Antimicrobial Activity of the Glucosinolates

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Abstract

The use of natural antimicrobial compounds is receiving much attention and is becoming very frequent by the importance that nowadays is given to natural resources. Natural components have been applied in several sectors such as agriculture, biomedicine and food preservation. The development of resistance to conventional antibiotic by pathogenic bacteria makes necessary to find alternative antimicrobials to eradicate these microorganisms. Many food products are perishable and require protection from spoilage to improve quality and shelf life. Numerous efforts are conducted to find safe natural alternatives to prevent microorganism growth in plants and food products, because of the consumer concern regarding synthetic pesticides and preservatives. Natural antimicrobials can be obtained from different sources including plants, animals, bacteria, algae, and fungi. Among them, glucosinolates and their derived products have been recognized for their benefits to human nutrition, plant defense, and as potent antimicrobial agents. This chapter describes the antimicrobial activity of glucosinolates and their hydrolysis products against different bacterial and fungal species, as well as the mechanism of action of these active compounds.

Keywords

Glucosinolates • Isothiocyanates • Antifungal activity • Antibacterial activity • Bioactive compounds

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Abbreviations

AAM	Allylamine
AC	Allyl cyanide
AITC	Allyl isothiocyanate
ASC	Ascorbigen
ATC	Ally thiocyanate
BAM	Benzylamine
BC	Benzyl cyanide
BITC	Benzyl isothiocyanate
CEPT	1-Cyano-2,3-epithiopropane
DIM	3,3′-Di-indolylmethane
EITC	Ethyl isothiocyanate
GLS	Glucosinolate
I3C	Indole-3-carbinol
IAN	Indole-3-acetonitrile
ITC	Isothiocyanate
MAP	Modified atmosphere packaging
MCT	Medium-chain triglyceride
MITC	Methyl isothiocyanate
PAM	2-Phenylethylamine
PEC	2-Phenylethyl cyanide
PEITC	Phenylethyl isothiocyanate
PITC	Phenyl isothiocyanate
SBO	Soybean oil
SFN	Sulforaphane
TC	Thiocyanate

Contents

1 Introduction

In agreement with the current trend to value the natural and renewable resources, the interest in the use of natural antimicrobial compounds is increasing for biomedical, agricultural, and especially food applications [1, 2].

Food products are perishable by nature and can be subjected to contamination by bacteria and fungi. Many of these microorganisms can cause undesirable

reactions that deteriorate flavor, odor, color, sensory, and textural properties of foods. Some of them can also potentially cause food-borne illness. For all these reasons, food products require protection from spoilage during their preparation, storage, and distribution to give them desired shelf life. Furthermore, the dramatic rise of antibiotic-resistant microorganisms is of concern and includes food-borne pathogens that are also more tolerant to several food processing and preservation methods. The consumer concern regarding synthetic products, such as food additives and pesticides, and the necessity to overcome the emergence of antibiotic-resistant pathogens led to the research of alternative compounds with potent antimicrobial activity which can reduce the impact of synthetic products on human and animal health [1].

Natural antimicrobials can be obtained from different sources including plants, animals, bacteria, algae, and fungi. To select the appropriate biocidal product, the microorganism strain must be identified and the spectrum of antimicrobial activity of the compound considered [2–4]. Several reports have demonstrated the efficacy of plant-derived compounds, most of all in food applications. Antimicrobials derived from plants are mostly secondary metabolites that possess various benefits including antimicrobial properties against pathogenic and spoilage microbes. The structural diversity of plant-derived compounds is immense, and the impact of antimicrobial action they produce against microorganisms depends on their structural configuration [5].

Among the potent natural antimicrobials, glucosinolates (GLS) are an important class of secondary plant products found in seeds, roots, stems, and leaves of cruciferous plants including 16 families of dicotyledonous angiosperms, mainly *Brassicaceae* [6]. There are about 120 different GLS identified, derived from amino acids (alanine, leucine, isoleucine, valine, phenylalanine, tyrosine, and tryptophan) and a number of chain-elongated homologues [7]. They are classified as aliphatic, aromatic, methylthioalkyl, and heterocyclic, which have a thioglucoside component in common structure and differ at their side chains [8].

Located within vacuoles, GLS are physically separated but accompanied by β-thioglucosidase enzymes known as myrosinases [9]. Following plant tissue disruption, the enzyme and GLS come into contact, which, in the presence of water, generates a hydrolysis forming an aglycone moiety, glucose, and sulfate. The aglycone moiety is unstable and rearranges to form three main groups of substances: nitriles, thiocyanates (TCs), and isothiocyanates (ITCs) (Fig. 1) [3, 10, 11].

GLS and their enzymatic hydrolysis products are responsible for a characteristic pungent flavor [12, 13]. These compounds have shown several biological activities including plant defense (against insects and microbial infections) and benefits to human health (anticarcinogenic, antioxidant, and antimicrobial properties) and might be potential natural agents for food preservation [14]. Their response to microbial population varies according to their structural characteristics. The biocidal effect of cruciferous tissues on other microorganisms has been attributed mainly to volatile degradation products of GLS, released from their plants. Among derived

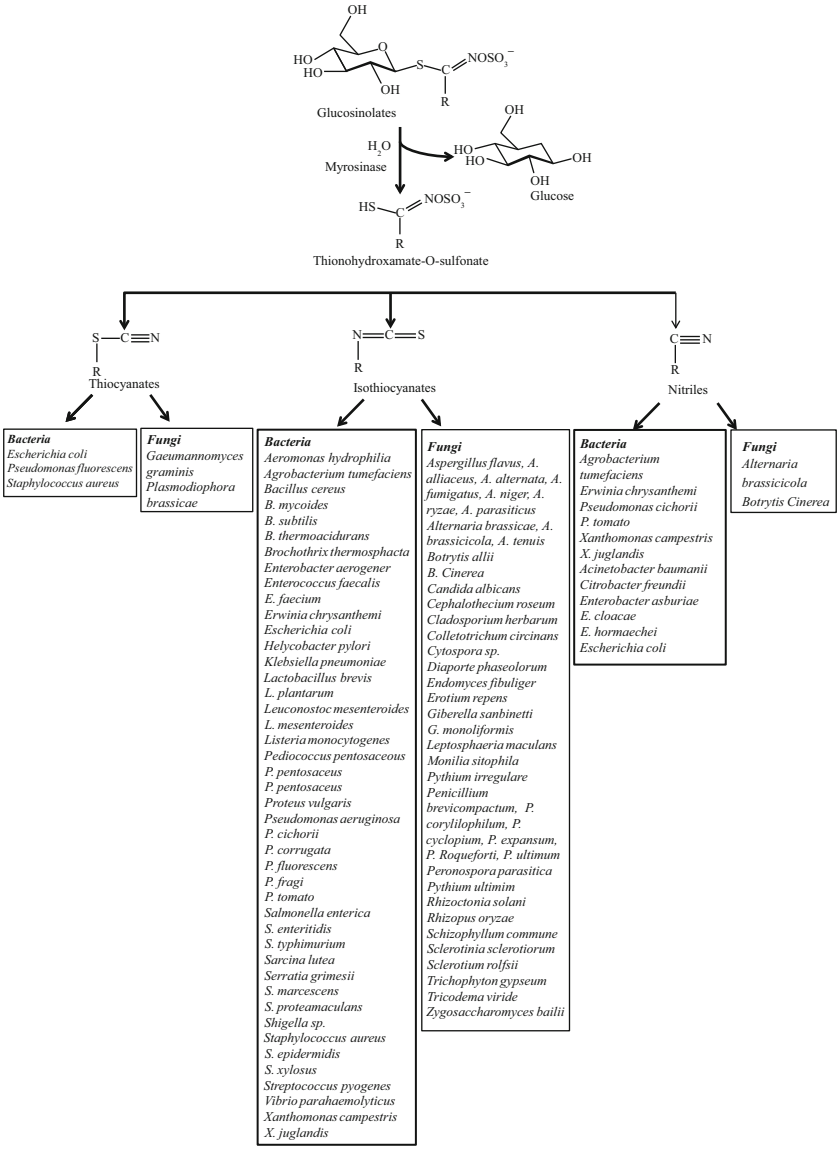

Fig. 1 Enzymatic degradation of glucosinolates and the antifungal spectrum activity of their derived products

products, ITCs are the major inhibitors of microbial activity, and they have been studied mainly for food preservation and plant pathogen control [5, 12]. ITCs are volatile substances that display an inhibitory effect on several microorganism species at low concentrations [15].

Therefore, the objective of this chapter was to present results of studies on antimicrobial activity of GLS and their enzymatically degradation products and highlight important aspects on the application.

2 Antibacterial Activity

GLS and, above all, their hydrolysis products elicit a wide spectrum of antimicrobial activity against a variety of bacteria. The concentrations of these compounds required to inhibit microorganisms are difficult to compare given differences in methodologies, materials, and test strains employed. There are considerably more data for the ITCs, and in particular for AITC, than others GLS products. Furthermore, the mode of delivery to target microorganisms has a large impact on the antibacterial effect. Dissolution of these compounds in liquid media can result in a weak antimicrobial activity, while lower concentrations in the vapor phase are sufficient to inhibit microorganisms [10]. On Table 1 are summarized some studies which report the antibacterial potential of these compounds.

Horseradish vapors, containing GLS hydrolysis products, showed stronger antibacterial activities against several bacterial strains [16, 17]. Later, also studied and compared were the bacteriostatic and bactericidal effects of AITC, EITC, and MITC against 10 strains. MITC was the most effective, in both solution and vapor phases, followed by AITC and EITC. *Escherichia coli* and *Staphylococcus aureus* appeared to be the more resistant strains, while the least resistant were *Bacillus subtilis*, *Bacillus mycoides,* and *Serratia marcescens*.

Virtanen [18] reported the antimicrobial activity of BITC and β-phenylethyl, m-methoxybenzyl, and methoxybenzyl ITCs against *S. aureus*. The activity of these ITCs was higher than the antimicrobial activity of a series of aliphatic ITCs. Zsolnai [19] demonstrated that the same concentration of AITC and PITC, used to severely inhibit the growth of yeasts and fungi, was not effective against *Streptococcus pyogenes*, *S. aureus*, and other Gram-negative bacteria. BITC was effective against *Staphylococci* but not on other bacteria.

Kanemaru and Miyamoto [20] studied the antibacterial activity of brown mustard and its major pungent compound, AITC, on the growth of *E. coli* 3301, *S. aureus* IFO 3761, *Proteus vulgaris* IFO 3851, *Pseudomonas fragi* IFO 3458, and *Pseudomonas aeruginosa* IFO 3755. To prepare the extract of black mustard extract was prepared as 20% mustard in ethanol (70%) after myrosinase treatment. AITC was also dissolved in 70% ethanol to form an equivalent concentration. The nutrient broth in which the bacteria were cultured contained the mustard extract or AITC and was stored at 30 °C on a shaker. Turbidimetry was used to determine bacterial growth. The results obtained evidenced that the antibacterial effect of mustard was mainly due to AITC. The concentrations of mustard in the medium that inhibited bacterial growth for 24 h were 0.138%, 0.104%, 0.064%, 0.043%, and 0.089% and those of AITC were 14.5, 12.3, 6.5, 3.6, and 7.2 ppm for *S. aureus*, *E. coli*, *P. vulgaris*, *P. fragi*, and *P. aeruginosa*, respectively. A bacteriostatic effect was

Table 1 Antibacterial potential of glucosinolate-derived products against several species on food products

Glucosinolate derivative	Bacterial strain	Food product	Reference
Allyl isothiocyanate	*Bacillus thermoacidurans*	Fresh apple juice	[44]
Allyl isothiocyanate	*Bacillus subtilis* IFO-13722 *Bacillus cereus* IFO-13494 *Staphylococcus aureus* IFO-12732 *Staphylococcus epidermidis* IFO-12993 *Escherichia coli* JCM-1649 *Salmonella typhimurium* A TCC-14028 *Salmonella enteritidis* JCM-189 *Vibrio parahaemolyticus* IFO-12711 P. *Pseudomonas aeruginosa* IFO-13275	Fresh beef Cured pork Sliced raw tuna Cheese Egg sandwich Noodles Pasta	[45]
Allyl isothiocyanate Phenethyl isothiocyanate Allyl thiocyanate	*Staphylococcus aureus*	Cooked roast beef	[46]
	Escherichia coli O157:H7		
	Staphylococcus typhimurium		
	Listeria monocytogenes		
	Serratia grimesii		
	Lactobacillus sake		
Allyl isothiocyanate Phenethyl isothiocyanate Allyl thiocyanate 1-Butane isothiocyanate	*Pseudomonas* spp. *Enterobacteriaceae* Lactic acid bacteria	Precooked roast beef slices	[47]
Methyl isothiocyanate, Allyl isothiocyanate	Rifampicin-resistant strain of *Salmonella,* Montevideo streptomycin-resistant strains of *Escherichia coli* O157:H7 and *Listeria monocytogenes* Scott A	Apples, tomatoes, Iceberg lettuce	[48]
Allyl isothiocyanate	*Escherichia coli* O157:H7	Fresh ground beef	[49]
Allyl isothiocyanate	*Pediococcus pentosaceus* *Staphylococcus carnosus* *Escherichia coli* O157:H7	Dry fermented sausage	[50]
Allyl isothiocyanate	*Lactobacillus algidus* *Leuconostoc mesenteroides* *Leuconostoc carnosum* *Carnobacterium maltaromaticum* *Carnobacterium divergens* *Brochothrix thermosphacta* *Serratia proteamaculans*	Marinated pork	[51]
Allyl isothiocyanate	*Salmonella*	Fresh cantaloupe	[52]

(*continued*)

Table 1 (continued)

Glucosinolate derivative	Bacterial strain	Food product	Reference
Allyl isothiocyanate	*Leuconostoc mesenteroides* *Lactobacillus plantarum*	Kimchi	[53]
Allyl isothiocyanate	*Escherichia coli* *Listeria monocytogenes*	Fresh cut onions	[54]
Allyl isothiocyanate	*Listeria monocytogenes* *Salmonella typhimurium*	Chicken breast	[56]
4-Hydroxybenzyl isothiocyanate	*Salmonella*	Sauce with particulates	[55]

shown by mustard on *S. aureus* and *E. coli* (0.8%), while the effect was bactericidal on *P. aeruginosa* at 0.2%

Shofran et al. [21] tested the antimicrobial activity of sinigrin and four sinigrin hydrolysis products, in broth culture, against different species of bacteria. Sinigrin is a GLS that, upon injury or mechanical disruption of plant tissue, is hydrolyzed by myrosinase producing up to four distinct compounds: AITC, allyl TC (ATC), allyl cyanide (AC), and 1-cyano-2,3-epithiopropane (CEPT). Sinigrin had little effect upon the growth of microorganisms [22], but its hydrolysis products were effective in inhibition of growth. The species of bacteria studied in the experiment were *E. coli* 33625, *E. coli* NC101, *Pseudomonas fluorescens* MD13, *Aeromonas hydrophilia* 7966, *S. aureus* 4220, *B. subtilis* IS75, *Pediococcus pentosaceus* FFL48, *Leuconostoc mesenteroides* FFL44, *Lactobacillus brevis* MD42, and *Lactobacillus plantarum* MOP3. Sinigrin, AC, and CETP at 1000 ppm did not show inhibitory effects against any of the bacteria tested. ATC was inhibitory to the growth of 3 strains of Gram-negative (*E. coli* 33625, *E. coli* NC101, *P. fluorescens* MD13) and 1 strain of Gram-positive bacteria (*S. aureus* 4220) with minimum inhibitory concentration (MIC) values ranged between 200 and 400 ppm. The antimicrobial activity of ATC was due to its conversion to AITC, sinigrin hydrolysis products with the highest antibacterial activity. AITC was effective against all the bacteria tested, except *L. plantarum* MOP3. The MIC of AITC against Gram-negative and Gram-positive non-lactic acid bacteria ranged between 100 and 200 ppm, while lactic acid bacteria were more resistant with MIC between 500 and 1000 ppm. It should be highlighted that the antimicrobial activity of AITC can be different if it is used in gaseous form or dissolved in broth culture. Furthermore, a lot of factors can influence the generation of AITC from sinigrin.

Kyung and Fleming [23] tested sinigrin and its derivate products against 15 species of bacteria: *Pediococcus pentosaceus* LA3, *P. pentosaceus* LA76, *L. mesenteroides* LA10, *L. mesenteroides* LA113, *L. plantarum* LA97, *L. plantarum* LA70, *L. brevis* LA25, *L. brevis* LA200, *Listeria monocytogenes* B67, *L. monocytogenes* B70, *S. aureus* B31, *E. coli* B34, *Enterobacter aerogenes*

B146, *B. subtilis* B96, and *Salmonella typhimurium* B38. Sinigrin itself was not antimicrobial because it did not inhibit growth up to 1000 ppm and microorganisms did not degrade it to its antimicrobial aglycones. AITC is known to be antimicrobial, and the MICs found ranged from 50 to 500 ppm for bacteria, including Gram-positive, Gram-negative, pathogenic, and lactic acid bacteria.

Delaquis and Sholberg [24] evaluate the microbistatic and microbicidal properties of gaseous AITC against bacterial cells of *S. Typhimurium* (ATCC 14028), *L. monocytogenes* (strain 81–861), *E. coli* O157:H7 (ATCC 43895), and *Pseudomonas corrugata* (isolated from lettuce). *S. typhimurium*, *L. monocytogenes*, and *E. coli O157:H7* were inhibited when exposed to 1000 μg L^{-1} AITC. *P. corrugata* failed to grow in the presence of 500 μg L^{-1}. Variations at different incubation temperatures were observed. Bactericidal activities varied with strain and increased with time of exposure. The most resistant bacterium was *E. coli*.

The antibacterial properties of the GLS and their hydrolysis products became of big interest and importance also in the eradication of pathogenic microorganisms that is complicated by the development of resistance to conventional antimicrobial agents. *Helicobacter pylori* is one of the most prevalent human pathogens in the world. Gastric infections with *H. pylori* are known to cause gastritis and peptic ulcers and dramatically enhance the risk of gastric cancer. Antibiotic therapy is recommended for infected patients with gastric or duodenal ulcers or gastric mucosa-associated lymphoid tissue lymphoma, but this treatment is not universally successful. Even with the combination of two or more antibiotics, *H. pylori* is difficult to eradicate due to the development of resistance of this bacteria to these antibiotics and the persistence of organisms within gastric epithelial cells and, furthermore, due to logistic, sociologic, and economic reasons. The ITC sulforaphane (SFN) appears to overcome all of these problems. SFN is abundant in certain varieties of broccoli and broccoli sprouts in the form of its GLS precursor called glucoraphanin. It has been demonstrated that SFN is a potent bacteriostatic agent against 3 reference strains and 45 clinical isolates of *H. pylori*. The MIC for 90% of the strains is <4 μg mL^{-1}, irrespective of their resistance to conventional antibiotics. It is a potent bactericidal agent against both extra- and intracellular *H. pylori* in vitro. Further, brief exposure to SFN eliminated intracellular *H. pylori* from a human epithelial cell line (HEp-2). Although higher concentrations are required to achieve bactericidal activity for the intracellular forms, SFN accumulates intracellularly to high levels, as its glutathione conjugate. It can be safely administered to humans because it is present in high concentrations in edible cruciferous vegetables and can be directly delivered to the stomach [25].

Haristoy et al. [26] evaluated the effect of SFN in vivo against *H. pylori* by using human gastric xenografts in nude mice. *H. pylori* was completely eradicated in 8 of the 11 SFN-treated grafts, after short-term administration of SFN at a dose that can be achieved in the human diet.

Haristoy et al. [27] analyzed the activities of 12 ITCs including sulforaphane on 25 strains of *H. pylori* using an agar dilution assay. The ITCs tested were iberin, cheirolin, erucin, $_{D,L}$-SFN, $_{D}$-SFN, $_{L}$-SFN, $_{L}$-sulforaphane, erysolin, berteroin, alyssin, hirsutin, PEITC, BITC, and 4-(α-L-rhamnopyranosyloxy)benzyl ITC.

Furthermore, the bactericidal activities of the six ITCs (cheirolin, $_{L}$-sulforaphane, erysolin, berteroin, hirsutin, and 4-(α-L-rhamnopyranosyloxy)benzyl ITC) that showed the lowest MICs were determined both directly and against intracellular bacteria in cultured human epithelial cells. The MIC_{90} values for these ITCs ranged between 4 and 32 μg mL^{-1}. It has been demonstrated that, in addition to SFN, four (cheirolin, berteroin, hirsutin, and 4-(α-L-rhamnopyranosyloxy)benzyl ITC) of the most active compounds exhibited high bactericidal activity against both extra- and intracellular bacteria.

Ono et al. [28] screened, isolated, and identified antibacterial compounds occurring in some common foods for bactericidal use, against *E. coli* and *S. aureus*. Among the different foodstuffs studied, wasabi stems, banana fruits, coriander leaves, and mustard seeds showed antibacterial activity. In particular, the lower minimal bactericidal concentration was obtained for wasabi stems, so their activity was highest. The compound with the antibacterial activity was identified as the 6-methyl-sulfinylhexyl ITC. The ethyl, butyl, hexyl, and octyl homologues of this ITC were determined in some *Cruciferae* plants. The main component contained in wasabi was the hexyl homologue, whereas horseradish contained the ethyl and hexyl homologues. Broccoli, Chinese cabbage, radish, and turnip almost exclusively contained the butyl homologue, and cabbage contained only the hexyl homologue. These homologues were also active against *E. coli* and *S. aureus* with minimal bactericidal concentration ranged between 0.1 and 2.0 mg mL^{-1}.

Liu and Yang [29] studied the stability and the antimicrobial activity of AITC in two medium-chain triglyceride (MCT) and soybean oil (SBO), dispersed in an oil-in-water system during long-term storage. It has been shown that the stability and antimicrobial activity were affected by the content, type, and oxidative stability of the oil. In particular, high oil content is favorable for AITC stability in the emulsion. AITC with MCT were more effective than AITC with SBO in inhibiting Gram-negative bacteria *E. coli* O157:H7, *Salmonella enterica*, and *Vibrio parahaemolyticus* and Gram-positive bacteria *S. aureus* and *L. monocytogenes*.

Luciano and Holley [30] evaluated the antibacterial activity of AITC against *E. coli* O157:H7 at different pH values and examined the inhibitory action of this compound against two enzymes important in the metabolism of this food-borne pathogen (thioredoxin reductase and acetate kinase). AITC showed greater antimicrobial activity at low pH values (4.5 and 5.5). Decomposition products of this ITC were also studied, and they did not show antibacterial activity toward *E. coli* O157:H7. Only AITC is antimicrobial in its original form. Furthermore, it has been demonstrated that only 1 μL L^{-1} of AITC could decrease the activity of thioredoxin reductase and AITC at 10–100 μL L^{-1} was able to significantly inhibit both thioredoxin reductase and acetate kinase.

The antimicrobial properties of different GLS autolysis products of *Hornungia petraea* were investigated against two isolates of *S. aureus*, *Salmonella enteritidis*, *Klebsiella pneumoniae*, *Sarcina lutea*, *E. coli*, *Shigella* sp., and *Bacillus cereus*. The tested compounds were showed to be active against all tested microorganisms, with the activity ranging from 1 to 1250 mg mL^{-1} for inhibitory and 1 to 5000 mg mL^{-1}

for microbicidal activity. In particular, the assays showed a very high antibacterial activity of the tested ITCs against *S. lutea* [31].

Olaimat and Holley [32] determined the minimum inhibitory and minimum bactericidal concentrations of AITC from mustard against five strains each of *Salmonella* and *L. monocytogenes* individually and combined. The MIC and MBC values of AITC ranged from 60 to 100 ppm and 120 to 180 ppm, respectively, at 37 °C and ranged from 10 to 40 ppm and 200 to 600 ppm, respectively, at 21 °C against both pathogens. AITC had no antimicrobial activity at low temperatures (4 °C or 10 °C) and alkaline pH over 10, but at neutral pH, *L. monocytogenes* is reduced. At acidic pH, AITC was more effective against *Salmonella*. However, AITC was more effective at combinations of 21 °C and neutral pH against *L. monocytogenes* and at combinations of higher temperature and acidic pH against *Salmonella*.

A lot of data are available about the antimicrobial activity of ITCs, but the results are difficult to compare. Accordingly, Wilson et al. [33] studied the antibacterial activity of a large number of ITCs on a wide range of microorganisms, using for all the same experimental conditions. Ten ITCs were tested, and, among them, six were investigated for the first time: SFN, iberin, AITC, BITC, MITC, PITC, PEITC-, propyl-, 3-methylthiophenyl-, and 3-methylthiopropyl-ITC. The bacteria tested were fourteen and included 8 Gram-positive species (*B. cereus* CIP 78.3, *B. subtilis* ATCC 6633, *Enterococcus faecalis* G9h, *Enterococcus faecium* ATCC 19434, *L. plantarum* DSM 9843 [299v], *L. monocytogenes* LC 10, *S. aureus* ATCC 6538, and *Staphylococcus xylosus* LC 57) and 6 Gram-negative species (*K. pneumoniae* DSM 681, *E. coli* ATCC 25922, *P. aeruginosa* DSM 1128, *S. enteritidis* LC 216, *S. typhimurium* LC 443, and *S. marcescens* LC 448). A turbidimeter was used to monitor the growth of bacteria, and the antimicrobial activity was expressed as antimicrobial efficacy index that is a function of the growth delay, the reduction in the maximum population, and the reduction in maximum specific growth rate. All the ITCs tested displayed antimicrobial activity, depending on the target bacteria and the structural features of the molecule considered. BITC showed the highest value of antimicrobial efficacy index, followed by PEITC. Different from other studies, AITC was the least active ITC, and not necessarily aromatic ITCs were more active than aliphatic compound. For example, 3-methylthiopropyl-ITC was much more active than PITC. Gram-negative bacteria were overall more sensitive to ITCs than Gram-positive bacteria, and considerable variations in sensitivity were evidenced between species even within the same Gram type.

AITC, BITC, and PEITC purified from cruciferous plants were evaluated against 15 isolates of methicillin-resistant *S. aureus* (MRSA) isolated from diabetic foot ulcer patients. In general, the AITC always presented the higher MIC values and thus lower antimicrobial activity, while BITC and PEITC presented the lowest MIC. Therefore, these ITCs showed the highest antimicrobial activity. The AITC and PEITC were essentially bacteriostatic, whereas BITC was bactericidal in 11 isolates of MRSA. Based on this, BITC is more effective in suppressing MRSA strains than PEITC. The antibacterial effectiveness of these compounds depends on the dose tested and on the chemical structure [34].

GLS and their derivate products are useful also in inhibiting the growth of pathogenic bacteria that can contaminate vegetable seeds. This contamination can occur at any point, from the field to the sprouting process and during subsequent handling of sprouts until they are consumed. Populations of *E. coli* O157:H7 have been reported to reach 10^6–10^7 cfu g^{-1} of sprouts produced from contaminated seeds. *E. coli* O157:H7 causes life-threatening hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura in the young, old, and immunocompromised. The efficacy of AITC in killing *E. coli* O157:H7 on dry and wet alfalfa seeds was investigated. AITC was lethal to *E. coli* inoculated onto agar disks, but, unfortunately, the enhanced effectiveness of AITC in killing the pathogen onto alfalfa seeds is offset by a dramatic reduction in seed viability. Nevertheless, the use of AITC for the purpose of killing *E. coli* O157:H7 in other fields and, perhaps, other pathogens on alfalfa seed holds promising [35].

GLS hydrolysis products also displayed antimicrobial activity against plant pathogenic microorganisms, and this feature reinforces the potential for using them as alternatives to the traditional chemical control of phytopathogenic bacteria. Aires et al. [36] evaluated the antibacterial effects of GLS hydrolysis products against six relevant plant pathogenic Gram-negative bacteria, using a disc diffusion assay: *Agrobacterium tumefaciens*, *Erwinia chrysanthemi*, *Pseudomonas cichorii*, *Pseudomonas tomato*, *Xanthomonas campestris*, and *Xanthomonas juglandis*. The GLS hydrolysis products used in the in vitro assay were AITC, AC, SFN, BITC, benzyl cyanide (BC), PEITC, 2-phenylethyl cyanide (PEC), indole-3-acetonitrile (IAN), indole-3-carbinol (I3C), and ascorbigen (ASC). A mix of AITC, BITC, and PEITC also was tested. The strongest inhibitory effect was showed by PEITC and SFN. Among the different GLS hydrolysis products studied, the ITCs were more efficient than the other products, and the antimicrobial effects were dose-dependent.

A transgenic *Arabidopsis thaliana* that overexpressed p-hydroxybenzyl GLS was used to evaluate the capacity of GLS and their breakdown products to influence and modify the natural rhizosphere community. It was showed that the proteobacteria and also the fungal community in the rhizosphere of the transgenic plant were significantly affected. Modification of the GLS content of the plant could be an alternative to the use of pesticides [37].

Aires et al. [38] evaluated the antimicrobial activity of intact GLS and their hydrolysis products and microbial catabolites, against human pathogenic or gastrointestinal tract bacteria: the Gram-positive *E. faecalis*, *S. aureus*, and *Staphylococcus saprophyticus* and the Gram-negative *Acinetobacter baumannii*, *Citrobacter freundii*, *Enterobacter asburiae*, *Enterobacter cloacae*, *Enterobacter hormaechei*, *E. coli* (two strains), *Hafnia alvei*, *Klebsiella oxytoca*, *K. pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, *P. aeruginosa*, *S. typhi*, and *Stenotrophomonas maltophilia*. The intact GLS examined were sinigrin, glucoraphanin, glucotropaeolin, gluconasturtiin, and indole glucobrassicin, while the enzymatic hydrolysis products were AITC, SFN, BITC, PEITC, I3C, AC, BC, PEC, and 3,3′-di-indolylmethane (DIM). Allylamine (AAM), benzylamine (BAM), and 2-phenylethylamine (PAM), which are microbial metabolites of GLS, were also tested. Among the compounds tested, only ITCs were effective, but GLS, nitriles,

and amines were ineffective at all the doses used. The highest activity was shown by SFN and BITC. IAN had some inhibitory activity against the Gram-negative bacteria. I3C had some inhibitory effects against the Gram-positive bacteria but had no effect, even at the highest dose, against the Gram-negative bacteria. The compound, the concentration used, and the microorganism tested influence the antimicrobial activity of the GLS hydrolysis products. Some of these were more effective than conventional antibiotics in inhibiting the growth of pathogenic microorganisms, such as ITCs. The data reported in this study demonstrate the potential for using these natural antimicrobials as an alternative or in combination with antibiotic-based therapies for treating infectious diseases.

Some ITCs display a synergy with conventional antibiotics. Tajima et al. [39] examined different hydroxy ITCs for antimicrobial synergism with various antibiotics against *E. coli* and *S. aureus*. It was demonstrated that 2-(4-hydroxyphenyl) ethyl ITC displayed antimicrobial synergism with aminoglycosides, such as streptomycin, against *E. coli* and *S. aureus* grown in glucose-containing medium. However, small changes in the concentrations of both ITC and streptomycin affect their combined action from synergism to suppression of antimicrobial activity. The mechanism of synergism and suppression remains unclear [40].

Palaniappan et al. [41] examined the synergistic interaction between natural antimicrobials and antibiotics to which the target bacteria were resistant. Among the agents studied, AITC was effective in reducing the MIC of erythromycin when tested against *S. pyogenes*.

The antibacterial effect in vitro of PEITC and its synergistic effect with antibiotics against different *E. coli* from human and animal were demonstrated by Freitas et al. [42].

Many of the older references about the antimicrobial properties of ITCs were often related to the use of these compounds as preservatives in foods. Tressler and Joslyn [43] suggested that the Romans added large quantities of mustard seed to crushed grape for preservative purposes. The use of mustard oils to fruit juices and wines has apparently been practiced for generations in some parts of the world. Kosker et al. [44] showed the possibility of using AITC as preservative in fresh apple cider at a concentration of 20 ppm. Furthermore, it was shown that the thermal resistance of *Bacillus thermoacidurans* can be greatly reduced using AITC 10 ppm in buffer and fruit juices. Kostova et al. [9] studied the use of AITC in the disinfection of eggs. It was reported that AITC could control the growth of microorganisms on the surface of goose and hen eggs by application of the solution or as vapor. This method was not pursued because the AITC was absorbed through the shell.

The major pungent component of black mustard (*Brassica nigra*) and brown mustard (*B. juncea*), which is the same as that of wasabi (*Eutrema wasabi* Maxim.), is AITC. The antimicrobial activity of brown mustard AITC vapor and the possibility of its use as modified atmosphere packing were studied by Isshiki et al. [45]. The bacteria used were *B. subtilis* IFO-13722, *B. cereus* IFO-13494, *S. aureus* IFO-12732, *Staphylococcus epidermidis* IFO-12993, *E. coli* JCM-1649, *S. typhimurium* A TCC-14028, *S. enteritidis* JCM-1891, *V. parahaemolyticus*

IFO-12711, and *P. aeruginosa* IFO-13275. First, the antibacterial activity of AITC vapor, against each microorganism, was evaluated in Petri dishes, and then application experiments were carried out with different foods. AITC vapor inhibited the growth of all microorganisms examined in the experiments. In the application experiments, none of the tested samples were spoiled after 7 days, while the controls grew sufficiently after 2 days [45].

Ward et al. [46] evaluated the effectiveness of different concentrations of a volatile distillate extracted from fresh horseradish root against the growth of spoilage and pathogenic bacteria inoculated on agar and roast beef slices at 12 °C. The distillate was composed by about 90% AITC and 9% 2-phenethyl ITC, and the bacteria tested were *S. aureus*, *E. coli* O157:H7, *S. typhimurium*, *L. m*onocytogenes, *Serratia grimesii*, and *Lactobacillus sake. L. sake* was the most resistant: 20000 nL distillate L^{-1} air were required to completely inhibit growth on agar. On the other side 4000 nL distillate L^{-1} air completely inhibited the growth of *S. aureus*, *E. coli* O157:H7, *S. typhimurium*, *L. monocytogenes,* and *S. grimesii* on agar for 7 days in aerobic storage at 12 °C. These bacteria were more resistant when inoculated on roast beef: 20,000 nL distillate L^{-1} were required to completely inhibit the growth, and *L. sake* was weakly inhibited at this concentration.

Delaquis et al. [47] determined the effect of vaporized horseradish essential oil (HEO) on microbial growth in precooked roast beef slices contaminated with *Pseudomonas* spp. and *Enterobacteriaceae* and lactic acid bacteria. The slices were stored 28 days at 4 ± 2 °C in air or 100% N_2 with and without HEO. The results showed that 20 μL L^{-1} of HEO inhibited the growth of most spoilage bacteria and *Pseudomonas* spp. And *Enterobacteriaceae* were strongly inhibited than lactic acid bacteria that were more resistant. The chemical changes and sensory properties of precooked roast beef treated with HEO were also evaluated and revealed that the development of off-flavors and odors derived from fat oxidation products was delayed by HEO.

The bactericidal activity of AITC and MITC was tested on iceberg lettuce inoculated with a rifampicin-resistant strain of *Salmonella* Montevideo and streptomycin-resistant strains of *E. coli* O157:H7 and *L. monocytogenes* Scott A in sealed containers at 4 °C for 4 days. MITC was more active against *L. monocytogenes* than the other bacteria, while AITC showed stronger activity against *E. coli* O157:H7 and *S.* Montevideo. Furthermore in this study, the AITC was tested also on tomato stem scars and skin contaminated with *S.* Montevideo and on apple stem scars contaminated with *E. coli* O157:H7. *S.* Montevideo inoculated on tomato skin was more sensitive to AITC than that on stem scars. Treatment with vapor generated from 500 mL of AITC caused an 8-log reduction in bacteria on tomato skin but only a 5-log reduction on tomato stem scars. The bactericidal activity of AITC was weaker for *E. coli* O157:H7 on apple stem scars; only a 3-log reduction in bacteria occurred when 600 mL of AITC was used [48].

The incorporation of mustard flour (non-deheated) as an ingredient in packaged ground beef to inactivate *E. coli* O157:H7 was tested by Nadarajah et al. [49]. The results showed that it is possible to use mustard flour at levels of between 5 and 10% to eliminate *E. coli* O157:H7 from fresh ground beef. The sensory evaluation of

cooked ground beef was carried out and showed that there were no significant differences between the overall sensory acceptability of ground beef formulated with 5% and 10% mustard [49].

Four sausage batters (17.59% beef, 60.67% pork, and 17.59% pork fat) were inoculated with *P. pentosaceus* and *Staphylococcus carnosus* and a five-strain cocktail of nonpathogenic variants of *E. coli* O157:H7. Microencapsulated AITC was added to three batters at 500, 750, or 1000 ppm to determine its antimicrobial effects. *E. coli* O157:H7 was reduced by 6.5 log10 CFU g^{-1} in sausages containing 750 and 1000 ppm AITC after 21 and 16 days of processing, respectively. *E. coli* O157:H7 numbers were reduced by 4.75 log10 CFU g^{-1} after 28 days of processing in treatments with 500 ppm AITC, and the organism was not recovered from this treatment beyond 40 days [50].

The antimicrobial activity of AITC against growth of typical spoilage bacteria (*Lactobacillus algidus*, *L. mesenteroides*, *Leuconostoc carnosum*, *Carnobacterium maltaromaticum*, *Carnobacterium divergens*, *Brochothrix thermosphacta*, *Serratia proteamaculans*) from marinated pork was also investigated in vacuum-packed pork meat. MICs for AITC were difficult to determine because of the absence of gastight barrier between the wells of a single plate used in the experiment. As AITC exerts antimicrobial activity in both liquid and gas phases, the addition of AITC to one well affected bacterial growth in adjacent wells. In fact, the addition of AITC completely inhibited the growth of *S. proteamaculans* and *B. thermosphacta* even in control wells containing no AITC. To determine the MIC for AITC in liquid phase, experiments with sealed wells would have to be carried out. The ability of AITC to exert antimicrobial effects in its gas phase even at low concentrations may make it more useful for applications in modified atmosphere-packaged foods [51].

AITC was also incorporated into chitosan coatings to develop an antimicrobial application against *Salmonella* that would improve the safety and extend shelf life of whole fresh cantaloupe. It has been demonstrated that with AITC concentrations increasing from 10 to 60 μL mL^{-1}, the antibacterial effects of coating treatments against *Salmonella* increased, and no visual changes in overall appearance and color of cantaloupe rind and flesh due to coating treatments were observed [52].

AITC was encapsulated using gum Arabic and chitosan to overcome the problem of its high volatility to investigate the effect of microencapsulated AITC as a natural additive on the shelf life and quality of Kimchi, a traditional Korean fermented vegetable food. Encapsulated AITC addition to Kimchi resulted in positive changes in pH, titratable acidity, and microbial analysis compared to that of control. The number of *Leuconostoc* and *Lactobacillus* species in Kimchi decreased with an increase in the concentration of AITC. However, with regard to sensory analysis, AITC concentrations of 0.10% or lower are recommended for manufacturing Kimchi [53].

The antimicrobial effect of AITC entrapped in alpha and beta cyclodextrin inclusion complexes (IC) against different target organisms, among them *Escherichia coli* and *Listeria monocytogenes*, was determined. AITC entrapped in beta IC exhibited a significantly better antimicrobial effect compared to unentrapped AITC. The antimicrobial effect of beta IC was determined during aerobic storage of

packaged fresh-cut onions. This application of beta IC (200 mL L^{-1}) to packaged fresh-cut onions effectively decreased numbers of *L. monocytogenes* [54]. ITCs are used in food active packaging to reduce, inhibit, or retard the growth of microorganisms on food products. White mustard essential oil (WMEO) showed antimicrobial activity against *Salmonella* recovered from inoculated frozen vegetables and chicken particulates. The antibacterial property was due to the production of 4-hydroxybenzyl ITC obtained by the hydrolysis of the GLS sinalbin, present in white mustard essential oil derived from white mustard seeds (*Sinapis alba L.*) [55].

AITC in combination with modified atmosphere packaging (MAP) was tested to control the growth of *L. monocytogenes* and *S. typhimurium* on fresh chicken breasts during refrigerated storage for 21 days. On day 21, the microbial counts in the products packaged with AITC and MAP were lower than ambient air and MAP, even if AITC was less effective against *L. monocytogenes* than *S. typhimurium*. Furthermore, vapor AITC has been found to be more effective than liquid AITC [56], but its strong odor can limit its use in food systems. The use of AITC as a flavoring substance has been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and by the EFSA (European Food Safety Authority) Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC). This report concluded that there were no safety concerns from AITC consumption at the estimated levels of intake [57].

3 Antifungal Activity

One of the first studies that demonstrated antifungal activity of cruciferous plant was carried out in the 1930s, when these authors demonstrated in vitro toxicity of volatile compounds (AITC, PITC, MITC, EITC, ethyl TC, allyl sulfide, ethyl sulfide, and sinigrin) toward certain fungi (*Colletotrichum circinans*, *Botrytis allii*, *Aspergillus niger*, *A. alliaceus*, and *Gibberella saubinetti*) [58]. The antifungal property was corroborated by Hooker et al. [59], and after this, many others investigations were followed. In general, volatile sulfur compounds demonstrate more potent inhibitory effects toward fungi than bacteria [60].

Studies have shown that GLS did not present antimicrobial activity in their intact form, only after enzymatic hydrolysis. Therefore, sinigrin which is one of the most important GLS present in oriental mustard presented no effect against *Paecilomyces fumosoroseus* [61]. Sinigrin also did not affect *Alternaria brassicae* (causative agent of black spot) in Czapek-Dox agar medium, as well as sclerotium formation of *Sclerotinia sclerotiorum* (causative agent of stem rot) [62]. Native GLS had no fungitoxic activity, whereas their hydrolytic products, in particular glucoiberin, glucoerucin, glucoheirolin, and glucotropaeolin, inhibited growth of *Rhizoctonia solani*, *S. sclerotiorum*, *Diaporthe phaseolorum*, and *Pythium irregulare* with different inhibitory responses depending upon the chemical nature of the hydrolytic products [3, 63].

The composition of hydrolysis products from GLS varies according to substrate, pH conditions, presence of ferrous ions, and specific protein factors. The chemical

nature of the breakdown products depends mainly on the structure of the GLS, plant species, and reaction conditions [64]. They are classified as nitriles, TCs, epithionitriles, oxazolidine-2-thiones, ITCs, and epithioalkanes with different antimicrobial activity [65].

From these groups, ITC is the major inhibitor of microbial populations, and differences in the potential are related to the nature of their side chain [66]. Assays with ITCs have been conducted directly as a component of a growth medium and a model food system and (generally more antimicrobial effective) in the gaseous form [49]. Volatiles released from GLS, predominantly 2-propenyl GLS, showed toxic effects to the blackleg fungus, *Leptosphaeria maculans*, in vitro [67]. AITC gaseous at 0.1 mg L^{-1} for 4 h showed a fungistatic effect against *Botrytis cinerea* (gray mold) reducing by over 45% the incidence of the gray mold on strawberries [68]. AITC (2 ppm) inhibited the growth of *Penicillium roqueforti*, *P. corylophilum*, *Eurotium repens*, *A. flavus*, and *Endomyces fibuliger* on rye bread slices in airtight environment [69].

Sellam et al. [70] demonstrate that ITCs were effective in vitro in different development stages of *A. brassicicola* and *A. brassicae*. Moreover, antifungal activity of 57 substituted derivatives of PEITC was determined on *A. niger*, *Penicillium cyclopium*, *Rhizopus oryzae*, *A. flavus*, *A. oryzae*, *A. fumigatus*, *P. brevicompactum*, *Cladosporium herbarum*, *Trichoderma viride*, *Alternaria tenuis*, *Monilia sitophila, Cytospora* sp., *Schizophyllum commune*, *Fusarium* sp., *Cephalothecium roseum*, and *Trichophyton gypseum* in culture medium. The authors describe that several PEITC derivatives, as well as the most active natural ITC analogues, represent remarkable antifungal compounds; however, there are some differences in their antifungal potential [13].

Several studies have been conducted using glucosinolate-derived products against molds and yeast (Table 2). These examples show the efficiency of ITCs against saprophytic and parasitic fungal species, usually applied at low levels in culture medium, food products, and plant defense. In agricultural sciences, ITCs such as AITC have been effective fumigants on the control of insects and fungal species [71]. Among ITCs, allyl isothiocyanate (AITC) is one of the most studied. Beneficial biological effects have been reported including antibacterial, antifungal, anti-nematode, and anti-insect activities [72]. Its uses as natural preservative have been growing because of its food origin and low toxicity [30]. The antimicrobial activity of AITC, as well as other ITCs, is related with the concentration of the compound applied, time of exposure, strains, microbial loading, temperature, food composition, pH conditions, water activity, and on diffusion of the vapor in food packaging systems [48, 71, 73]. However, its use on food products usually is limited by the interference of organoleptic characteristics, its poor aqueous solubility, instability at high temperature, and intrinsic food compounds.

GLS-derived products have also been presenting antibiocidal potential against yeast. Kyung and Fleming [23] reported that AITC showed antifungal effects against fermentative yeasts on culture media with an MIC $\leq$ 4 ppm. *Candida albicans*, a fungus potentially pathogenic to human, was inhibited by fresh cauliflower juice (*Brassica oleracea* var. *botrytis*) [74]. In the use of BITC and 3- and

Table 2 Antifungal potential of glucosinolate-derived products against several species on food products

Glucosinolate derivative	Fungal strain	Food product	Reference
4-Hydroxybenzyl isothiocyanate	*Zygosaccharomyces bailii*	Acidified fruit drink	[75]
Yellow and oriental mustard (based on allyl isothiocyanate and p-hydroxybenzyl isothiocyanate)	*Aspergillus parasiticus CECT 2681*	Peanut, cashew, almonds, walnut, pistachio, hazelnut	[81]
Allyl isothiocyanate	*Penicillium roqueforti, P. corylophilum, Eurotium repens, A. flavus, Endomyces fibuliger*	Rye bread slices	[69]
Allyl benzyl phenyl isothiocyanates	*Gibberella moniliformis strains 2983, 5847, 5850*	Bread	[78]
Allyl isothiocyanate	*Botrytis cinérea*	Strawberries	[68]
Allyl isothiocyanate	*Aspergillus parasiticus*	Fresh pizza crust	[77]
Allyl isothiocyanate	*Aspergillus parasiticus, Fusarium poae*	Wheat flour	[80]
Benzyl isothiocyanate	*Alternaria alternata*	Tomato	[79]
Ethyl isothiocyanate	*Penicillium expansum*	Apple	[71]

4-methoxybenzyl ITCs, antifungal effects against *Aspergillus fumigatus* and *C. albicans* were revealed with MIC of 1 μg mL^{-1} (Radulovic et al., 2012). An essential oil obtained from white mustard seeds containing 25 mg L^{-1} of 4-hydroxybenzyl isothiocyanate (4HBITC)/L was able to stabilize an acidified fruit drink against acid-tolerant bacteria (*Gluconobacter* species) and preservative-resistant yeast (*Zygosaccharomyces bailii*) for 28 days at ambient temperature [75].

Thus, the ability of ITCs to reduce mycotoxigenic molds and mycotoxins was also investigated. *P. expansum* (patulin producer) was inhibited with $>$ 50 mg of AITC, whereas *A. parasiticus* (aflatoxin producer) in culture medium was sensible to doses $>$ 5 [76]. *Aspergillus parasiticus* was inactivated in fresh pizza crust after 30 days of AITC exposition and suppressed aflatoxin formation [77]. AITC, BITC, and PITC inhibited the growth of *Gibberella moniliformis* strains 2983, 5847, and 5850 and reduced 2.1–89.7% of the mycelium size. ITCs also reacted with FB_2 in bread reducing the levels by 73–100% [78]. Benzyl-ITC showed antifungal activity against *Alternaria alternata* on tomato [79] and ethyl-ITC against *P. expansum* on apple [71], both patulin producers. AITC gaseous at 0.1 μL L^{-1} was investigated to reduce aflatoxin produced by *A. parasiticus* and beauvericin and enniatins produced by *Fusarium*. The authors observed reduction of 6.9% to 23% mycotoxin levels while at 10 μL L^{-1}; AITC completely inhibited the production of mycotoxins for 30 days [80]. In a commercial packaging simulation, GLS present in yellow and oriental mustard flours reduced aflatoxin B_1, B_2, G_1, and G_2 in nuts (peanut, cashew, almonds, walnut, pistachio, and hazelnut). This reduction ranged from 83.1 to 87.2%

in the oriental mustard flour, whereas it was 27.0–32.5% in the yellow flour [81]. AITC reacted with beauvericin in solution, reducing from 20% to 100%, and in a food system, beauvericin was reduced from 10% to 65%, in a dose-dependent manner [82]. AITC, BITC, and PITC diminished fumonisin B_1 (FB_1) and B_2 (FB_2) levels in solution from 42% to 100%, and on fumigation treatment (50, 100, and 500 μL L^{-1}), ITCs were able to reduce 53–96% of FB_1 and 29–91% of FB_2 contained in corn products, with four reaction products identified through the reaction [83].

3.1 Plant Protection

GLS-derived products have been recognized as antimicrobial agents, and several studies demonstrated the ability to control soil-borne plant pathogens [84–87]. The GLS content in plant reaches about 1% (highly variable) of dry weight in some tissues of *Brassica* vegetables [88]. Plant species and age are the major determinants of GLS composition [89], but also other factors such as nutritional status of the plant, fungal infection, and insect damage have significant effect on the content in growing plants [64].

Qualitative and quantitative differences of GLS composition vary also among plant organs [89]. GLS are found mainly in seeds, siliques, and young leaves, while intermediate contents are detected in leaves, stems, and roots [90]. Indole GLS and their hydrolysis products found in large amounts in roots may be related to their higher stability in the soil than air [91]. These compounds play a role in the development of root disease, caused by *Plasmodiophora brassicae* [92]. Volatile compounds from macerated *Brassicae* root tissue inhibited the fungal pathogen of wheat, *Gaeumannomyces graminis* [87]. Nevertheless, roots of a transgenic *Arabidopsis thaliana* had altered the profile of GLS compared with non-transgenic, with influence in the microbial community on roots and active populations in the rhizosphere [37]. The rhizospheric strains of *Fusarium* showed a protective effect on *Lepidium sativum* against *Pythium ultimum*. Accumulation of ITCs in roots not only increases resistance of the plant but also gives a competitive advantage to *Fusarium* strains [93].

Degradation products of GLS showed an inhibition of *L. maculans* at concentrations greater than 40 μg mL^{-1} [94]. Cauliflower plants (*Brassica oleracea* var. *botrytis*) infected by *Peronospora parasitica* resistant to downy mildew presented higher sinigrin content than the susceptible variety. The susceptible seedlings exhibited a 12% decrease in glucobrassicin and a 25% increase in methoxyglucobrassicin when compared with healthy ones six days after treatment whereas no difference in glucobrassicin and a 10% increase in methoxyglucobrassicin were observed in healthy and inoculated resistant seedlings [95].

The disease resistance may be dependent on fungal pathogen species and the composition of GLS-derived products present in the plant [91]. *Arabidopsis thaliana* mutant extracts were investigated on *B. cinerea* and *Alternaria brassicicola* isolates. *A. brassicicola* was more affected by aliphatic GLS and ITCs, while *B. cinerea*

isolates showed variable composition-dependent sensitivity to GLS and their hydrolysis products [96]. Propenyl ITC and EITC demonstrated fungistatic potential at 0.3 μL, which inhibited mycelial growth and completely suppressed conidial and chlamydospore germination of four *Fusarium oxysporum* isolates. EITC, BITC, and PEITC were fungitoxic to *F. oxysporum* conidia and chlamydospores [97]. ITCs released from cabbage tissues were effective toward *P. parasitica*, *P. ultimum*, and *Sclerotium rolfsii* [98]. PEITC inhibited the growth of a range of fungi, oomycetes, and bacteria [99]. Pedras and Sorensen [100] observed that 5-(methylsulfamyl)-pentyl-1-ITC, 6-(methylsulfamyl)-hexyl-1-ITC, and 6-(methylsulfinyl)-hexyl-1-ITC inhibited spore germination of *Phoma lingam* virulent isolate BJ 125 at a concentration of 5×10^{-4} M. *Alternaria* infection was positively correlated with GLS content in 33 oilseed rape lines (*Brassica napus* L. ssp. *oleifera*) [101].

4 Structure Activity Relationships

The mechanism of ITC antimicrobial action is unclear, but some hypotheses have been proposed. The central electrophilic carbon of ITCs (R-N = C = S) undergoes rapid reaction with hydroxyls, amines, and thiols, generating products such as carbamates, thiourea, and thiocarbamates, respectively [102, 103]. Thereby, AITC reacted with glutathione, amino acids, proteins, water, alcohol, and sulfites [104, 105], and it was able to disintegrate the cysteine disulfide bond through an oxidative process [104, 106].

Zsolnai [19] reported that thioglycolate and cysteine could diminish the antibacterial action of ITCs. The study also describes that the antimicrobial action of ITCs may be linked to the inhibition of sulfhydryl enzymes. This finding is consistent with those observations of Luciano et al. [106], who reported that AITC was able to react with glutathione and cysteine naturally present in meat, which interfered on their antimicrobial activity. In addition, the presence of proteic substances reduced genotoxic activity of AITC, PEITC [107], and MITC [108], on which the compounds were able to cause DNA damage in *Salmonella*, *E. coli*, and human cells (Hep G2) [109].

Kojima and Ogawa [110] suggested that ITCs act by inhibiting the oxygen uptake by yeast through the uncoupler action of oxidative phosphorylation in the mitochondria of yeast, inhibiting the coupling between the electron transport and phosphorylation reactions, thus hindering the ATP synthesis. However, the levels to achieve both enzymatic and oxygen uptake inhibitions used in the study were 200 times greater than the actual MIC of the ITCs for those organisms [30].

It is not clear if AITC crosses membranes and enters the cytoplasm of prokaryotic and eukaryotic cells or if it has an effect on cell membranes. Inside a cell, AITC can react with glutathione, sulfites, amino acids, oligopeptides, proteins, and water [111]. Delaquis and Mazza [10] suggest that AITC might cause inactivation of essential intracellular enzymes through oxidative cleavage of disulfide bonds. Lin et al. [112] observed damages on the bacteria cell by exposition to AITC, creating pores on cell membranes and inducing leaking of cellular substances. AITC was able

to modify the internal structure of *L. monocytogenes* when compared to non-treated cells when analyzed by transmission electron microscopy [104]. On the other hand, Ahn et al. [113] observed no damage in cell wall or leakage of ATP when AITC was tested against *L. monocytogenes*. The reduction of ATP could be the result of inhibition of enzymes related to ATP formation or depletion of proton motive force.

The mechanism of fungal death by ITCs was investigated by Calmes et al. [114]. Exposure of AITC, PEITC, and BITC in *A. brassicicola* decreased oxygen consumption rate, intracellular accumulation of reactive oxygen species (ROS), and mitochondrial membrane depolarization. The two major regulators of the response to oxidative stress, MAP kinase AbHog1 and the transcription factor AbAP1, were activated in the presence of ITCs. Once activated by ITC-derived ROS, AbAP1 may promote the expression of different oxidative-response genes. Besides, fungal strains deficient in AbHog1 or AbAP1 were hypersensitive to ITCs, and it might be useful to understand the mechanism of fungal resistance. In other studies, the authors [13] suggest some differences on the mode of action of 57 ITCs and related compounds investigated against *A. niger*, *P. cyclopium*, *Rhizopus oryzae*, and other species. These variations occurred in compounds in which -NCS group is directly bound on the aromatic moiety compared with the bounds on aliphatic radical. Normally, aromatic ITCs are more toxic than aliphatic, and the fungal toxicity of aliphatic ITCs decreased with the increasing length of the side chain [64].

Furthermore, it may be considered that AITC degraded in aqueous solution at 37 °C, generating allyl dithiocarbamate, diallyl tetra- and pentasulfide, sulfur, and N, N'-diallylthiourea, dependent on temperature and pH conditions [104] However, there is no information relating this degradation to ITC's antimicrobial potential [30].

5 Conclusions

With the current trend, natural compounds are preferred and widely studied. Considering the data from several studies carried out, it may be observed that glucosinolates demonstrate a biocidal effect after their enzymatic hydrolysis. These breakdown products show a huge antibacterial and antifungal capacity, and they may be used on food preservation as well as plant defense. Several studies have demonstrated that the structure of glucosinolates and the microbial strain are responsible for their antimicrobial potential. Among the GLS hydrolysis products, ITCs are the main group that demonstrated an efficiency to reduce microbial growth. Allyl isothiocyanates are the most investigated ITCs against microorganisms, and its use as a fumigant agent on food preservation has been investigated.

There is not enough information regarding the mechanism behind the antimicrobial activity of GLS. Studies indicated that the central electrophilic carbon of ITCs may react with hydroxyls, amines, and thiols. However, it is not clear if ITC crosses the membrane and enters the cytoplasm or if they have an effect on cell membranes. Thus, further studies are necessary to clarify the mechanism of these active

compounds on microorganisms and evaluate the feasibility application of GLS products as food preservative through fumigation treatment.

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