological effects, such as the antibacterial activity of *Allium* species in food systems, have been proven.

The addition of garlic essential oil to minced beef contributed to its storage stability by decreasing native bacterial counts (APC, *Enterobacteriaceae,* coliform bacteria and *S. aureus*) (Salem et al. 2010). However, all garlic oil supplements (0.5%, 1.0% and 1.5%) demonstrated fair enhancements of sensory attributes in storage periods of 3 days, while the samples treated with 0.5% garlic oil demonstrated the lowest sensory enhancement after 6 days. Wong and Kitts (2002) showed that the pre-seasoning of steak with garlic caused a reduction in the psychrotroph population in both non-irradiated and irradiated steaks after refrigerated storage for 2 weeks.

Yin and Cheng (2003) revealed the antibacterial properties of four thiosulfinates (diallyl sulfide, DADS, s-ethyl cysteine and n-acetyl cysteine) in minced beef. APC was significantly reduced and the growth of five inoculated pathogenic bacteria, *S.* Typhimurium*, E. coli* O157:H7, *L. monocytogenes*, *S. aureus* and *Campylobacter jejuni* was inhibited by the presence of DAS and DADS in ground beef. Park et al. (2008) reported an antimicrobial effect which inhibited the growth of APC and *Enterobacteriaceae* in pork belly and loin cuts by the injection of solutions containing 5% of garlic or onion powder into the cuts and the inhibition was superior compared to sodium ascorbate injection. Aydin et al. (2007) investigated the antimicrobial effect of chopped garlic in ground beef and raw meat balls (cig kofte, a traditional Turkish raw meat product). The addition of garlic at 5% or 10% to the raw meatball mix decreased the microbial count, in terms of APC and yeast and mold counts. However, it was not as effective in ground beef as much as it was in raw meat ball mix.

Various garlic products, garlic oil, garlic powder and fresh garlic were investigated for their antibacterial efficiency in chicken sausage. The addition of fresh garlic (30 g/kg) or garlic powder (9 g/kg) significantly reduced the APC and, subsequently,

Table 4.3 *In vitro* antifungal activity of thiosulfinates

Antifungal organosulfur compounds	Fungi	MIC value (μg/mL)	Reference
Ajoene	*A. niger*	16.6	Yoshida et al. (1987)
	Candida albicans	7.6	
Ajoene	*C. albicans*	13	Naganawa et al. (1996)
	S. cerevisiae	12	
Allicin	*A. niger*	30.9	Yoshida et al. (1987)
	C. albicans	17.3	
Z-10-devinylajoene	*S. cerevisiae*	80	Yoshida et al. (1999)
Z-ajoene	*S. cerevisiae*	20	Yoshida et al. (1998)
Z-10-devinylajoene	*S. cerevisiae*	75	Yoshida et al. (1998)
Allicin	*C. albicans*[a]	0.3–0.8	Ankri and Mirelman (1999)
	C. parapsilosis	0.15	

MIC Minimum inhibitory concentration
[a]Two different isolates of *Candida albicans* were tested

the shelf-life of the product was extended to 21 days. However, the addition of garlic oil resulted in no additional reduction in APC when compared with control samples (Sallam et al. 2004).

4.2.2.2 Antifungal Activity

The importance of fungi is highlighted by their harmful effects, such as food spoilage or toxin production as well as by their beneficial properties such as food fermentation. The antifungal activity of thiosulfinates has been extensively reported by a variety of *in vitro* and *in vivo* studies. Yamada and Azuma (1977) first reported the antifungal activity of allicin against *Candida, Cryptococcus, Trichophyton, Epidermophyton* and *Microsporum*. These researchers also stated that the MIC of allicin against various pathogenic fungi was affected considerably by differences in experimental conditions, e.g., incubation time, inoculum size, type of medium and medium pH. Moreover, alike in antibacterial activity, the breakdown products of allicin, including DATS, DADS, DAS and ajoene are the major active antifungal agents of onion and garlic extracts and have a greater antifungal effect than allicin (Tansey and Appleton 1975; Corzo-Martinez et al. 2007). Table 4.3 lists the results of a number of *in vitro* studies showing the antifungal properties of thiosulfinates in *Allium* vegetables.

Several models for mode of antifungal action of thiosulfinates were suggested by various researchers. Yamada and Azuma (1977) reported that allicin in the concentration of 3.13 μg/mL inhibited swelling and germination of fungi spores in nutritious media. Barone and Tansey (1977) showed that garlic and allicin suppressed *C. albicans* cell metabolism by the inactivation of proteins, competitive inhibition of sulfhydryl compounds, or by the noncompetitive inhibition of enzyme function by oxidation. In another study, the deformation and distortion of yeast cells, cell collapse and cytoplasmic debris were observed in cells grown in the presence of garlic aqueous extract (Ghannoum 1988). Adetumbi et al. (1986) proposed that garlic extract inhibited the protein and nucleic acid synthesis of *C. albicans* in parallel with growth inhibition, but inhibited lipid synthesis completely.

The antifungal effects of *Allium* extracts have also been reported by many researchers. Yin and Tsao (1999) found that garlic bulb extract exhibited the best inhibitory effect among the extracts of seven different *Allium* vegetables on *Aspergillus niger, A. flavus* and *A. fumigatus* with the MIC values of 35, 75 and 104 μg/mL, respectively. Benkeblia (2004) showed the dose-dependent *in vitro* inhibition of five different essential oil extracts of three types of garlic and onion on *A. niger, Penicillium cyclopium* and *Fusarium oxysporum*. Lower concentrations of onion extracts (50 and 100 mL/L) did not cause a significant inhibitory effect compared to control. Shams-Ghahfarokhi et al. (2006) also investigated the antifungal activities of aqueous extracts of garlic and onion on *Malassezia furfur* (25 strains), *C. albicans* (18 strains), and other *Candida* spp. (12 strains) as well as 35 strains of various dermatophyte species. They showed that the extracts were able to inhibit the growth of all fungi tested in a dose-dependent manner. The activity of aqueous garlic extract against *M. furfur, C. albicans*, other *Candida* spp. and the dermatophytes was 64, 8, 4 and 32–128 fold that of the aqueous onion extract, respectively.

Heated garlic as well as fresh garlic and mustard is already used in some foods as preservatives. Kim and Kyung (2003) investigated the antifungal activity of heated garlic and alliin against different strains of *C. albicans, C. utilis*, *Saccharomyces cerevisiae*, *Pichia membranifaciens, Zygosaccharomyces bisporus* and *Z. rouxii*. Alliin heated in distilled water showed an antifungal activity pattern similar to that of heated garlic, suggesting that the compound(s) thermally generated from alliin were the main antifungal compound(s) of heated garlic. The antifungal activity was increased as the time of heating increased up to 45 min at 121°C, and the activity did not change when garlic was further heated for up to 120 min.

4.2.2.3 Antiviral Activity

Relatively few studies have shown antiviral properties of thiosulfinates. Weber et al. (1992) tested the antiviral potentials of not only fresh garlic extract but also its sulfur components, allicin, allyl methyl thiosulfinate, methyl allyl thiosulfinate, ajoene, alliin, deoxyalliin, DADS, and DATS against selected viruses including, herpes simplex virus type 1, herpes simplex virus type 2, parainfluenza virus type 3, vaccinia virus, vesicular stomatitis virus, and human rhinovirus type 2. The order for virucidal activity was generally found as: ajoene≥allicin≥allyl methyl thiosulfinate≥methyl allyl thiosulfinate while no activity was observed for alliin, deoxyalliin, DADS or DATS. Ajoene, allyl alcohol and DADS were shown to be active against HIV (Human immunodeficiency virus) -infected cells (Tatarintsev et al. 1992; Shoji et al. 1993). Chen et al. (2011) studied the antiviral activity of the powders of five *Allium* plants (garlic, onion, leek, shallot and green onion) and allicin against two adenovirus isolates (ADV41 and ADV3). They found that shallots exhibited the highest level of antiviral activity for both ADV41 and ADV3, followed by garlic and onions. Shallots exhibited the highest level of antiviral activity against ADV3 and ADV41 infection from 0 to 2 h, during the early period of virus replication.

4.2.2.4 Antiparasitic Activity

The antiparasitic effects of freshly crushed garlic have been known since ancient times. Chinese people traditionally use the alcoholic extract of garlic cloves to cure intestinal diseases. Only a few reports are available regarding the antiparasitic activity of *Allium* vegetables or their sulfur compounds. It was reported that allicin (30 μg/mL) could efficiently inhibit the growth of some such as *Giardia lamblia*, *Leishmania major, Leptomonas colosoma* and *Crithidia fasciculate* (Ankri and Mirelman 1999). Ankri et al. (1997) also showed that allicin inhibited cysteine proteinases and the cytopathic effects of *Entamoeba histolytica*. Lun et al. (1994) tested the antiparasitic activity of DATS, known as dasuasu in China, against several important protozoal parasites *in vitro*. The IC_{50} (concentration to inhibit metabolism or growth of parasites by 50%) for *Trypanosoma brucei brucei, T. b. rhodesiense, T. b. gambiense, T. evansi, T. congolense* and *T. equiperdum* was found in the range of 0.8–5.5 μg/mL. IC_{50} values were 14 and 59 μg/mL for *E. histolytica* and *G. lamblia*, respectively.

4.3 Glucosinolates

Glucosinolates (GLSs) are a class of organic compounds which are formed from glucose and an amino acid and contain sulfur and nitrogen (Du and Halkier 1998). Cruciferous or *Brassica* vegetables are rich sources of GLSs and all the members of these plants contain these organosulfur compounds naturally (Song et al. 2005; Higdon et al. 2007). However, GLSs do not only exist in crucifers; at least 500 non-cruciferous species were reported to contain one or more forms of GLSs (Fahey et al. 2001). Similar to cruciferous vegetables, many plants belonging to other plant families such as *Capparaceae* also contain GLSs (Whitmore and Naidu 2000).

Brassicaceae is a large family including 338 genera and 3,709 species (Warwick et al. 2006). Most cultivated *Brassica* species include mustards, cabbage (derived from *Brassica oleracea*, Capitata, Pekinensis, Chinensis and Acephala groups), cauliflower (derived from *B. oleracea* var. *botrytis*), Brussels sprouts (*B. oleracea* var. *gemmifera* Zenker), broccoli (derived from *B. oleracea*), kohlrabi (*B. oleracea* L. var. *gongylodes* L.), kale (*B. oleracea* L. var. a*cephala group ornamental*), horseradish (*Armoracia rusticana* G. M. Sch.), wasabi or Japanese horseradish (*Wasabia japonica* Matsum or *Wasabia tenuis* Matsum), and radishes (*Raphanus sativus* L. var. *radicula* Pers.) (Whitmore and Naidu 2000).

Table 4.4 GLSs, their degradation products and precursors (Pengelly 2004)

Glycosinolate	Degradation product	Precursor	Plant
Sinigrin	Allyl isothiocyanate	Homo-thionine	*Brassica nigra, B. juncea, B. oleracea*
Sinalbin	p-hydroxybenzyl thiocyanate	Tyrosine	*Brassica alba, Sinapis alba*
Gluconasturtiin	Phenylethyl thiocyanate	Phenylalanine	*Armoracia rusticana, Nasturtium officinalis*
Glucobrassicin	3-indolylmethyl isothiocyanate	Tryptophan	*B. oleracea, B. sativus* var. *niger*
Glucotropaeolin	Benzyl isothiocyanate	Phenylalanine	*Tropaeolum majus, Lepidium sativum*
Progoitrin	2-hydroxy-3-butenyl isothiocyanate	Tyrosine	*B. oleracea*

4.3.1 Occurrence and Chemistry of Glucosinolates

GLSs are amino acid derived secondary plant metabolites which contain a sulphate and a thioglucose (Halkier and Du 1997). These compounds are mainly found in the seeds, although they may also be present in other tissues of the plants. Various amino acids such as tyrosine, phenylalanine and tryptophan, are precursor components and are converted to GLSs by decarboxylation. Table 4.4 lists the GLSs, their degradation products and precursors.

Cruciferous plants may contain total GLSs of about 1% of dry weight but the content is rather variable. For example, broccoli sprouts or seeds may possess 70–100 μmol/g total GLSs of fresh weight. More than 120 individual GLS compounds have been identified in different plant families so far (Fahey et al. 2001). More than 80% of all GLSs identified are found in the *Brassicaceae* family (Kjaer 1976; Whitmore and Naidu 2000) although *Arabidopsis thaliana* was found to contain 20 different GLSs (Brown et al. 2003).

GLSs are classified in aliphatic, aromatic and indolyl groups on the basis of whether they are derived from aliphatic amino acids (often methionine), phenylalanine or tyrosine, or tryptophan, respectively (Halkier and Du 1997). The structural variation of GLSs results from chain elongations of amino acids before the formation of the GLS core structure and secondary modifications of the GLSs side chain (e.g. thiol oxidation, desaturation, hydroxylation, and esterification) and/or glucose moiety (esterification) (Wittstock and Halkier 2002). Fahey et al. (2001) grouped GLSs under ten different chemical structures (sulfur-containing side-chains; aliphatic, straight-chain; aliphatic, branched-chain; olefins; aliphatic, straight and branched-chain alcohols; aliphatic, straight-chain ketones; aromatic; benzoates; indole; multiply glycosylated and others).

GLSs are chemically and thermally stable to a certain extent and therefore generally enzymatic hydrolysis occurs (Polat 2010). Myrosinase is a specific class of β-thioglucosidases located in idioblasts (myrosin cells) distributed in most tissues

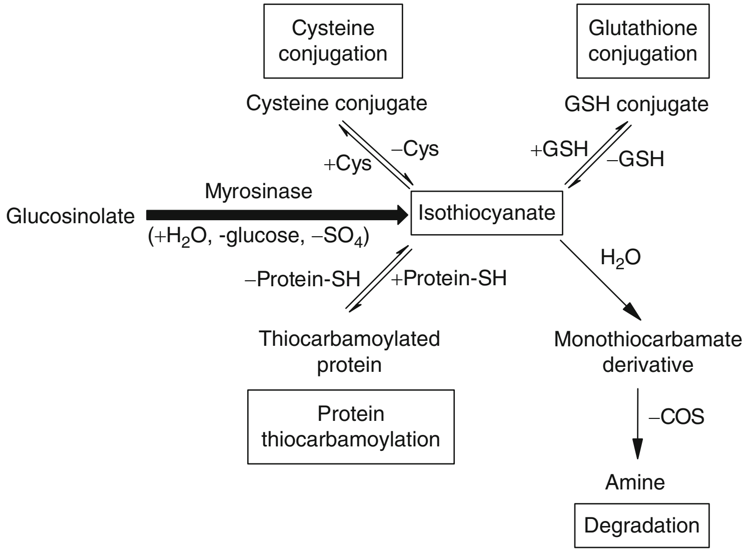

Fig. 4.2 Main hydrolysis mechanism of glucosinolates (Song et al. 2005)

of GLS producing plants (Grubb and Abbel 2006). Reaction kinetics or the enzyme myrosinase may differ depending on the plant species and an individual plant may contain multiple forms of myrosinase (James and Rossiter 1991). By the enzyme myrosinase releases in consequence of tissue damage caused by bruising, wounding, mastication, harvest, shipping and/or handling, relatively nonreactive GLSs are hydrolyzed (Fahey et al. 2001) (Fig. 4.2). The hydrolysis products are thioglucose, sulphate and an unstable derivative which is converted to several degradation products, many of which have pronounced biological effects. These degradation products include isothiocyanates (ITCs), nitriles and thiocyanates. However, epithionitriles and oxazolidine-2-thiones are also produced depending on several factors such as pH and side-chain structures (Halkier and Du 1997) (Fig. 4.2). Stable ITCs are usually formed at pH 6–7, while nitriles are the main hyrolysis products under acidic conditions. Although the majority of hydrolysis products are relatively very stable, indole GLSs such as glucobrassicin yield unstable ITCs and further hydrolysis occurs to give 3-indolemethanol, 3-indoleacetonitrile and 3,3′-diindolylmethane and subsequently condenses into dimers, trimers or tetramers (Holst and Williamson 2004; Gilbert and Senyuva 2008).

Sinigrin is one of the most common mustard oil GLSs found in many cruciferous species and a few other plant families. When plant tissues are damaged, this glucoside is hydrolyzed to release allyl thiocyanate (ATC), a volatile mustard oil (Erickson and Feeny 1974) (Fig. 4.3). Sinalbin is the predominant GLS of yellow mustard and yields mainly nonvolatile 4-hydroxybenzyl ITC responsible for the hot mouthfeel within the hydrolysis by myrosinase (Choubdar et al. 2010). Glucobrassicin and

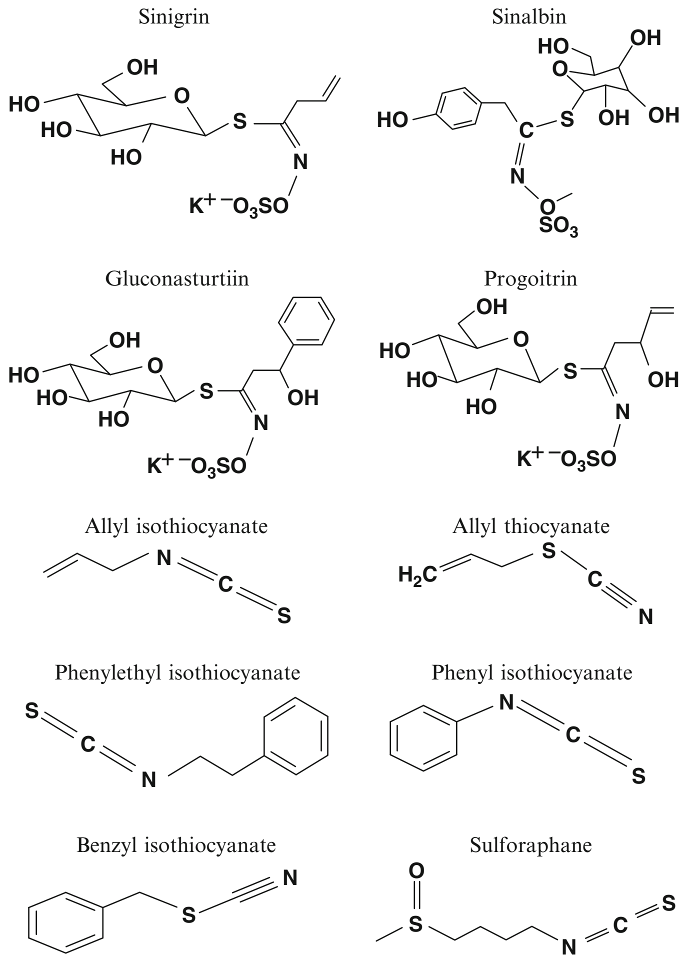

Fig. 4.3 The chemical structures of main glucosinolates and their hydrolysis products

glucoiberin are found in all types of *B. oleracea*, although *B. oleracea* can contain a diversity of GLSs (Cartea and Velasco 2008) (Fig. 4.3).

ITCs are the main hydrolysis products of GLSs. Figure 4.3 shows the chemical structures of main ITCs formed by the hydrolysis of GLSs. Cabbage species contain 4–146 ppm allyl isothiocyanate (AITC), 0–2.8 ppm and 1–6 ppm phenylethyl isothiocyanate (PEITC). The pulps and seeds of ripe papaya fruit contain 4 and 2,910 ppm benzyl isothiocyanate (BITC), respectively (Van Etten et al. 1976; Whitmore and Naidu 2000). Sulforaphane (SFN – generally known as a major ITC converted from glucoraphenine in radish) contents in *Baemuchae* and radish sprouts were found to be 172.6 ± 11.23 and 131.3 ± 4.08 1 μg/g, respectively (Lim et al. 2009). BITC is produced by the hydrolysis of glucotropaeolin and the most abundant

ITC found naturally in several plants such as *Carica papaya* L., Indian cress (*Tropaeolum majus* L.) and garden cress (*Lepidium sativum* L.) (Troncoso-Rojas et al. 2005) (Fig. 4.3).

Amounts of GLSs and ITCs in *Brassica* vegetables may be influenced from various storage, processing and cooking conditions. Storage at ambient temperature or in cold conditions does not cause significant loss of GLSs while finely shredded vegetables may be exposed to a remarkable decline in GLS levels. Cooking by steaming, microwaving and stir-fry does not result in significant loss of GLSs whereas boiling shows significant losses by leaching into cooking water (Song and Thornalley 2007).

4.3.2 Antimicrobial Properties of Glucosinolates and Isothiocyanates

The biological properties of cruciferous plants such as cabbage, broccoli, cauliflower, Brussels sprouts and kale, have mainly been associated with GLSs and their breakdown products such as ITCs, nitriles, thiocyanates and other indole compounds. First of all, GLSs are the defensive compounds of these plants against herbivores, insects and other harmful organisms (Fahey et al. 2001). Over the past 30 years, a number of secondary metabolites including GLSs, and specifically their breakdown products has been considered as important bioactive tools (i.e. anticarcinogenic, antioxidant) in the diet and therapy (Aires et al. 2009). Moreover, the pesticidal activity of crucifers primarily depends on the breakdown products, while intact GLSs have generally less biological activity (Vaughn 1999). Volatile ITCs are considered as the major inhibitor allelochemicals of *Brassica* plants. For example, methyl isothiocyanate (MITC) can inhibit the growth of various weeds such as pigweed, dandelion and crabgrass (Whitmore and Naidu 2000). AITC, ATC, allyl isocyanate (AIC) and allyl cyanide (AC) are all considered as potent fumigant materials against house fly (Tsao et al. 2002). On the other hand, in view of their antimicrobial activity, it is well recognized that GLSs and their breakdown products exhibit inhibitory effects against a wide spectrum of bacteria and fungi. Generally, GLSs are not themselves active; the hydrolysis products are the main antimicrobial agents. Moreover, chemical structure influences biological activities, particularly antimicrobial activity. For instance, aromatic ITCs are more effective than aliphatic ITCs (Aires et al. 2009).

4.3.2.1 Antibacterial Activity

Cruciferous compounds that possess inhibitory effects against spoilage and pathogenic bacteria as well as intestinal microbiota have been well studied in recent years. Table 4.5 summarizes the antibacterial activity of GLSs and their hydrolysis products, primarily ITCs. Previous studies showed that Gram-negative bacteria were

Table 4.5 Antibacterial activity of GLSs and their derivatives

Antibacterial organosulfur compound	Explanation	Bacteria	Antibacterial level (%) or (MIC)	Reference
MSITC	50% lethal dose concentration (µM) was measured	*E. coli*	282	Tierens et al. (2001)
		Xanthomonas campestris	136	
		Pseudomonas syringae	28	
		Sarcina lutea	294	
AITC	MIC value (ppm) was measured	*Ped. pentosaceus*	300–400	Kyung and Fleming (1997)
		Leu. mesenteroides	500	
		Lactobacillus plantarum	300–400	
		Lb. brevis	300	
		L. monocytogenes	200	
		S. aureus	100	
		E. coli	50	
		Enterobacter aerogenes	300	
		Bacillus subtilis	50	
		S. Typhimurium	100	
AITC	The percentage of relative inhibition zone (%) at 3.0 µM dose was calculated[a]	*Citrobacter freundii*	48.5	Aires et al. (2009)
		E. coli	51.1	
		Enterobacter hormaechei	48.2	
		Hafnia alvei	46	
		K. pneumoniae	50	
		K. oxytoca	66.7	
		Morganella morganii	74.1	
		P. aeruginosa	66.1	
		Proteus mirabilis	54.9	
		S. Typhi	52.7	

AITC	The minimum inhibitory dose (MID) (μg/dish) was defined as the dose at which no growth was observed in petri dishes	*B. subtilis*	420	Isshiki et al. (1992)
		B. cereus	360	
		S. aureus	420	
		S. epidermidis	420	
		E. coli	110	
		S. Typhimurium	210	
		S. Enteritidis	420	
		Vibrio parahaemolyticus	210	
		P. aeruginosa	210	
AITC	MIC value (ppm) was measured	*E. coli*	100–200	Shofran et al. (1998)
		P. fluorescens	200	
		Aeromonas hydrophila	200	
		S. aureus	200	
		B. subtilis	200	
		Ped. pentosaceus	1000	
		Lc. mesenteroides	500	
		Lb. brevis	1000	
		Lb. plantarum	–	
SFN	The percentage of relative inhibition zone (%) at 3.0 μM dose was calculated[a]	*A. baumanii*	175	Aires et al. (2009)
		Citrobacter freundii	127.9	
		E. coli	155.2–183.2	
		Enterobacter asburiae	188.2	
		E. hormaechei	127.8	
		E. cloacae	144.4	
		Hafnia alvei	181.7	
		K. pneumoniae	116	
		K. oxytoca	183.3	
		Morganella morganii	123.5	
		P. aeruginosa	138.1	
		Proteus mirabilis	168.6	
		S. Typhi	50.2	

(continued)

Table 4.5 (continued)

Antibacterial organosulfur compound	Explanation	Bacteria	Antibacterial level (%) or (MIC)	Reference
ATC	MIC value (ppm) was measured	*E. coli*	200–400	Shofran et al. (1998)
		P. fluorescens	200	
		S. aureus	200	
BITC	The percentage of relative inhibition zone (%) at 3.0 μM dose was calculated[a]	*A. baumanii*	112.5	Aires et al. (2009)
		C. freundii	94.8	
		E. coli	198.2–206.4	
		Enterobacter asburiae	65.6	
		E. hormaechei	150	
		E. cloacae	55.6	
		Hafnia alvei	231.8	
		K. pneumoniae	79.7	
		K. oxytoca	83.3	
		M. morganii	No growth	
		P. aeruginosa	46.1	
		Proteus mirabilis	388.2	
		S. Typhi	153.3	
PEITC	The percentage of relative inhibition zone (%) at 3.0 μM dose was calculated[a]	*A. baumanii*	58.3	Aires et al. (2009)
		E. coli	56.8–61.7	
		Enterobacter asburiae	55	
		E. hormaechei	46.3	
		Hafnia alvei	54.6	
		K. pneumoniae	52.4	
		K. oxytoca	61.1	
		M. morganii	100	
		P. aeruginosa	44	
		Proteus mirabilis	62.8	
		S. Typhi	62.5	

PEITC	Inhibition zone was measured at 2.0 mg/disc concentration by paper disc agar diffusion method	*Clostridium difficile*	>30	Kim and Lee (2009)
		C. perfringens	>30	
		E. coli	16–20	
BITC	Inhibition zone was measured at 1.0 mg/disc concentration by paper disc agar diffusion method	*C. difficile*	21–30	Kim and Lee (2009)
		C. perfringens	>30	
		E. coli	21–30	
BOITC	Inhibition zone was measured at 5.0 mg/disc concentration by paper disc agar diffusion method	*C. difficile*	>30	Kim and Lee (2009)
		C. perfringens	>30	
		E. coli	>30	

MSITC 4-methylsulphinylpropyl isothiocyanate, *AITC* Allyl isothiocyanate, *ATC* Allyl thiocyanate, *SFN* Sulforaphane, *BITC* Benzyl isothiocyanate, *PEITC* Phenylethyl isothiocyanate, *BOITC* Benzoyl isothiocyanate, *MIC* Minimum inhibition concentration

[a]Percentage of relative inhibition zone diameter = (Inhibition zone diameter of sample – Inhibition zone of negative control)/Inhibition zone of antibiotic standard × 100

more susceptible than Gram-positive against ITCs (Isshiki et al. 1992; Lin et al. 2000). Several of the antibacterial actions of ITCs have been reported. AITC alters protein structures and thereby inhibits microbial growth (Kawakishi and Kaneko 1987). Lin et al. (2000) observed the antibacterial mechanism of AITC against *S.* Montevideo, *E. coli* O157:H7 and *L. monocytogenes* Scott A. They showed that AITC was effective in both the exponential and stationary phases of the bacterial growth. AITC affected cell membranes causing leakage of cellular metabolites and increased β-galactosidase activity of bacteria. Delaquis and Mazza (1995) reported that AITC might damage the essential intracellular enzymes of microorganisms through oxidative cleavage of disulfide bonds.

SFN was found to be effective in the inhibition of *Helicobacter pylori*, the cause of human gastritis and stomach cancer (Fahey et al. 2002; Haristoy et al. 2003, 2005; Yanaka et al. 2009). SFN was also reported as both bacteriostatic and bactericidal against all strains of bacteria. Broccoli sprouts are relatively rich sources of ITCs with a content of 20–50 times more precursor GLSs than mature broccoli. Broccoli sprouts were suggested as a potential digestive eradicator of *H. pylori* from the human gastrointestinal system (Galan et al. 2004).

Sinigrin, a thioglucoside of cruciferous plants and its hydrolysis products AITC, AC, ATC and 1-cyano-2,3-epithiopropane (CETP) were tested for their MIC in broth against 9 species of bacteria including the strains of *E. coli*, *Pseudomonas fluorescens*, *Aeromonas hydrophila, S. aureus*, *B. subtilis, Pediococcus pentosaceus*, *Leu. mesenteroides*, *Lb. brevis* and *Lb. plantarum* (Shofran et al. 1998). Sinigrin, AC and CETP were found to be ineffective in the inhibition of any of the bacteria. ATC showed an inhibitory effect against only *E. coli*, *Pseudomonas fluorescens* and *S. aureus*. *E. coli* was the most sensitive pathogen to AITC with the MIC value of 100 ppm. In this study, the effect of pH on the antimicrobial activity of AITC was also tested and the MIC values of AITC varied from 60 to 140 ppm against *E. coli* and 120 to 220 ppm for *S. aureus*, respectively. Kyung and Fleming (1997) tested the susceptibility of 15 different bacterial strains to sinigrin and some other sulfur compounds. As same in the previous study, they found sinigrin to be inactive against the bacteria while the MIC values of AITC varied from 50 to 500 ppm.

The balance of intestinal microflora is of importance because it helps to maintain human health, contributes to pathogen resistance and interacts with the host immune system (Lahtinen et al. 2009). Several studies have been conducted on the effect of GLSs and derivatives on intestinal microflora. Kim and Lee (2009) reported that PEITC isolated from *Sinapis alba* L. (white mustard) strongly inhibited the growth of *C. difficile* and *C. perfringens* at 1 mg/disc and moderately inhibited the growth of *E. coli* at a dose of 2 mg/disc *in vitro* while it did not exhibit any inhibitory effect on the growth of beneficial microbiota including bifidobacteria and lactobacilli. Aires et al. (2009) showed *in vitro* antibacterial activity of GLS hydrolysis products and specifically SFN and BITC against Gram-positive and Gram-negative pathogenic bacteria isolated from the human intestinal tract. On the other hand, several bacterial strains associated with GLS hydrolysis and belonging to *E. coli, Bifidobacterium longum, Enterobacter cloacae, Bacteroides thetaiotaomicron* and *Bacteroides vulgatus* species have been identified in the intestinal flora.

These strains degrade GLS during 24–48 h cultivation and cause a decline in medium pH from 7.1 to 5.2 (Traka and Mithen 2009).

Ogawa et al. (2000) investigated the combined effects of hydrostatic pressure, temperature and addition of AITC on the inactivation of different strains of *E. coli in vitro*. They found that the antibacterial effects of pressurization with the addition of AITC at 4°C and 40°C were greater than at 20°C and all bacteria tested were effectively killed at 200 or 250 MPa with 10–80 μg/mL of AITC.

The favorableness of GLSs and hydrolysis products for extending the shelf-life of foods, especially meat products, has been investigated. Muthukumarasamy et al. (2003) reported that AITC (about 1,300 ppm) completely eliminated *E. coli* O157:H7 in low inoculated ground beef and reduced the viability >4.5 log cfu/g at high inoculated beef sample by the end of the storage period for 25 days at 4°C. Nadarajah et al. (2005) also obtained similar results in ground beef patties. In another study, Chacon et al. (2006) used microencapsulated AITC to eliminate *E. coli* O157:H7 and total aerobic bacteria (TAB) in finely chopped beef samples. AITC levels lower than 1,000 ppm were ineffective in reducing *E. coli* O157:H7 numbers while both low and high levels of inoculated *E. coli* O157:H7 were completely eliminated by 4,980 ppm AITC addition after 15 and 18 days of storage, respectively. 4,980 ppm AITC kept APC levels <3 log cfu/g during 18 days of storage. Shin et al. (2010) investigated the efficiency of the AITC-MAP (modified atmosphere packaging) system to control the growth of *L. monocytogenes* and *S.* Typhimurium on raw chicken breast during refrigerated storage for up to 21 days. Both release rates of 0.6 and 1.2 μg/h plus MAP systems significantly reduced the population of *S.* Typhimurium whereas *L. monocytogenes* was weakly inhibited at the lower release rate of AITC.

Fresh fruit and vegetables are at the risk of outbreaks of foodborne illnesses resulting from the contamination of pathogens during pre- and post-harvest stages. ITCs have been investigated as natural antimicrobial agents to sanitize fresh produce. Obadiat and Frank (2009a) compared the effectiveness of AITC, carvacrol and cinnamaldehyde in the vapor phase to eliminate strain cocktails of *Salmonella* and *E. coli* O157:H7 from sliced and whole tomatoes. AITC exhibited the highest antimicrobial activity and lowest level of AITC (8.3 μL/liter of air) inactivated *Salmonella* on sliced tomatoes by 1.0 and 3.5 log at 4°C and 10°C, respectively, in 10 days and by 2.8 log at 25°C in 10 h. AITC also reduced the *E. coli* O157:H7 level on sliced tomatoes by 3.0 log at 4°C and 10°C in 10 days, but there was no inactivation at 25°C in 10 h. The same researchers also tested those antimicrobial compounds in vapor phase on the intact and damaged portions of lettuce and spinach leaves against *E. coli* O157:H7 (Obadiat and Frank 2009b). On intact lettuce surface, 4 μL/liter of air AITC inactivated >4 log of *E. coli* O157:H7 at 0°C and 4°C in 4 days and at 10°C in 2 days. Pathogen inactivation on spinach surface was lower than on lettuce by 1 log. Higher concentrations of AITC were required when the surfaces were damaged by cutting. Lin et al. (2000) showed that lettuce inoculated with *E. coli* O157:H7 and *S.* Montevideo (each 10^4 cfu/g) showed no detectable bacterial counts after exposure to 400 μL of AITC and MITC for 24 and 48 h, respectively. *L. monocytogenes* was reduced to undetectable levels after the treatment with 400 μL of MITC for 2 days whereas 400 μL of MITC failed to achieve the same bactericidal effect.

4.3.2.2 Antifungal Activity

Although cruciferous plants produce inducible chemical defensive systems, they may be exposed to infection by a wide range of fungi. It was demonstrated that GLSs and their breakdown products, the major defensive compounds of crucifers, exhibit antimicrobial activity against fungal pathogens both *in vitro* and *in planta* (Sellam et al. 2007). The inhibitory effects of GLSs and their derivatives against various fungal species are shown in Table 4.6.

Postharvest fungal growth becomes a threat for many crops during their storage under unfavorable conditions and causes considerable economic losses worldwide. The antifungal effects of vapor-phase ITCs have been demonstrated in many studies. Mari et al. (2002) reported that the best control of blue mold (*P. expansum*) on pears was obtained by exposing fruits in a 5 mg/L AITC-enriched atmosphere for 24 h. Wu et al. (2011) tested the potential use of AITC and EITC as fumigants in *in vitro* and *in vivo* trials to determine their effects on *P. expansum* Link and *Botrytis cinerea* Persl. infection in apples. A 3:1 ratio of AITC:EITC showed the best inhibitory activity on *in vitro* spore germination of *P. expansum* and *B. cinerea*. In *in vivo* trials on artificially infected apples, AITC, EITC and their combination reduced the incidence by more than 85% after 3–4 days of apple incubation at 20°C. Kurt et al. (2011) investigated *in vitro* and *in vivo* antifungal activity of synthetic pure ITCs against *Sclerotinia sclerotiorum*, a necrotrophic pathogen causing *Sclerotinia* stem and root rot of tomato and other economically important crops. MITC, AITC and BITC completely inhibited mycelial growth of the mold in the volatile phase. Butyl ITC (BUITC) and BITC reduced the apothecial production of *S. sclerotiorum* at their highest concentrations. In *in vivo* assay, AITC and PEITC reduced disease incidence by 76.7% and 70%, respectively.

Goncalves et al. (2009) investigated the antifungal efficiency of AITC sachets in cottage cheese preservation. The sachets produced by the incorporation of AITC were attached to the inner surface of the lids of cups containing cheese and the cups were stored for 35 days at 5 ± 2°C and 10 ± 2°C. After 7 days, the AITC sachets were found to be effective in reducing yeast and mold counts.

4.4 Conclusion

Organosulfur compounds in intact vegetables of the *Allium* and *Brassica* spp. and particularly their hydrolysis products are considered as key compounds of these vegetables. They give bioactive properties to these vegetables such as antimicrobial, anticarcinogenic, antitumor etc. while also giving them their pungent flavor and odor. Garlic is the most studied *Allium* vegetable as regards the antimicrobial activity

Table 4.6 Antifungal activity of GLSs and their derivatives

Antifungal agent	Explanation	Fungi	Antifungal level	Reference
MSITC	50% lethal dose concentration (μM) was measured	*Alternaria brassicicola*	>1130	Tierens et al. (2001)
		Botrytis cinerea	>1130	
		Fusarium culmorum	124	
		F. oxysporum	325	
		Neurospora crassa	271	
		Nectria hematococca	260	
		Plectosphaerella cucumerina	147	
		Penicillium expansum	294	
		Verticillium dahlia	215	
AITC	MIC value (ppm) was measured	*Saccharomyces cerevisiae*	4	Kyung and Fleming (1997)
		Torulopsis etchellsii	1	
		Hansenula mrakii	4	Shofran et al. (1998)
		Pichia membranefaciens	2	
		Torulospora delbrueckii	4	
		Zygosaccharomyces rouxii	–	
AITC	The minimum inhibitory dose (MID) (μg/dish) was defined as the dose at which no growth was observed in petri dishes	*S. cerevisiae*	62	Isshiki et al. (1992)
		Hansenula anomala	124	
		T. delbrueckii	50	
		Z. rouxii	31	
		Candida tropicalis	62	
		C. albicans	62	
		Aspergillus niger	124	
		A. flavus	124	
		Penicillium islandicum	62	

(continued)

Table 4.6 (continued)

Antifungal agent	Explanation	Fungi	Antifungal level	Reference
		P. citrinum	62	
		P. chrysogenum	250	
		Fusarium oxysporum	62	
		F. graminearum	31	
		F. solani	110	
		Alternaria alternata	62	
		Mucor racemosus	250	
AITC (gas)	MIC value (µg/L) was measured	*A. flavus*	100	Delaquis and Sholberg (1997)
		Botrytis cinerea	100	
		P. expansum	100	
BITC	MIC value (mg/mL) was measured	*Alternaria alternata*	0.1	Troncoso-Rojas et al. (2005)
MITC	MIC values (µM/L) of ITCs in the volatile phase were measured	*Sclerotinia sclerotiorum*	732	Kurt et al. (2011)
AITC			714	
BUITC			840	
EITC			550	
PITC			836	
BITC			525	
PEITC			670	
MITC	MIC values (µM/L) of ITCs in the contact phase were measured	*S. sclerotiorum*	4387	Kurt et al. (2011)
AITC			3065	
BUITC			1688	
EITC			2358	
PITC			1672	
BITC			750	
PEITC			2011	

MSITC 4-methylsulphinylpropyl isothiocyanate, *AITC* Allyl isothiocyanate, *ATC* Allyl thiocyanate, *SFN* Sulforaphane, *BITC* Benzyl isothiocyanate, *PEITC* Phenylethyl isothiocyanate, *BOITC* Benzoyl isothiocyanate, *MIC* Minimum inhibition concentration since it is the richest source of antimicrobial organosulfur compounds among the *Allium* ssp. whereas all species of crucifers incorporate organosulfur materials. Allicin and other thiosulfinates such as DAS, DADS, DATS and ajoene present in *Allium* plants are the principal organosulfur compounds to exhibit antimicrobial activity. In the *Brassica* family, GLSs are not generally active themselves; their hydrolysis products are the main antimicrobial agents. Volatile ITCs like AITC, ATC, MITC and SFN have been shown to be the most effective antimicrobial compounds formed by the hydrolysis of GLSs in cruciferous plants.

Overall, it can be concluded that organosulfur compounds have the importance not only for the plant but also in the food industry in virtue of their health benefits. These compounds as well as various extracts of edible parts of organosulfur-source plants might be used in food product formulations for health and/or antimicrobial purposes. Although specific flavors/odors of these materials adversely affect organoleptic properties of most foods used, they might be considered as convenient ingredients of especially some meat and dairy products. Furthermore, several studies should be focused on preventing the come into forefront of the negative flavor/odors of them in foods when they are consumed.

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