

Chemical and biochemical mechanisms underlying the cardioprotective roles of dietary organopolysulfides

Article

Published Version

Creative Commons: Attribution 4.0 (CC-BY)

Open Access

Tocmo, R. ORCID: <https://orcid.org/0009-0007-4850-977X>,
Liang, D., Lin, Y. and Huang, D. (2015) Chemical and
biochemical mechanisms underlying the cardioprotective roles
of dietary organopolysulfides. *Frontiers in Nutrition*, 2. 1. ISSN
2296-861X doi: <https://doi.org/10.3389/fnut.2015.00001>
Available at <https://centaur.reading.ac.uk/117263/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

To link to this article DOI: <http://dx.doi.org/10.3389/fnut.2015.00001>

Publisher: Frontiers

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online



Chemical and biochemical mechanisms underlying the cardioprotective roles of dietary organopolysulfides

Restituto Tocmo¹, Dong Liang¹, Yi Lin² and Dejian Huang^{1,2*}

¹ Food Science and Technology Programme, Department of Chemistry, National University of Singapore, Singapore, Singapore

² National University of Singapore (Suzhou) Research Institute, Jiangsu, China

Edited by:

Sascha Rohn, University of Hamburg, Germany

Reviewed by:

Marcin Szymanski, Poznan University of Medical Sciences, Poland
Franziska S. Hanschen, Leibniz-Institute of Vegetable and Ornamental Crops Großbeeren/Erfurt e.V., Germany

*Correspondence:

Dejian Huang, Food Science and Technology Programme, Department of Chemistry, National University of Singapore, Science Drive 2, 117543 Singapore
e-mail: chmhj@nus.edu.sg

Foods that are rich in organosulfides are highly regarded for their broad range of functions in disease prevention and health promotion since ancient time yet modern scientific study, particularly clinical studies could not agree with traditional wisdom. One of the complexities is due to the labile nature of organosulfides, which are often transformed to different structures depending on the processing conditions. The recent evidence on polysulfides as H₂S donors may open up a new avenue for establishing structure and health promotion activity relationship. To put this development into perspective, we carried out a review on the recent progress on the chemistry and biochemistry of organopolysulfides with emphasis on their cardioprotective property. First, we briefly surveyed the foods that are rich in polysulfides and their structural diversity. This is followed by in-depth discussion on the chemical transformations of polysulfides under various processing conditions. We further reviewed the potential action mechanisms of polysulfides in cardioprotection through: (a) hydrogen sulfide releasing activity; (b) radical scavenging activity; and (c) activity in enzyme inhibition and intervention of gene regulation pathways. Based on the literature trend, we can conclude that the emerging concept of organopolysulfides as naturally occurring H₂S donors is intriguing and warrants further research to establish the structure and activity relationship of the organopolysulfides as H₂S donors.

Keywords: *Alliums*, stinky beans, dietary organopolysulfides, hydrogen sulfide, cardioprotection

INTRODUCTION

According to the World Health Organization data, cardiovascular disease (CVD) is the number one cause of deaths and there will be estimated 23.3 million people who will die due to CVD by 2030 globally, which is more than the total population of Australia (1). Finding effective means for reducing the risk factors of CVDs has been a long-standing research theme for decades and much progress has been made and healthy diet is critically important. Fruits and vegetable are the key components of a healthy diet in part because they are rich in bioactive phytochemicals such as polyphenolic antioxidants, anti-inflammatory agents, and poly unsaturated fatty acids, particularly omega-3 fatty acids. For example, flavanols are shown to improve endothelium-mediated vasodilation (2) and European Food Safety Authority has approved the claim that consumption of 200 mg flavanols from cocoa daily can improve blood flow (3). In addition, cumulative evidence has suggested that dietary organosulfur compounds have a wide range of bioactivity, particularly cardiovascular health (4).

Cruciferous vegetables and the *Allium* family are known for their rich contents of bioactive organosulfur compounds (Figure 1). Cruciferous vegetables are rich in glucosinolates, which undergo hydrolysis by thioglucosidase (myrosinase) to isothiocyanates (5) including sulforaphane in the broccoli (6), benzyl isothiocyanate in garden cress (7), and phenyl-ethyl isothiocyanate in watercress (8). While isothiocyanates from cruciferous vegetables have received great attention because of their potential

anti-cancer activity through modulation of phase II enzyme activities (9, 10), the organosulfides in *Alliums* are well known for their broad spectrum of health promoting benefits, including antimicrobial, anti-cancer, and cardioprotective effects (4, 11). Yet, there is lack of consistent human clinical evidence to support the traditional wisdom. The chemistry of dietary organosulfur compounds is particularly complex because of their sensitivity to structural transformation mediated by the enzymes in the vegetables or during food processing. Consequently, it is a challenge to establish structure and bioactivity relationships. The bioactivity of allicin from garlic has been extensively studied and reviewed. However, human clinical trials in garlic found that allicin has no effect on reducing cholesterol level (12). The other important organosulfides in *Alliums* are volatile polysulfides readily formed when garlic is processed. They have shown potential for cardiovascular health promotion. Of all the *Allium* species currently known, garlic (*Allium sativum*) and onion (*Allium cepa*) are the most popular species due to various health benefits associated with their consumption. Garlic and onions have been greatly valued for their medicinal uses throughout the history of civilization. A large amount of literature have shown that the organosulfur compounds in these two species are associated with their biological functions against chronic ailments, including cancer, diabetes, and CVDs (4, 11). Yet, the labile chemistry of dietary organosulfides makes it challenging to establish structure and activity relationship. In this review, we summarized recent literatures regarding the

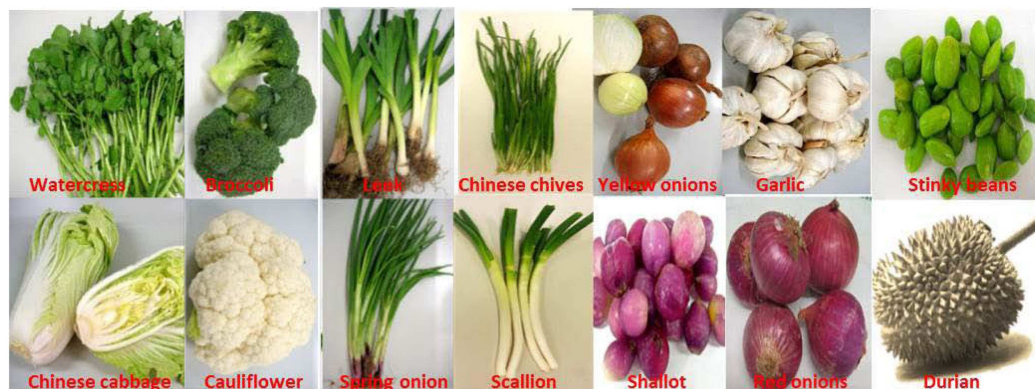


FIGURE 1 | Organosulfide-rich fruits and vegetables.

organopolysulfide chemistry, biochemistry, and potential action mechanisms for promoting cardiovascular health, particularly as natural donor of H_2S . Based on this, the future research directions of dietary organopolysulfides are suggested.

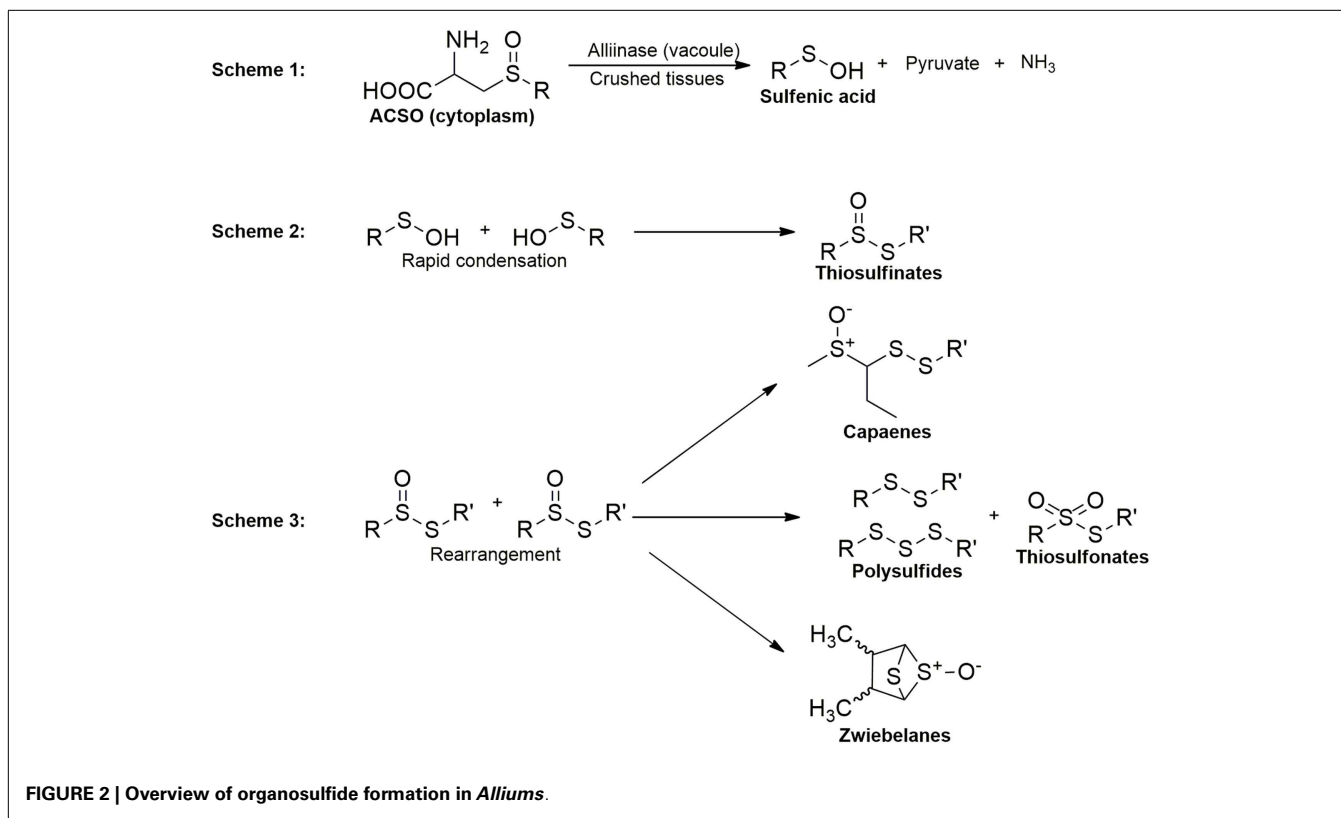
BRIEF SURVEY OF ORGANOSULFIDES RICH FOODS

The organosulfides in *Alliums* are classified into two major groups: (1) oil-soluble polysulfides and (2) water-soluble thiosulfonates, the intermediate formed upon the reaction of the vacuolar enzyme alliinase with the non-volatile *S*-alk(en)yl-L-cysteine-sulfoxides (ACSOs) present in the cell cytoplasm when *Alliums* are crushed. Thiosulfonates from garlic and onions are known for their wide biological activities, including antithrombotic, antihypertensive, antioxidant, antibacterial, and antifungal effects and these biological properties have been reviewed elsewhere (13, 14). Organopolysulfides (di-, tri-, and tetrasulfides) are the major OSCs in the oil-soluble components of *Alliums*. The formation of these lipophilic compounds starts with the alliinase-ACSO reaction, which produces highly unstable intermediates sulfenic acids, pyruvate, and ammonia. Condensation of sulfenic acids leads to the formation of thiosulfonates, which undergo further rearrangements into polysulfides and other OSCs, including cepaenes and zwiebelanes (Figure 2) (15). Alliinase is the key enzyme that facilitates the formation of the oil-soluble OSCs. Due to the compartmentalization of this enzyme and the ACSOs, cell rupture by cutting or maceration is necessary to facilitate their release (16) for the reaction to take place.

Aside from glucosinolates and isothiocyanates, *S*-methyl-L-cysteine sulfoxide (MCSO, or methiin), is present in vegetables of genus *Brassica* (17). MCSO significantly contributes to the typical spicy and pungent aromas of culinary processed *Brassica* (18). Similar to *Alliums*, enzymatic catabolism of MCSO in *Brassicaceae* generates other sensory-active sulfur compounds including dimethyl thiosulfonate, dimethyl thiosulfinate, and dimethyl sulfides (19). The enzyme responsible, termed cystine lyase (EC 4.4.1.8), behaves similarly as the alliinase in garlic, except that it can also hydrolyze L-cystine (20). Hydrolysis of MCSO generates highly reactive methyl sulfenic acid, which condenses to generate methyl methanethiosulfinate. Subsequently, thermal degradation

of methyl methanethiosulfinate forms volatile polysulfides, majority of which are composed of dimethyl disulfide and dimethyl trisulfide (18). The occurrence, concentration, and distribution of MCSO in cruciferous vegetables are well documented (18, 19, 21, 22) and its biological functions have recently been reviewed in Ref. (23). In general, MCSO is found at about 1–2% dry weight in vegetables that belong to *Brassicaceae*, especially those of the genus *Brassica* (24). Although MCSO is universally present in *Brassicaceae*, factors associated to species and varietal differences influence the concentration and distribution of MCSO in these plants (23). Other factors affecting MCSO concentrations include environmental conditions, nutrient availability, harvest timing, and storage practices (25–27). While it is generally accepted that MCSO and other thermally generated breakdown products such as *S*-methyl methanethiosulphinates and *S*-methyl methanethiosulfonates, contribute to the typical flavor of processed cruciferous vegetables, their cardiovascular effect, although limited, has been reported (28, 29).

Garlic has been used for centuries as a traditional remedy to treat infectious diseases (30, 31). One of the main sulfur-containing compounds present in raw garlic is γ -glutamylcysteine. It has been proposed that γ -glutamylcysteine, along with glutathione are the starting compounds, which undergo hydrolysis and oxidation leading to the biosynthesis of ACSOs (13). In garlic, *S*-allyl-L-cysteine sulfoxide (alliin) is the predominant ACSO, while *S*-methyl-L-cysteine sulfoxide (methiin) and *S*-propenyl-L-cysteine sulfoxide (isoalliin) are present in smaller amounts (32). Another major compound formed upon hydrolysis of garlic is *S*-allylcysteine (SAC), a water-soluble thiosulfonate produced during aqueous garlic extraction catalyzed by the enzyme γ -glutamyl transpeptidase (33). Some of the health-beneficial functions of garlic are attributed to its organosulfur components, including the main ACSO precursor alliin and allicin, a thiosulfonate resulting from the lyses of alliin by alliinase. However, allicin is a transient compound that rapidly undergoes non-enzymatic decomposition into numerous oil-soluble polysulfides such as diallyl monosulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), diallyl tetrasulfide, and allyl methyl trisulfide (34, 35). The main components of garlic oil (Table 1) obtained by steam



and hydrodistillation are DADS, DATS, allyl methyl trisulfide, and 2-vinyl-4H-1,3-dithiin (36–40). All eight thiosulfinates containing combinations of methyl, 1-propenyl, and 2-propenyl substituents are present in garlic homogenates, which explains the presence of lipid-soluble polysulfides with similar substituent combinations in distilled garlic oil (41).

The organosulfide profile of onion oil includes polysulfides with different combinations of methyl, propyl, and 1-propenyl substituents, except for the compound with 1-propenyl and 1-propene substituents on either side of the $-\text{S}-\text{S}(=\text{O})-$ group of a thiosulfinate (60). 1-Propenyl 1-propenethiosulfinate ($\text{CH}_3-\text{CH}=\text{CH}-\text{SS}(\text{O})\text{CH}=\text{CH}-\text{CH}_3$) has not been detected in *Allium* tissues possibly due to its oxidation to bisulfine or cyclization to zwiebelanes and a possible reaction with sulfenic acids to form capaenes (15, 32).

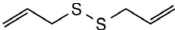
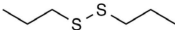
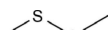
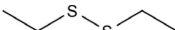
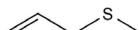
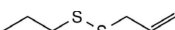


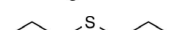

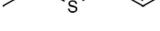
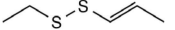
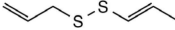
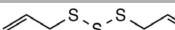


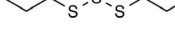
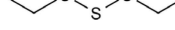
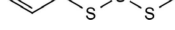
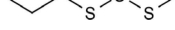

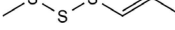
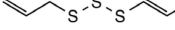
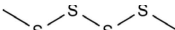
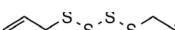
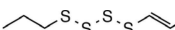

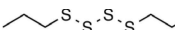
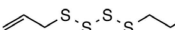
Distilled oils and solvent extracts from other *Allium* species are also known for their predominantly high contents of organosulfur compounds. These species include Chinese chive (*Allium tuberosum* Rottl. ex.), leek (*Allium porrum* L.), shallot (*Allium cepa* L. Aggregatum Group), rakkyo (*Allium chinense* G. Don), scallion (*Allium fistulosum* L. var *caespitosum*), and Welsh onions (*Allium fistulosum* L. var *maichuon*) (Figure 1). Summarized in Table 2 are the concentrations of the various oil-soluble polysulfides from their respective sources. Apparently, the abundance of the individual compounds depends on which *Allium* species they are found, although it is well known that garlic oil is rich in allyl polysulfides while onion oil is typically characterized by their high amounts of polysulfides with propyl substituents. While varietal differences

may have effects on the levels of polysulfides, it is rather premature to conclude on this yet due to limited literature available.

Shallot is an important *Allium* species commonly used in many Asian diets. High amounts of organosulfides have been reported in the distilled and solvent extracted oils of shallot (42). Data from our lab also shown that hydrodistilled oil from shallot originating from Vietnam contain high amounts of onion-type polysulfides (52). Distilled oil and solvent extracts of Welsh onions and scallions consists 82–87% of organosulfides (50, 62). Similarly, organosulfides from the essential oils of Chinese chive and rakkyo comprised 88–94% of their total volatiles (44). Twelve varieties of solvent extracted oil from Chinese chive were found to contain disulfides, trisulfides, and vinylthiins formed enzymatically from methiin as their precursor (46). The individual polysulfides in these less popular *Allium* species are listed in Table 1.

Organosulfur compounds are also present in the seeds of *Parkia speciosa* Hassk, commonly known as “petai” or stinky bean because of its unpleasant smell. In addition to its culinary uses, stinky bean is believed to have anti-microbial (65, 66), antioxidant (67–69), hypoglycemic (70), antiulcer (71), and antihypertensive (69) effects. Cyclic polysulfides are the major components of cooked petai (63). Earlier reports indicate that cyclic polysulfides [1,2,4-trithiolane (1), 1,3,5-trithiane (2), 1,2,4,6-tetrathiepane (5), and 1,2,3,5,6-pentathiepane (lenthionine) (7), 1,2,4,5,7,8-hexathionine] were the major constituents of stinky bean (Figure 3) (65, 68). In another study, hydrogen sulfide was found as the most abundant (41.3%) headspace constituent of stinky bean (64). Other cyclic polysulfides that

Table 1 | Acyclic and cyclic organopolysulfides from the oil-soluble components of common dietary sources.

Organosulfur compound	Structure	Source	Reference
ACYCLIC DISULFIDE			
Diallyl disulfide		Garlic, shallot, onion, leek, Chinese chive, rakkyo	(42–48)
Dipropyl disulfide		Onion, shallot, leek, scallion, Welsh onion, rakkyo	(42, 45, 48–52)
Dimethyl disulfide		Shallot, garlic, scallion, Welsh onion, Chinese chive, rakkyo	(43, 44, 46, 47, 49–51, 53)
Diethyl disulfide		Durian	(54–58)
Allyl methyl disulfide		Garlic, Chinese chive, Rakkyo	(40, 44, 46, 47, 53)
Allyl propyl disulfide		Garlic	(53)
Methyl propyl disulfide		Leek, onion, shallot, scallion, Welsh onion, Rakkyo, Chinese chive	(42–45, 49–51)
Methyl ethyl disulfide		Durian, Rakkyo, Chinese chive	(44, 59)
Ethyl propyl disulfide		Durian, onion	(49, 59)
Methyl 1-propenyl disulfide		Leek, garlic, onion, shallot, scallion, Welsh onion, Chinese chive, rakkyo	(40, 42–46, 49–51, 53)
Ethyl 1-propenyl disulfide		Rakkyo, Chinese chive	(44)
Allyl 1-(E)-propenyl disulfide		Chinese chive	(46)
Propyl 1-propenyl disulfide		Leek, onion, shallot, scallion, Welsh onion, Chinese chive, rakkyo	(42–45, 49–51)
ACYCLIC TRISULFIDE			
Diallyl trisulfide		Garlic, rakkyo, Chinese chive	(40, 44, 47, 53)
Dimethyl trisulfide		Leek, garlic, shallot, onion, scallion, Welsh onion, Chinese chive, rakkyo	(42, 43, 45, 46, 49–52)
Dipropyl trisulfide		Leek, shallot, Chinese chive, onion, rakkyo	(42, 44, 45, 48, 49, 52)
Diethyl trisulfide		Durian	(54–57, 59)
Allyl methyl trisulfide		Garlic, Chinese chive, rakkyo	(40, 44, 46, 47, 51, 53)
Methyl propyl trisulfide		Onion, shallot, Chinese chive, rakkyo	(42–44, 49, 51, 52)
Ethyl methyl trisulfide		Rakkyo, Chinese chive	(44)
Methyl 1-propenyl trisulfide		Onion, shallot, Rakkyo, Chinese chive	(42–44, 51)
Allyl 1-propenyl trisulfide		Garlic, rakkyo	(51)
Propyl 1-propenyl trisulfide		Onion, shallot, Chinese chive	(42–44, 49)
ACYCLIC TETRASULFIDE			
Dimethyl tetrasulfide		Shallot, onion, rakkyo, Chinese chive	(43, 44, 51)
Diallyl tetrasulfide		Garlic	(40)
Propyl 1-propenyl tetrasulfide		Chinese chive, rakkyo	(44)
Methyl pentyl tetrasulfide		Chinese chive	(44)
Dipropyl tetrasulfide		Chinese chive, leek	(44, 48)
Allyl propyl tetrasulfide		Chinese chive	(44)

(Continued)

Table 1 | Continued

Organosulfur compound	Structure	Source	Reference
CYCLIC POLYSULFIDES			
3-Vinyl-[4H]-1,2-dithiin		Garlic, Chinese chive	(46, 51, 53)
2-Vinyl-[4H]-1,3-dithiin		Garlic, Chinese chive	(46, 51, 53)
[3H,4H]-1,2-dithiin		Chinese chive	(46)
[2H,4H]-1,3-dithiin		Chinese chive	(46)
3,5-Diethyl-1,2,4-trithiolane		Leek, garlic, scallion, Welsh onion, rakkyo	(45, 50, 51, 53)
3,5-dimethyl-1,2,4-trithiolane		Durian, shallot	(52, 59)
3-Methyl-5-ethyl-1,2,4-trithiolane		Scallion, Welsh onion, rakkyo	(50)
3-Ethyl-1,2-dithi-4-ene		Onion, shallot	(43, 49, 52)
3-Ethyl-1,2-dithi-5-ene		Onion, shallot	(43, 52)

have been found from stinky bean, include 3,5-dimethyl-1,2,4-trithiolane (3), 1,2,4,5-tetrathiane (4), 1,2,4,5-tetrathiocane (6), 1,2,3,4,5,6-hexathiepane (8), and 1,2,4,5,7,8-hexathionane (9).

Of the edible fruits, durian (*Durio zibethinus* Murray) is perhaps the only tropical fruit known for its organosulfur contents. Durian, dubbed as the king of tropical fruits, is an exotic fruit with extensive popularity in Southeast Asia due to its distinct taste, odor, and texture. Of the 108 volatiles compounds determined in durian, 18 organosulfides were identified, making sulfurous compounds the second major volatile constituents, after ester group (56). Durian from Indonesia was reported to contain 17 (55) and 43 (72) organosulfides in two individual studies, with some common organosulfides detected such as *S*-ethyl thioacetate, diethyl disulfide, and 3,5-dimethyl-1,2,4-trithiolane. The volatile constituents of three different durian varieties D15, D28, and D74 were compared and it was found that D28 has the highest organosulfides content (54). In a separate study, organosulfides were the predominant compounds in durian in terms of quantity, contributing to more than 50% of the total volatiles (57).

Overall, there are various natural organosulfide sources including durian, the *Allium* vegetables, and stinky bean with characteristically high amounts of organosulfides. The formation of garlic- and onion-type OSCs follow a similar reaction mechanism with the ACSO-alliinase reaction as the main step after *Alliums* are crushed. Except for garlic and onions, the biological functions of the OSCs from these dietary sources are not yet fully explored.

TRANSFORMATIONS OF DIETARY ORGANOSULFIDES UNDER DIFFERENT PROCESSING CONDITIONS

Unlike fruits, vegetables are commonly cooked before they are consumed, except in the case where they are added as ingredients to salads. In general, cooking methods that involve heating (i.e., boiling, steaming, and microwaving) are commonly used to prepare homemade dishes. The most common commercially available form of *Allium* products is the raw form. However, other forms of *Allium* products have increased their popularity and availability in the international market (11). Common *Allium* products available in the market include powdered or dried garlic flakes, garlic and onion essential oils, naturally fermented garlic or aged garlic extract (AGE), pickled fermented and unfermented garlic, and pastes (73). An overview of the various processed forms of *Allium* products is shown in Figure 4.

The study on the chemical transformation of single organosulfide would shed some light on the key reaction principles and the factors governing the transformation in complex food matrix. Sulfoxides are known to undergo β -elimination reaction upon heating and this property has been applied in organic synthesis of olefins sulfenic acids as by-products (74). In principle, ASCOs can form sulfenic acid through thermal elimination perhaps at higher temperature cooking in food matrix. However, this has not been reported in the literature yet. Instead, the focus is on alliinase catalyzed β -elimination reaction at room temperature. The resulting sulfenic acid quickly dehydrates to form allicin, which

Table 2 | Continued

Organosulfur compound	Source	Concentration (GC% area)
[2H,4H]-1,3-dithiin	Garlic	1.85 ²
3,5-Diethyl-1,2,4-trithiolane	Garlic	1.00 ¹ , 0.50 ³
	Welsh onion	11.39 ⁸
	Rakkyo	8.42 ⁸
3,5-Dimethyl-1,2,4-trithiolane	Durian	2.30 ⁹ , 3.91 ¹⁰
3-Ethyl-1,2-dithi-4-ene	Onion	0.76 ⁵
3-Ethyl-1,2-dithi-5-ene	Onion	0.70 ⁵
1,2,4-trithiolane	Stinky bean	50.68 ¹¹ , 4.75 ¹²
1,3,5-trithiane	Stinky bean	0.21 ¹²
1,2,4,5-tetrathiane	Stinky bean	2.53 ¹¹ , 0.34 ¹²
1,2,4,6-tetrathiepane	stinky bean	11.21 ¹¹
1,2,3,5,6-pentathiepane	stinky bean	3.78 ¹¹

References: ¹(38); ²(40); ³(37); ⁴(44); ⁵(61); ⁶(42); ⁷(50); ⁸(62); ⁹(59); ¹⁰(58); ¹¹(63); ¹²(64).

GC Column used: ¹SE-52 on Chromosorb B; ^{2,3}HP-5MS capillary column; ⁴DB-1 fused silica capillary column; ⁵DB-5 fused silica column; ⁶SCOT Carbowax 20M; ^{7,8}HP-1 fused silica capillary column; ⁹DB-FFAP column; ¹⁰SPB-5 column; ¹¹SPB-1 column; ¹²TC-WAX capillary column.

may undergo β -elimination again to regenerate sulfenic acid and 2-propenethial. The latter leads to the formation of cyclic sulfides through Diels–Alder reaction (75). Formation of disulfides and polysulfides from allicin was proposed to be mediated by acid (76) (Figure 5). Beta-elimination from methyl substituted sulfoxide, S-methyl-L-cysteine sulfoxide, were shown to give rise to primarily dimethyl disulfides, which are responsible for the characteristic flavors of *Alliums* (18). Cyclic sulfides from stinky beans are not as common. Chemically, they are likely to be formed from formaldehyde and hydrogen sulfide followed by oxidation. In biological system, their formation mechanisms remain to be elucidated (63–65).

In home cooking, cloves or whole onions are often cut into small pieces or crushed into pastes. Biotransformation of organosulfides in *Alliums* begins when cytoplasmic ACSOs are released and mixed with the vacuolar alliinase. When garlic cloves or onion are cooked as whole, deactivation of the alliinase would limit the transformation of ACSOs, whereas fresh macerates of garlic or onion would have high contents of thiosulfonates. Thiosulfonates were found to be present in *Allium* homogenate with stability lasting for 26 h under room temperature, except for considerable losses observed in those represented by $\text{MeCH}=\text{CHS}(\text{O})\text{SR}$ and $\text{MeS}(\text{O})\text{SMe}$ (60). Thiosulfonates can decompose to generate other form of organosulfides (i.e., linear polysulfides) (15). This decomposition is greatly affected by typical processing parameters such as temperature, pH, and storage time (77, 78). Commercial *Allium* products are available in the form of macerates (e.g., crushed garlic in oil) or homogenates, but the more commonly available ones are freeze-dried powder, essential oil, or cloves in fermented or acidified brine (11, 79). In garlic or onion essential oils, thermal decomposition of thiosulfonates generates various mono-, di- and trisulfides as a result of different distillation processes (11, 13).

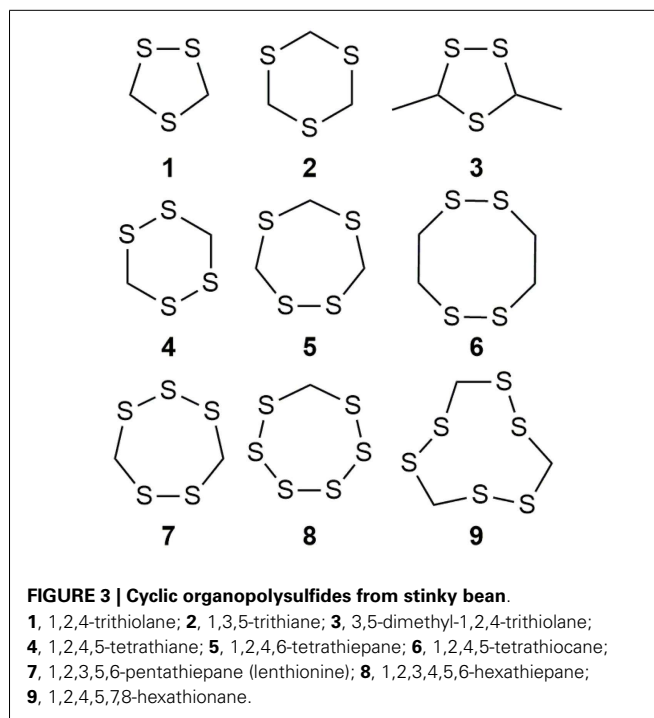
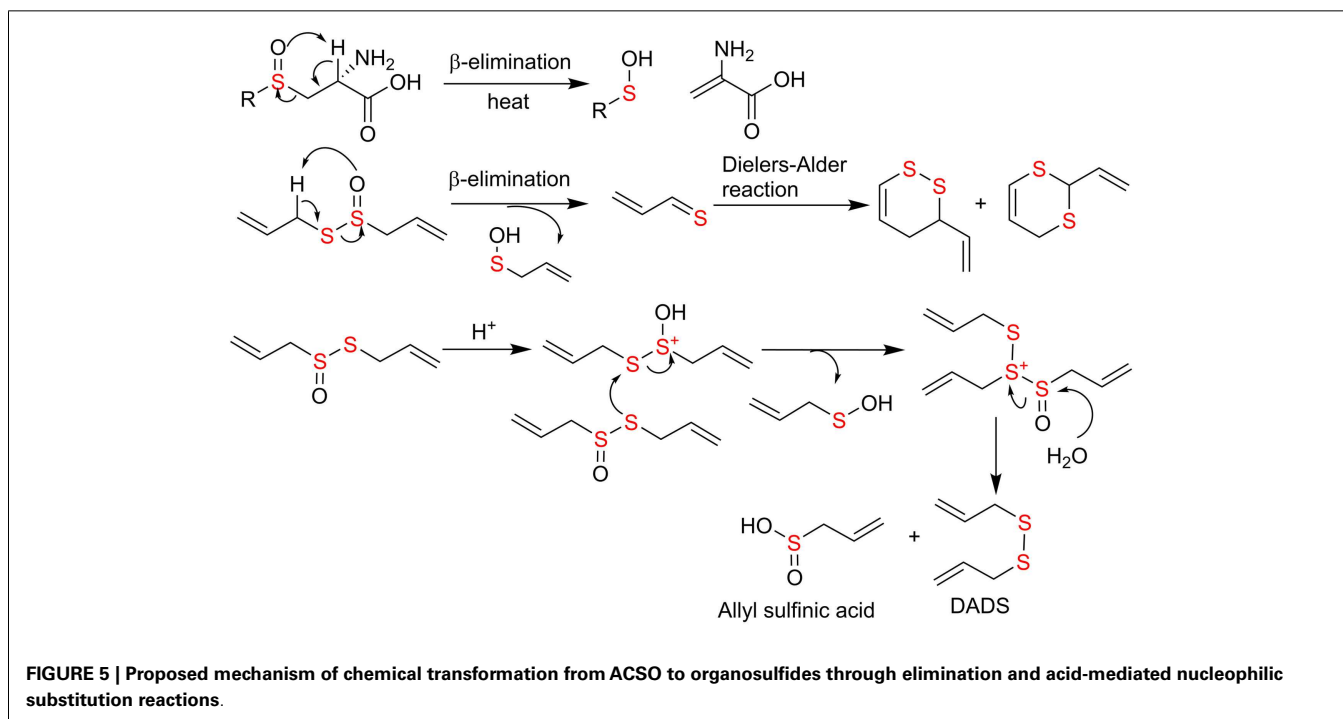
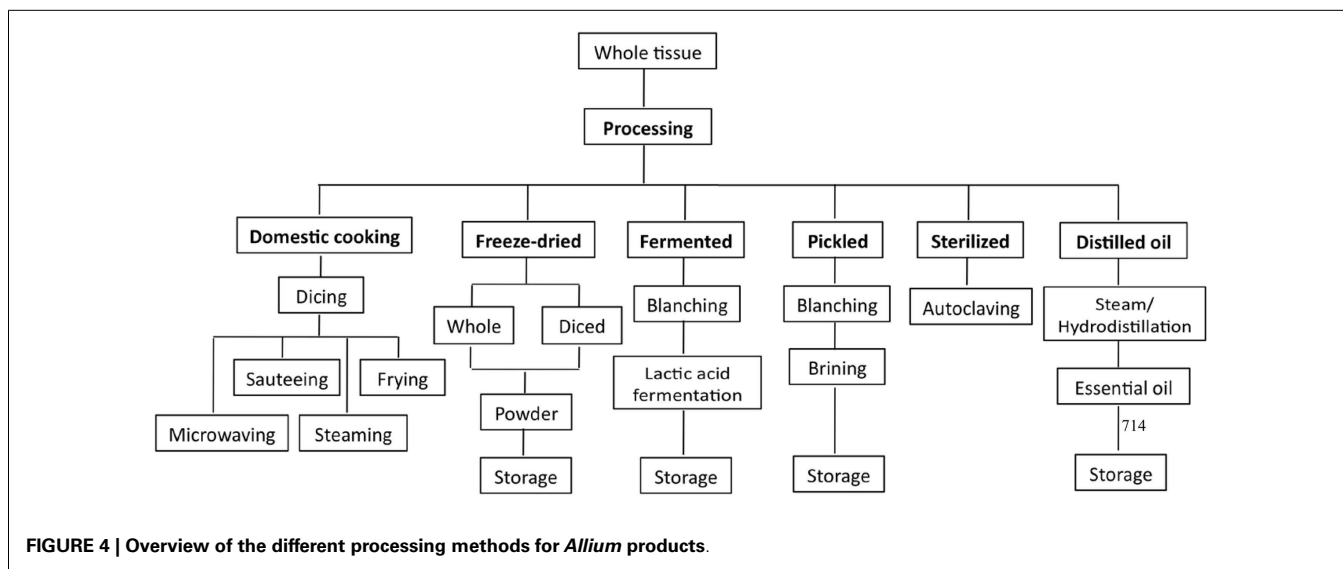


FIGURE 3 | Cyclic organopolysulfides from stinky bean.

1, 1,2,4-trithiolane; 2, 1,3,5-trithiane; 3, 3,5-dimethyl-1,2,4-trithiolane; 4, 1,2,4,5-tetrathiane; 5, 1,2,4,6-tetrathiepane; 6, 1,2,4,5-tetrathiocane; 7, 1,2,3,5,6-pentathiepane (lenthionine); 8, 1,2,3,4,5,6-hexathiepane; 9, 1,2,4,5,7,8-hexathionane.

Indeed, GC-MS analysis of *Allium* essential oil has revealed predominance of mixed polysulfides (Table 1). Essential oils from *Alliums* are highly valued for their medical and biological functions because of their contents of organopolysulfides, including DADS, DATS, allyl methyl trisulfide, diallyl tetrasulfide in garlic distilled oil and dipropyl disulfide, dipropyl trisulfide, methyl propenyl disulfide, and methyl propyl trisulfide in distilled onion oil (11). An overview of the organosulfide transformations in *Alliums* as affected by various processing conditions is illustrated in Figure 6.

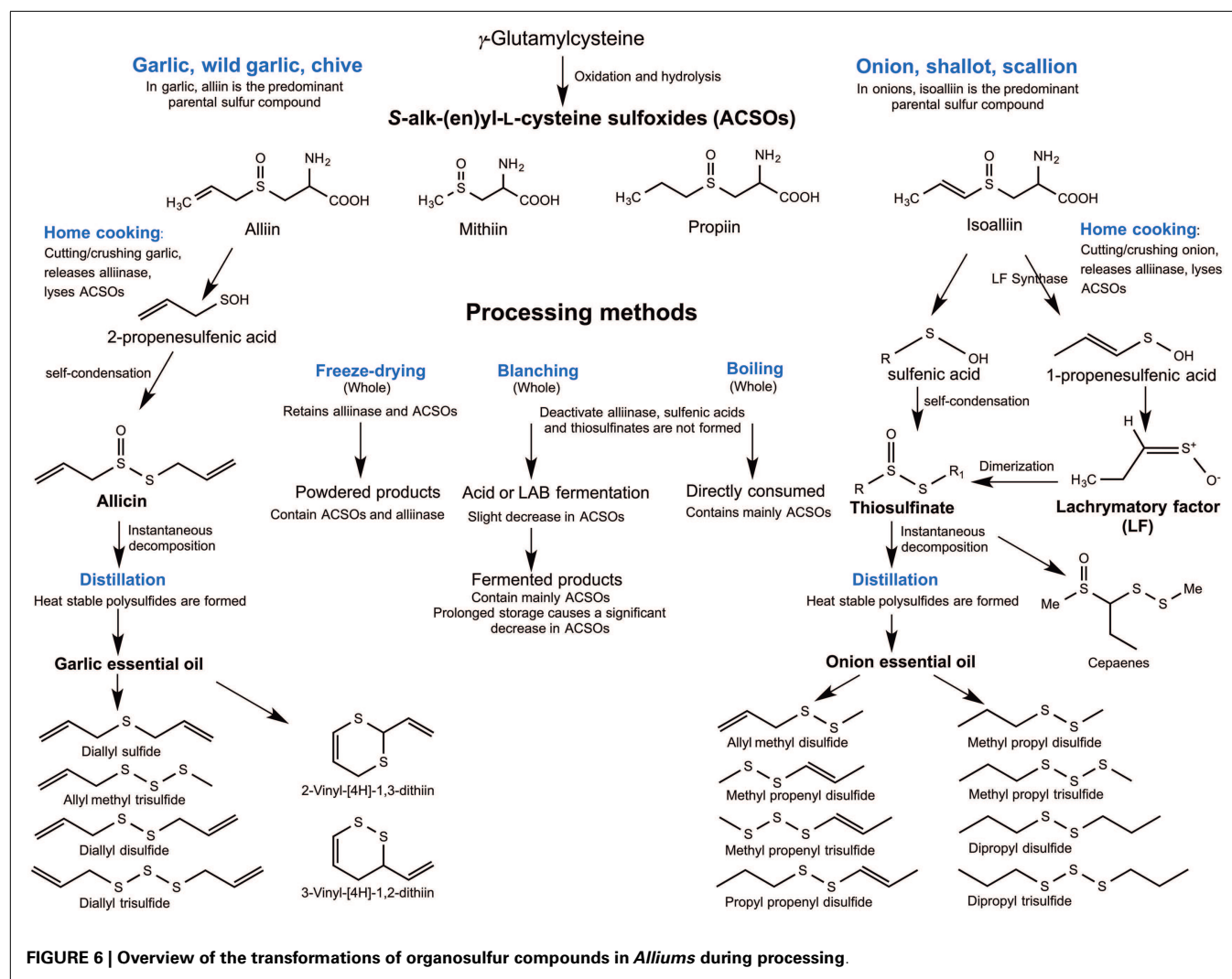
Aside from distilled oils, powdered form of garlic or onion is widely sold in the market. Freeze-drying is a major processing step employed in making this product. Since cloves or whole onions are typically used during freeze-drying, tissues remain intact; hence, hydrolysis of ACSOs by alliinase may not occur. Therefore, the powder basically has similar content of ACSO profile as the fresh ones. Freeze-drying of high-pressure processed (HPP) onion resulted in variable effects on the concentrations of major polysulfides (80). Significant decreases ($P < 0.05$) in dipropyl disulfide (59–82%), dipropyl trisulfide (62–85%), and trans-propenyl propyl disulfide (38–65%) were observed, retaining the levels of dimethyl trisulfide and methyl 1-propenyl disulfide. In our recently published work, we found that freeze-drying preserved most of the individual organosulfides in shallot, although total organosulfur compounds decreased due to reduction in the concentrations of only a few compounds (52). Moreover, freeze-drying showed retention of polysulfides comparable with other drying methods (air- and over-drying) (40). These observations indicate that freeze-drying is a good alternative method in producing dried *Allium* products with comparable polysulfide contents as those of the fresh.



Other drying methods have also been shown to have direct effects on the organosulfides of different *Allium* species. The effects of microwave drying and cabinet drying on the volatile components of hydrodistilled oil derived from garlic powder have been compared (81). DADS and diallyl tetrasulfide increased with both drying methods, however, DATS and allyl methyl trisulfide decreased, with microwave drying showing greater reduction. The observed reduction of polysulfides with microwave drying can be attributed to the effect of heat generated that might have driven off the volatiles and deactivated alliinase. It has been previously demonstrated that heating at 80°C for 10 min can partially deactivate alliinase (82).

The effect of long-term frozen storage (−20°C) of leek slices showed significant reduction of 14 sulfur compounds after 12 months (45). Some polysulfides including, propyl (E)-propenyl di- and trisulfides and dipropyl disulfide, increased after 2 weeks but compounds such as dimethyl disulfide and 2-propenyl disulfide were not detected after 4–6 months. The increase in concentration after 2 weeks of frozen storage implies that alliinase maintains its catalytic activity in sliced leeks even during 2 weeks of freezing.

Due to the sensitivity of alliinase to heat, boiled *Alliums* are expected to have no heat sensitive thiosulfonates, which have their purported bioactivity. For instance, the loss in the *in vitro*



anti-aggregatory activity (IVAA) of garlic was suggested to be due to the sensitivity of thiosulfates to heat after 10 min of boiling (100°C) (83). Similar results were observed in onion thiosulfates. Boiling for 46 min completely suppressed IVAA, which the author correlates with the decrease in thiosulfates (84). Minimal duration of boiling (<3 min) may preserve thiosulfates as indicated by a maintained IVAA of both onion and garlic (83, 84). In another study, heat treatment by microwave (500 W) and convection oven (200°C) generally decreased thiosulfate content as indicated by a reduction in IVAA (78). However, the extent to which these antiplatelet agents are destroyed varied depending on the method (whole, quarters, and crushed) of sample preparation with whole showing longer retention of IVAA (after 30 min) as compared to quarters (20 min) and crushed samples (10 min).

Among the *Allium* products in the market, there appears to be a fair acceptance of pickled blanched garlic, which comes as either fermented or unfermented. The main step in the process is blanching (90°C) of garlic cloves, which may deactivate alliinase and, therefore, have direct implication in the formation of other organosulfur compounds. The next step is brining whereby

garlic cloves are immersed in an acidified brine containing lactic acid (85). Fermented pickled garlic involves subjecting the blanched garlic cloves to lactic acid bacteria fermentation, typically *Lactobacillus plantarum* or *Lactobacillus pentosus* (86). The final pH of the brine is around 3.9 with a titratable acidity of 1.2% (as lactic acid) (86). It was found that only SAC and GSAC slightly decreased after blanching (79). Therefore, blanching for 5 min is not that detrimental to the major OSC precursors in garlic such as alliin. However, after pickling and fermentation, the contents of alliin, isoalliin, and cycloalliin significantly decreased and became even much lower (23–28% loss) after 1 year of storage at refrigerated temperature (79). The blanching step is carried out mainly to deactivate alliinase. Consequently, the contents of oil-soluble polysulfides will be impacted by blanching. Pickling and fermenting garlic may eliminate the pungent flavor brought about by formation of thiosulfates. Flavor improvement using these processing techniques could be useful as a marketing strategy. However, these processing methods may have critical implications on the formation of other bioactive organosulfur compounds in *Alliums*.

The sensitivity of organosulfides to processing methods used pose a challenge to processing *Alliums* so that the end products have optimal bioactive organosulfides. This would require research work that can delineate the structure and activity relationship through mechanistic studies.

ACTION MECHANISMS OF POLYSULFIDES: AS H₂S DONORS

Perhaps the most interesting and promising aspects of dietary organosulfur compounds are their potentials to be precursors of hydrogen sulfide (H₂S), a colorless, flammable, and toxic gas with the characteristic foul odor of rotten eggs. For quite a long time, H₂S attracted attention mainly because of its toxicity, however, in the past decades H₂S has been found as a gaseous signaling molecule, which plays important roles in many physiological and pathological conditions (87). H₂S is the third and newest member of gasotransmitter family, along with nitric oxide (NO) and carbon monoxide (CO) (88, 89).

Since the discovery that endogenous H₂S selectively enhance *N*-methyl-D-aspartate (NMDA) receptor (a glutamate receptor controlling synaptic plasticity and memory function) mediated response in 1996 (90), tremendous progress has been made in the understanding of H₂S physiology. H₂S is associated with a wide yet still expanding range of physiological events – their cardiovascular benefits attracting the most attention (87, 91). The first paper concerning the cardiovascular effect of H₂S was published in 1997 (92), in which H₂S was found to induce smooth muscle relaxation *in vitro*. Four years later it was reported that the H₂S was a K_{ATP} channel activator, which was responsible for the vasorelaxation effect *in vivo* (93). Since then, the beneficial effects of H₂S have been shown in many other studies (94). Hydrogen sulfide dilates blood vessel (95), protects against ischemia-reperfusion injury in myocardium (96), protects against heart failure by reducing oxidative stress, increasing myocardial vascular density, and preserving mitochondrial function (97). Furthermore, H₂S prevents atherosclerosis by reducing smooth muscle cell proliferation and inhibiting adhesion molecule expression (98).

Hydrogen sulfide is produced endogenously in mammalian tissues mainly by enzymatic metabolism of L-cysteine. Currently, four enzymes, including cystathionine γ -lyase (CSE), cystathionine β -synthase (CBS), 3-mercaptopyruvate sulfur transferase (3-MST), and cysteine aminotransferase (CAT) have been found to be involved in its biological production (87, 99, 100). CSE and CBS are believed to be the major enzymes responsible for endogenous H₂S synthesis. CSE has been reported in various organs including kidney, liver, uterus, and placenta but it is predominantly found in the vasculature and liver; while CBS is expressed in the liver and central nervous system.

Some non-enzymatic pathways that lead to H₂S generation also exist, for example, the reaction between naturally occurring polysulfides and biological thiols, mainly through thiol-disulfide exchange reactions. The generation of H₂S from the reaction between glutathione and calicheamicin γ 1 (a natural antitumor agent with an allyl trisulfide group) was reported in 1994, when Myers et al. were trying to elucidate how calicheamicin γ 1 initiated the DNA cleavage process (101). H₂S was also generated upon the reaction between glutathione and 7-methylbenzopentathiepin, an analog of naturally occurring antibiotics varacin (102),

however the H₂S generation in these studies attracted little attention.

In 2007, Benavides et al. reported that DATS and DADS can be converted to H₂S by human red blood cells or rat aorta ring, and the H₂S produced exerts a vasorelaxant effect on rat aorta (103). Hence, they suggested that H₂S mediates the vasoactivity of garlic. They also demonstrated that H₂S production was through the reaction between DADS/DATS and glutathione. Since the conventional thiol-disulfide exchange between DADS and GSH does not produce H₂S, they proposed that DADS undergo nucleophilic substitution at α carbon, producing the key intermediate allyl perthiol, which subsequently reacts with GSH to release H₂S (103). They also found that the H₂S releasing activity of organosulfurs from garlic is higher for those with allyl substituents and increased with increasing number of tethering sulfur atoms (103). Another study found that DATS treatment can significantly increase the H₂S level, reduce the infarct size, and preserve cardiac function in mice after myocardial ischemia-reperfusion. This study substantiated the notion that DATS may be cardioprotective via H₂S-related pathway (104).

Besides these polysulfide H₂S donors, another group of organosulfur compounds that exert cardioprotective effects through H₂S mediated pathways are cysteine derivatives, including SAC and its synthetic analog *S*-propargyl cysteine (R)-2-amino-3-(2-propynylthio) propanoic acid, SPRC. Instead of releasing H₂S by themselves or by reacting with other compounds, they are believed to function by mediating endogenous H₂S production. Increased CSE gene expression, elevated plasma H₂S level and decreased mortality, infarct size, and ventricular hypertrophy were observed in acute myocardial infarction mice treated with SAC (105) or SPPC (106). The abolishment of these beneficial effects by a CSE inhibitor propargylglycine substantiated that their cardioprotective functions are H₂S dependent.

Although many organosulfur compounds have been found from dietary source, only a few of them have been studied for H₂S releasing activity. From a chemical point of view, polysulfides with more than two tethering sulfur atoms should be able to release H₂S through thiol-disulfide exchange with GSH. Research on the H₂S releasing activity by these compounds might provide new explanations for their purported cardiovascular benefits, and, therefore, warrants in-depth investigation.

ORGANOPOLYSULFIDES AS REACTIVE OXYGEN SPECIES SCAVENGERS

The implication of reactive oxygen species (ROS) in the development of CVDs has been shown in numerous studies in the past decades. The most important ROS in cardiovascular system are superoxide anion radical (O₂^{•-}) and hydrogen peroxide (H₂O₂). O₂^{•-} is produced in vascular cells by a wide variety of oxidases, including the predominant NADPH oxidases, as well as lipoxygenases, xanthine oxidase, cytochrome P450, uncoupled mitochondrial electron transfer chain, and uncoupled endothelial nitric oxide synthase (eNOS) (107). O₂^{•-} undergoes dismutation by superoxide dismutase (SOD) to generate H₂O₂. Superoxide anion rapidly would react with NO to generate peroxynitrite (ONOO⁻). This reaction consumes NO and reduces its activity in maintaining healthy cardiovascular functions. On the

contrary, the resulting product, ONOO^- , is an important lipid oxidation mediator that will lead to the oxidation of low-density lipoprotein (LDL), forming strong proatherogenic oxidized LDL (ox-LDL) (108). Besides, ROS contributes to cardiovascular injury through the modulation of multiple cellular responses, including monocyte adhesion, platelet aggregation, vascular smooth muscle cell apoptosis, migration and proliferation, inflammatory gene expression, and dysfunction of endothelium dependent relaxation (109).

Sulfur atoms in dietary organosulfides are electron rich and ready to donate electrons in a redox reaction; therefore, they are supposed to be good oxidant scavengers. AGE and garlic oil containing high amounts of organosulfurs have been shown to scavenge ROS and prevent damage caused by oxidative stress (110–113). However, very limited reports studied the scavenging activities of individual organosulfur compounds to certain types of free radicals. Those reported works are inconsistent and incomparable likely because different evaluating methods and sample concentrations were employed. In addition, nearly all of the studies were *in vitro* tests carried out in simple chemical systems because of the lack of analytical methods that can selectively target a specific free radical *in vivo*. But the general trend is, SAC, the water soluble, major organosulfur compound in AGE, possess strong scavenging activities against peroxynitrite (114, 115) and hydroxyl radical (114–116), but has negligible effects toward O_2^- or H_2O_2 (114, 116). However, the major lipophilic polysulfides in garlic oil DADS and DATS can inhibit O_2^- as much as SOD/ascorbic acid can (114) and their activity increases with the number of sulfur atoms; besides, they are good ONOO^- scavengers but have very limited effects on hydroxyl radical or H_2O_2 (114). The potent hypochlorite acid scavenging effect of SAC, DADS, and DATS as well as some other thioallyl compounds such as allyl mercaptan, allyl methyl sulfide, dipropyl sulfide, and allicin has been reported in by several studies (115, 117, 118). Allicin is a good scavenger against O_2^- , OH^\cdot , and ONOO^- (116, 117), but their activity seems to be attributed to the sulfenic acid formed from the copper elimination of allicin (119).

The cardioprotective effects of organosulfur compounds against oxidative damage in cell lines or in animals have been shown in several studies. SAC, which was able to inhibit LDL oxidation, was also reported to dose-dependently inhibit the H_2O_2 formation in ox-LDL challenged human umbilical vein endothelial cells (HUVEC) (120). Similar protective effect was also found in bovine pulmonary artery endothelial cells (121). DATS and DADS are reported to decrease the cellular peroxide level in ox-LDL-treated HUVEC cells by as much as 50 and 43%, respectively (122). DATS was shown to decrease the ROS and O_2^- levels in H9c2 cells induced by high-glucose treatment, and protect cardiac myocytes from apoptotic cells death in culture medium as well as in diabetic rats (123) through ROS related pathways. Similar effects were also found in rats treated with DADS and garlic oil (124). However, it needs to be pointed out that these protective effects against ROS damage observed in cell lines or in experimental animals might come from the combination of a wide range of physiological pathways such as enhancement of antioxidant enzyme expression or inhibition of peroxidation enzymes production activity, instead of direct free radical scavenging.

ENZYME ACTIVATION/INACTIVATION AND GENE REGULATION

Recently, a number of studies have suggested that the beneficial effects of organosulfides in cardiovascular health are associated with their ability to modulate antioxidant genes and enzyme expression (Table 3). In this section, we discuss these mechanisms and present an overview of the important biochemical pathways associated in the cardioprotective effects of organosulfides (Figure 7).

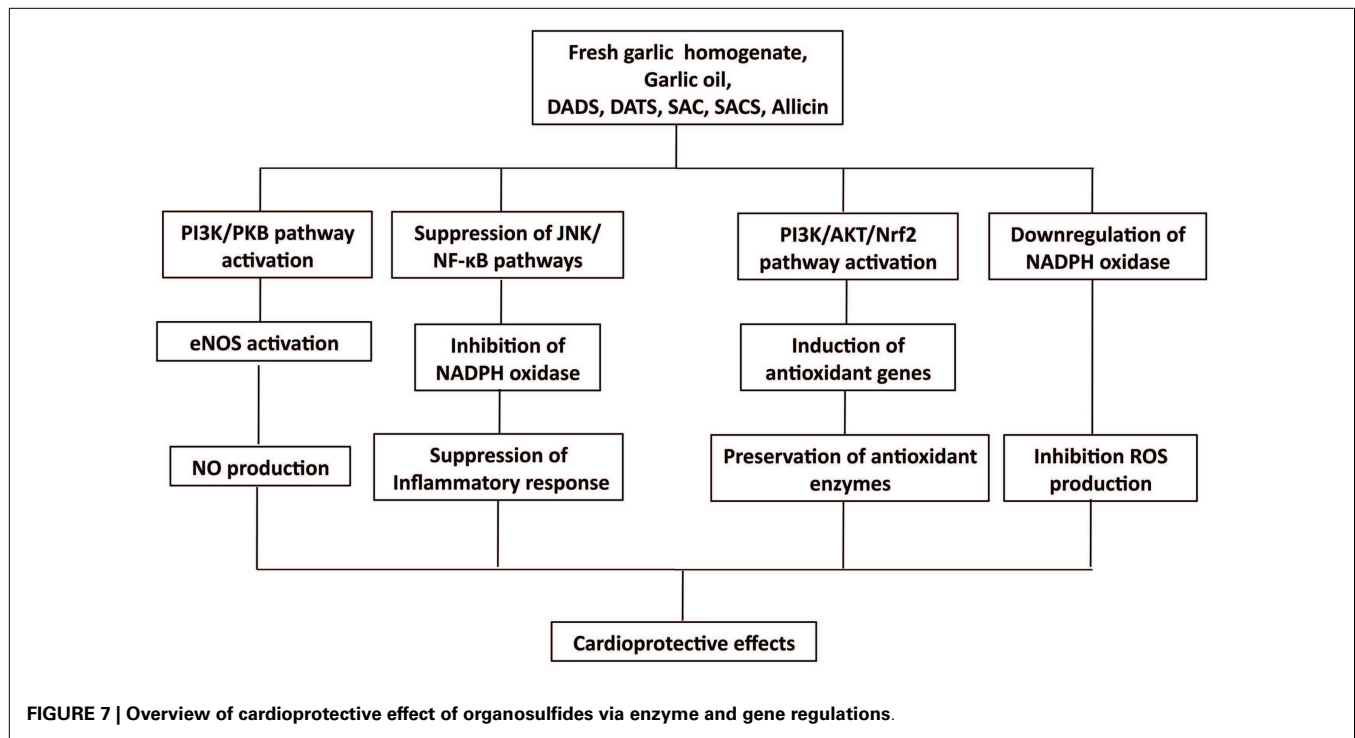
Nrf2 ACTIVATION AND ANTIOXIDANT GENE MODULATION

Oxidative stress, viewed at the molecular level, is linked to the activities of nuclear factor-E-2-related factor (Nrf2) in the nucleus, which upregulates genes that encode for the expression of antioxidant enzymes (132). It has been demonstrated that Nrf2 coordinates the expression and upregulation of various antioxidant enzymes, including heme oxygenase-1 (HO-1), glutathione S-transferase (GST), and SOD (133, 134). These enzymes collectively protect cardiomyocytes from oxidative stress (135, 136). For example, SOD-1, one of the three SOD, works together with catalase to detoxify superoxides and hydrogen peroxide (H_2O_2) (137). Induced upregulation or downregulation of *SOD-1* genes and expression of the antioxidant enzyme SOD-1 are often evaluated as an indicator of ROS stress.

The induced Nrf2-mediated antioxidant gene activation by DATS is linked to several pathways, including MAPK, PKC, and PI3K/AKT (138). Tsai et al. showed that the cytoprotective effect of DATS against oxidative stress in high-glucose exposed neonatal cardiomyocytes and streptozotocin-induced diabetic rats is by activation of the PI3K/Akt/Nrf2 pathway (125). Expression of Nrf2 proteins was upregulated by DATS in a time-dependent manner resulting in a dose-dependent improvement of the expression of antioxidant genes, including *HO-1* and *SOD2*. Moreover, PI3K-specific siRNA and Nrf2-specific siRNA transfected cells showed normal levels of superoxide than those administered with DATS alone, indicating that the antioxidative effect of DATS is suppressed by Nrf2 and PI3K siRNA, further suggesting that the cytoprotective effect of DATS against high-glucose-induced oxidative stress is by the activation of PI3K/Akt/Nrf2 pathway (125). The ability of DATS to induce Nrf2 activation was further demonstrated in a study that specifically looked at Keap1 cysteine 288 residue, one of the cysteine residues that is essential in regulating Nrf2 activation (139). It was found that mono-allyl mono-sulfide may attach to the Keap1 peptide fragment containing Cys288, indicating that DATS specifically interacts with this cysteine residue accounting for its ability to induce Nrf2 activation (139). Recently, raw garlic homogenate administration to fructose fed-diabetic rats showed increased myocardial Nrf2 expression, along with increased Mn-superoxide dismutase (*Mn-SOD*) gene expression and, elevated myocardial SOD, catalase, and glutathione peroxidase (GPx) activities (126). In addition to enhanced antioxidant gene and enzyme expressions, this study also showed that garlic homogenate can increase the levels of phospho-PI3K and phospho-AKT, indicating that PI3K/AKT pathway plays a major role in cardiac hypertrophy and oxidative stress by activating Nrf2, which, in turn regulates the activation of antioxidant defense enzymes during cardiovascular complications (126). In another study, administration of garlic oil

Table 3 | *In vivo* and *in vitro* studies showing the effect of organosulfides on enzyme and gene regulation and the underlying mechanisms.

Organosulfide	Study	Enzyme/Gene	Experimental object	Dose	Organosulfide preparation	Mechanism	Reference
DATS	<i>In vitro</i>	Antioxidant enzymes (HO-1, SOD-1, SOD-2)	H9c2 high-glucose cardiomyoblast cells	10 μ M	Garlic oil (40% DATS)	Upregulate the P13K/Ak/Nrf2 pathway; activate antioxidant enzyme system	(125)
	<i>In vivo</i>	Genes (<i>HO-1</i> , <i>SOD-2</i> , <i>yGCS</i>)	Streptozotocin-induced diabetic male Wistar rats				
Allicin, alliin, S-allyl-L-cysteine, deoxyalliin, vinylthiin	<i>In vivo</i>	Myocardial catalase, myocardial SOD, glutathione peroxidase (GPx) <i>MnSOD</i> gene	Sprague-Dawley rats	250 $\text{mg}^{-1}\text{kg}^{-1}\text{day}^{-1}$	Raw garlic homogenate	Activation of endogenous antioxidant defenses and reduction of oxidative stress and cardiac hypertrophy as a result of increased myocardial Nrf2 expression; Increased Mn-SOD expression, myocardial SOD, catalase, and GPx	(126)
DADS, DATS	<i>In vivo</i>	<i>SOD-1</i> gene	Weanling male Wistar rats	40 $\text{mg}^{-1}\text{kg}^{-1}$ BW DADS and DATS; 100 $\text{mg}^{-1}\text{kg}^{-1}$ BW GO	Garlic oil and pure compounds	Increased expression of <i>SOD-1</i> preserved antioxidant action against oxidative stress during diabetic cardiomyopathy	(124)
DATS	<i>In vivo</i>	Superoxide dismutase (SOD), glutathione peroxidase (GPx)	Obese diabetic rat; high-glucose-induced endothelial cell	Animal: 5.0 $\text{mg}^{-1}\text{kg}^{-1}\text{day}^{-1}$ Cell: 25-100 $\mu\text{mol/L}$	Pure compound	Elevated the activities of SOD and GSH-Px in mitochondrion	(127)
DATS	<i>In vitro</i>	c-Jun N-terminal kinases (JNKs)	H9c2 cardiomyoblast cells; neonatal cardiomyocytes	1-10 μM	Garlic oil (40% DATS)	Suppression of ROS-stimulated downstream JNK/NF- κ B signaling	(123)
DATS, DADS	<i>In vitro</i>	Endothelial NOS (eNOS)	LDL-treated HUVEC	200 μM DADS 50 μM DATS	Pure compounds	Preserved the interaction of eNOS with calveolin-1; suppressed the reduction of the cellular eNOS protein by ox-LDL	(128)
Not specified	<i>In vivo</i>	Myocardial catalase, GPx, mitochondrial enzymes i.e., citrate cynthase and B hydroxyacyl CoA dehydrogenase	Male Swiss albino mice	250-500 $\text{mg}^{-1}\text{kg}^{-1}\text{day}^{-1}$	Saline and aqueous garlic homogenate	Garlic homogenate preserved expression of antioxidant enzymes and attenuated isoproterenol-induced cardiac changes. GO preserves activity of antioxidant defense enzymes and their interaction with NO pathway	(129)
DATS	<i>In vivo</i>	eNOS	MI/R mice model	200 μg	Pure compound	Activates eNOS and improved NO bioavailability	(104)
Allicin	<i>In vivo</i>	SOD	Wistar rats	6-10 $\text{mg}^{-1}\text{kg}^{-1}\text{day}^{-1}$	Pure compound	Increase in SOD helps inhibit lipid peroxidation by hyperhomocysteinemia and regulates the excretion and balance of plasma endothelin and NO	(130)
SACS	<i>In vivo</i>	SOD, catalase	Female Wistar albino rats	0.111-0.222 $\text{mg}^{-1}\text{kg}^{-1}$ SACS; 125-250 $\text{mg}^{-1}\text{kg}^{-1}$ garlic homogenate	Fresh garlic homogenate and pure compound	Restoring SOD and catalase to normal levels	(131)



and pure compounds of DADS and DATS increased level of SOD-1 expression and enhanced the PI3K/AKT signaling in diabetic rats (124).

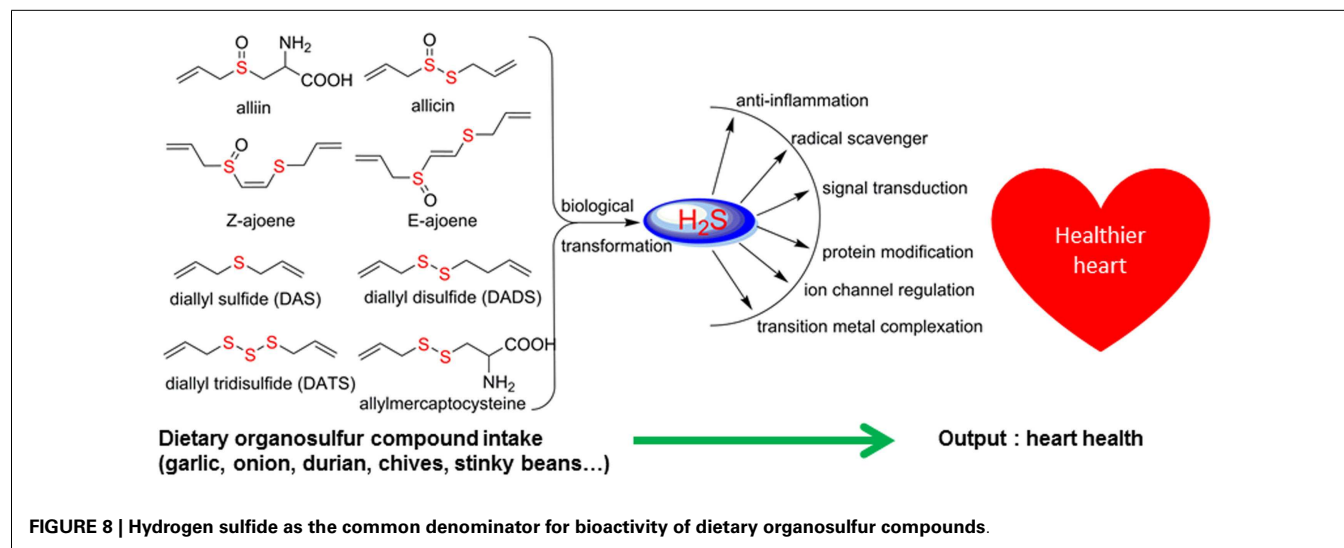
MODULATION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE

Oxidized LDL is an important factor in the pathogenesis of atherosclerosis (108). Ox-LDL promotes vascular dysfunction in various mechanisms, one of which is its inhibition of eNOS activity resulting in the alteration of the NO-regulated responses in the endothelial cells (140, 141). Organosulfur compounds from garlic are involved in the modulation of eNOS activity and are responsible for garlic's anti-atherogenic effect. Lei et al. studied garlic's role against ox-LDL in HUVEC and found that the protective role of DADS and DATS in eNOS activation and NO production is associated with the PI3K/PKB pathway (128). Ox-LDL was found to decrease PKB and eNOS phosphorylation but this effect was abolished by DADS and DATS pretreatment along with restored production of NO. Moreover, treatment with PI3K inhibitor wortmanin attenuated the protective effect of DADS and DATS in eNOS activation and recovery of NO production (128). Serine phosphorylation and blocking of eNOS activation may result from the inhibition of PI3K/PKB pathway or from PKB site mutation on the eNOS protein (at serine 1177) signifying the importance of PI3K/PKB pathway in regulating eNOS activity (142). In another study, garlic homogenate ($250 \text{ mg kg}^{-1} \text{ day}^{-1}$ for 30 days) treatment on isoproterenol-induced myocardial infarction mice model relieved oxidative stress by significantly increasing release of GPx and catalase activities (129). This study also demonstrated the positive association of NOS activation, NO production, and antioxidant enzyme activity protection with garlic treatment suggesting that the maintenance of redox balance through protecting the

activation of NOS and, hence, production of NO, explain garlic's protective role against myocardial damage. Moreover, eNOS activation was demonstrated by Predmore et al., showing that DATS treated rat myocardial tissue had an increased eNOS phosphorylation at Ser¹¹⁷⁷ and elevated levels of NO metabolites, including nitrite and nitrate (104).

MODULATION OF ANTIOXIDANT ENZYMES AND INHIBITION OF NADPH OXIDASE ACTIVITY

The protective effect of DATS against hyperglycemia-induced oxidative stress was demonstrated in an *in vivo* model of obese diabetic rats and in high-glucose-treated endothelial cells (127). The reduction of mitochondrial oxidative stress was suggested to be the action mechanism because attenuation of endothelial cell impairment was observed along with enhanced activities of SOD and GPx in the mitochondria upon administration of DATS. SOD and GPx are antioxidative enzymes with detoxifying actions against mitochondrial ROS (143). DATS attenuated the hyperglycemia-induced NADPH oxidase and its related ROS production in the mitochondria mainly by preserving but not upregulating the activities of SOD and GPx (127). In another study, the effect of low ($6 \text{ mg kg}^{-1} \text{ day}^{-1}$) and high ($10 \text{ mg kg}^{-1} \text{ day}^{-1}$) allicin doses was demonstrated in hyperhomocysteinemia-induced vascular endothelial dysfunction animal model (130). Hyperhomocysteinemia is another cause of oxidative stress that may lead to vascular endothelial dysfunction and injury (144, 145). Formation of H_2O_2 may result from the self-oxidation of homocysteine at the active free sulfhydryl group. Homocysteine level in rats with hyperhomocysteinemia was reduced by treatment with allicin along with an increase in SOD activity (130). Similar results were demonstrated in another study employing fructose-induced hypertensive



rat models (131). SOD and catalase were restored to normal levels after treatment with *S*-allyl cysteine sulfoxide (SACS) isolated from fresh garlic homogenate.

In a recent paper, expression of p22phox and gp91phox, the subunits comprising the membrane-bound component cytochrome b_{558} , and the subunits responsible for the activity of NADPH oxidase increased along with the production of superoxide free radicals ($O_2^{\cdot-}$) in high-glucose-treated H9c2 cells but was attenuated by treatment with DATS (1–10 μ M). Production of ROS by NADPH oxidase is related to the activation of c-Jun N-terminal kinases (JNK) signaling and the transcriptional factor NF- κ B (146). Activation of JNK leads to their translocation into the nucleus where they phosphorylate transcription factors including c-Jun and p53 that are involved in the regulation of apoptosis (147, 148). Treatment with DATS (1–10 μ M) dose-dependently inhibited the high-glucose activation of JNK, which was suggested to be associated with the inhibition of NADPH oxidase-regulated ROS generation in H9c2 cells and neonatal primary cardiomyocyte (123). Treatment with DATS (1–10 μ M) inhibited the nuclear translocation of NF- κ B in H9c2 cells and reduced the protein levels of NF- κ B in streptozotocin-induced diabetic rats (40 mg/kg BW DATS) (123). Hyperglycemia is not only known to induce ROS generation but also inflammation, which could activate transcription regulators, including NF- κ B, which, in turn, regulates intracellular apoptosis (149, 150). Treatment with DATS protects high-glucose-treated neonatal cardiomyocytes and H9c2 cells from ROS damage by inhibiting the activation of JNK/NF- κ B pathways (123).

CONCLUSION

There are significant scientific research papers suggesting that dietary organosulfurs have broad range of bioactivity including cancer chemoprevention and promotion of cardiovascular health. While there is strong evidence to suggest that isothiocyanates may be the active form in cruciferous vegetables for their cancer chemopreventive property, the daily intake of such compounds could be very low because cooking of these vegetables will prevent their formation. For other organosulfides, it remains a challenge to

pinpoint the compounds responsible for their purported health promoting effects. Allicin was considered as the active components of garlic and has been used as a marker compound in garlic supplement standardization. The null results from clinical trials make us reconsider other potential compounds. The discovery of DATS and DADS as donors of hydrogen sulfide opens up a new avenue for establishing evidence of organosulfide action mechanisms on promoting cardiovascular health. It remains to be seen whether H_2S donating activity can be the unifying mechanism for dietary organopolysulfides to exert their health benefits. H_2S is a “double-edged sword” as it is toxic at high concentrations. Rapid burst of H_2S from organosulfides may lead toxic effects *in vivo* but it may be needed for their anti-microbial activity. For cardiovascular health, slow (or controlled) release of H_2S from dietary polysulfides would be desired. There are many other organosulfides in our diet that have not been investigated yet. The outstanding ones include cyclic polysulfides founds in stinky beans and mushrooms. While these polysulfides are important flavoring molecules (sometime smelly!), little is known on their health promoting activity. The high sulfur loading of them (i.e., compounds shown in Figure 3) would make them ideal reservoirs of H_2S , if they can be biotransformed to release H_2S in human body. Research work in the future shall be focused on establishing the structure and H_2S releasing potentials and rates of individual dietary polysulfides in cell line and animal model systems. Since processing conditions can greatly alter the polysulfide profiles in foods, an H_2S releasing activity guided optimization of processing conditions would lead to optimal effectiveness of supplements or functional foods based on *Alliums* and stinky beans for their cardioprotective effects (Figure 8). The research is just at the beginning on taming the pesky dietary organosulfides for human health promoting and disease prevention.

ACKNOWLEDGMENTS

The authors thank the Agency of Science, Technology and Research (A*Star) of Singapore for financial support (Grant Number: 112 177 0036) and the support of a Jiangsu Province Grant to NUSRI for Food Science and Technology (platform2).

REFERENCES

- WHO. *Cardiovascular Disease*. World Health Organization (2014). Available from: <http://www.who.int/cardiovascular_diseases/en/>
- Davison K, Coates AM, Buckley JD, Howe PRC. Effect of cocoa flavanols and exercise on cardiometabolic risk factors in overweight and obese subjects. *Int J Obes* (2008) **32**:1289–96. doi:10.1038/ijo.2008.66
- EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific opinion on the modification of the authorisation of a health claim related to cocoa flavanols and maintenance of normal endothelium-dependent vasodilation pursuant to Article 13(5) of Regulation (EC) No 1924/2006 following a request in accordance with Article 19 of Regulation (EC) No 1924/2006. *EFSA J* (2014) **12**:3654. doi:10.2903/j.efsa.2014.3654
- Tsai CW, Chen HW, Sheen LY, Lii CK. Garlic: health benefits and actions. *BioMedicine* (2012) **2**:17–29. doi:10.1016/j.biomed.2011.12.002
- Moriarty RM, Naithani R, Surve B. Organosulfur compounds in cancer chemoprevention. *Mini Rev Med Chem* (2007) **7**:827–38. doi:10.2174/138955707781387939
- Alvarez-Jubete L, Smyth TJ, Valverde J, Rai DK, Barry-Ryan C. Simultaneous determination of sulphoraphane and sulphoraphane nitrile in *Brassica* vegetables using ultra-performance liquid chromatography with tandem mass spectrometry. *Phytochem Anal* (2014) **25**:141–6. doi:10.1002/pca.2480
- Kassie F, Rabot S, Uhl M, Huber V, Qin HM, Helma C, et al. Chemoprotective effects of garden cress (*Lepidium sativum*) and its constituents towards 2-amino-3-methyl-imidazo[4,5-f]quinoline (IQ)-induced genotoxic effects and colonic preneoplastic lesions. *Carcinogenesis* (2002) **23**:1155–61. doi:10.1093/carcin/23.7.1155
- Rose P, Yen KW, Choon NO, Whiteman M. β -Phenylethyl and 8-methylsulphonyloctyl isothiocyanates, constituents of watercress, suppress LPS induced production of nitric oxide and prostaglandin E2 in RAW 264.7 macrophages. *Nitric Oxide* (2005) **12**:237–43. doi:10.1016/j.niox.2005.03.001
- Houghton CA, Fassett RG, Coombes JS. Sulforaphane: translational research from laboratory bench to clinic. *Nutr Rev* (2013) **71**:709–26. doi:10.1111/nure.12060
- Cui J, Li S. Inhibitors and prodrugs targeting CYP1: a novel approach in cancer prevention and therapy. *Curr Med Chem* (2014) **21**:519–52. doi:10.2174/09298673113206660277
- Corzo-Martínez M, Corzo N, Villamiel M. Biological properties of onions and garlic. *Trends Food Sci Technol* (2007) **18**:609–25. doi:10.1016/j.tifs.2007.07.011
- Gardner CD, Lawson LD, Block E, Chatterjee LM, Kiazand A, Balise RR, et al. Effect of raw garlic vs commercial garlic supplements on plasma lipid concentrations in adults with moderate hypercholesterolemia: a randomized clinical trial. *Arch Intern Med* (2007) **167**:346–53. doi:10.1001/archinte.167.4.346
- Rose P, Whiteman M, Moore PK, Yi ZZ. Bioactive S-alk(en)yl cysteine sulfoxide metabolites in the genus *Allium*: the chemistry of potential therapeutic agents. *Nat Prod Rep* (2005) **22**:351–68. doi:10.1039/b417639c
- Benkeblia N, Lanzotti V. *Allium* thiosulfinates: chemistry, biological properties and their potential utilization in food preservation. *Food* (2007) **1**:193–201.
- Block E. The organosulfur chemistry of the genus *Allium* – Implications for the organic chemistry of sulfur. *Angew Chem Int Ed Engl* (1992) **31**:1135–78. doi:10.1002/anie.199211351
- Wafler U, Shaw ML, Lancaster JE. Effect of freezing upon alliinase activity in onion extracts and pure enzyme preparations. *J Sci Food Agric* (1994) **64**:315–8. doi:10.1002/jsfa.2740640311
- Manchali S, Chidambara Murthy KN, Patil BS. Crucial facts about health benefits of popular cruciferous vegetables. *J Funct Foods* (2012) **4**:94–106. doi:10.1016/j.jff.2011.08.004
- Kubec R, Drhová V, Velišek J. Thermal degradation of S-methylcysteine and its sulfoxide – important flavor precursors of *Brassica* and *Allium* vegetables. *J Agric Food Chem* (1998) **46**:4334–40. doi:10.1021/jf980379x
- Kubec R, Svobodová M, Velišek J. Gas-chromatographic determination of S-methylcysteine sulfoxide in cruciferous vegetables. *Eur Food Res Technol* (2001) **213**:386–8. doi:10.1007/s002170100384
- Hamamoto A, Mazelis M. The C-S lyases of higher plants: isolation and properties of homogeneous cystine lyase from broccoli (*Brassica oleracea* var botrytis) buds. *Plant Physiol* (1986) **80**:702–6. doi:10.1104/pp.80.3.702
- Marks HS, Hilson JA, Leichtweis HC, Stoewsand GS. S-methylcysteine sulfoxide in *Brassica* vegetables and formation of methyl methanethiosulfinate from brussels sprouts. *J Agric Food Chem* (1992) **40**:2098–101. doi:10.1021/jf00023a012
- Kyung KH, Lee YC. Antimicrobial activities of sulfur compounds derived from S-Alk(en)yl-L-cysteine sulfoxides in *Allium* and *Brassica*. *Food Rev Int* (2001) **17**:183–98. doi:10.1081/FRI-100000268
- Edmands WMB, Gooderham NJ, Holmes E, Mitchell SC. S-methyl-L-cysteine sulfoxide: the Cinderella phytochemical? *Toxicol Res* (2013) **2**:11–22. doi:10.1039/c2tx20030a
- Seigler DS. *Plant Secondary Metabolism*. Boston, MA: Kluwer Academic (1998).
- Griffiths DW, Macfarlanesmith WH, Boag B. The effect of cultivar, sample date and grazing on the concentration of S-methylcysteine sulfoxide in oilseed and forage. *J Sci Food Agric* (1994) **64**:283–8. doi:10.1002/jsfa.2740640307
- Kopsell DE, Randle WM, Eiteman MA. Changes in the S-alk(en)yl cysteine sulfoxides and their biosynthetic intermediates during onion storage. *J Am Soc Hortic Sci* (1999) **124**:177–83.
- Montaño A, Beato VM, Mansilla F, Orgaz F. Effect of genetic characteristics and environmental factors on organosulfur compounds in garlic (*Allium sativum* L.) grown in Andalusia, Spain. *J Agric Food Chem* (2011) **59**:1301–7. doi:10.1021/jf104494j
- Fujiwara M, Itokawa Y, Uchino H, Inoue K. Anti-hypercholesterolemic effect of a sulfur containing amino acid, S-methyl-L-cysteine sulfoxide, isolated from cabbage. *Experientia* (1972) **28**:254–5. doi:10.1007/BF01928671
- Komatsu W, Miura Y, Yagasaki K. Suppression of hypercholesterolemia in hepatoma-bearing rats by cabbage extract and its component, S-methyl-L-cysteine sulfoxide. *Lipids* (1998) **33**:499–503.
- Delaha EC, Garagusi VF. Inhibition of mycobacteria by garlic extract (*Allium sativum*). *Antimicrob Agents Chemother* (1985) **27**:485–6. doi:10.1128/AAC.27.4.485
- Arora DS, Kaur J. Antimicrobial activity of spices. *Int J Antimicrob Agents* (1999) **12**:257–62. doi:10.1016/S0924-8579(99)00074-6
- Briggs WH, Xiao H, Parkin KL, Shen C, Goldman IL. Differential inhibition of human platelet aggregation by selected *Allium* thiosulfinates. *J Agric Food Chem* (2000) **48**:5731–5. doi:10.1021/jf0004412
- Iciek M, Kwiecien I, Włodek L. Biological properties of garlic and garlic-derived organosulfur compounds. *Environ Mol Mutagen* (2009) **50**:247–65. doi:10.1002/em.20474
- Lawson LD, Hughes BG. Characterization of the formation of allicin and other thiosulfinates from garlic. *Planta Med* (1992) **58**:345–50. doi:10.1055/s-2006-961482
- Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura Y. Intake of garlic and its bioactive components. *J Nutr* (2001) **131**:955S–62S.
- Calvo-Gómez O, Morales-López J, López MG. Solid-phase microextraction-gas chromatographic-mass spectrometric analysis of garlic oil obtained by hydrodistillation. *J Chromatogr A* (2004) **1036**:91–3. doi:10.1016/j.chroma.2004.02.072
- Kimbaris AC, Siatis NG, Daferera DJ, Tarantilis PA, Pappas CS, Polissiou MG. Comparison of distillation and ultrasound-assisted extraction methods for the isolation of sensitive aroma compounds from garlic (*Allium sativum*). *Ultrason Sonochem* (2006) **13**:54–60. doi:10.1016/j.ultsonch.2004.12.003
- Sowbhagya HB, Purnima KT, Florence SP, Appu Rao AG, Srinivas P. Evaluation of enzyme-assisted extraction on quality of garlic volatile oil. *Food Chem* (2009) **113**:1234–8. doi:10.1016/j.foodchem.2008.08.011
- Li R, Chen WC, Wang WP, Tian WY, Zhang XG. Extraction of essential oils from garlic (*Allium sativum*) using ligarine as solvent and its immunity activity in gastric cancer rat. *Med Chem Res* (2010) **19**:1092–105. doi:10.1007/s00044-009-9255-z
- Dziri S, Casabianca H, Hanchi B, Hosni K. Composition of garlic essential oil (*Allium sativum* L.) as influenced by drying method. *J Essent Oil Res* (2014) **26**:91–6. doi:10.1080/10412905.2013.868329
- Lawson LD, Wang ZY, Hughes BG. Identification and HPLC quantitation of the sulfides and dialk(en)yl thiosulfinates in commercial garlic products. *Planta Med* (1991) **57**:363–70. doi:10.1055/s-2006-960119
- Wu JL, Chou CC, Chen MH, Wu CM. Volatile flavor compounds from shallots. *J Food Sci* (1982) **47**:606–8. doi:10.1111/j.1365-2621.1982.tb10133.x
- Hanum T, Sinha NK, Guyer DE, Cash JN. Pyruvate and flavor development in macerated onions (*Allium cepa* L.) by γ -glutamyl transpeptidase and exogenous C-S lyase. *Food Chem* (1995) **54**:183–8. doi:10.1016/0308-8146(95)00027-G

44. Pino JA, Fuentes V, Correa MT. Volatile constituents of Chinese chive (*Allium tuberosum* Rottl. ex Sprengel) and rakkyo (*Allium chinense* G. Don). *J Agric Food Chem* (2001) **49**:1328–30. doi:10.1021/jf9907034
45. Studsgaard Nielsen G, Larsen LM, Poll L. Formation of aroma compounds and lipoxygenase (EC 1.13.11.12) activity in unblanched leek (*Allium ampeloprasum* Var. Bulga) slices during long-term frozen storage. *J Agric Food Chem* (2003) **51**:1970–6. doi:10.1021/jf0202921o
46. Yabuki Y, Mukaida Y, Saito Y, Oshima K, Takahashi T, Muroi E, et al. Characterisation of volatile sulphur-containing compounds generated in crushed leaves of Chinese chive (*Allium tuberosum* Rottler). *Food Chem* (2010) **120**:343–8. doi:10.1016/j.foodchem.2009.11.028
47. Kim NY, Park MH, Jang EY, Lee J. Volatile distribution in garlic (*Allium sativum* L.) by solid phase microextraction (SPME) with different processing conditions. *Food Sci Biotechnol* (2011) **20**:775–82. doi:10.1007/s10068-011-0108-4
48. Casella S, Leonardi M, Melai B, Fratini F, Pistelli L. The role of diallyl sulfides and dipropyl sulfides in the *in vitro* antimicrobial activity of the essential oil of garlic, *Allium sativum* L., and leek, *Allium porrum* L. *Phytother Res* (2013) **27**:380–3. doi:10.1002/ptr.4725
49. Kallio H, Salorinne L. Comparison of onion varieties by headspace gas chromatography-mass spectrometry. *J Agric Food Chem* (1990) **38**:1560–4. doi:10.1021/jf00097a029
50. Kuo MC, Ho CT. Volatile constituents of the distilled oils of welsh onions (*Allium fistulosum* L. variety Maichuon) and scallions (*Allium fistulosum* L. variety Caespitosum). *J Agric Food Chem* (1992) **40**:1111–7. doi:10.1021/jf00022a036
51. Mochizuki E, Yamamoto T, Komiya Y, Nakazawa H. Identification of *Allium* products using flame photometric detection gas chromatography and distribution patterns of volatile sulfur compounds. *J Agric Food Chem* (1998) **46**:5170–6. doi:10.1021/jf9803076
52. Tocmo R, Lin Y, Huang D. Effect of processing conditions on the organosulfides of shallot (*Allium cepa* L. Aggregatum Group). *J Agric Food Chem* (2014) **62**:5296–304. doi:10.1021/jf500739n
53. Yu TH, Wu CM, Chen SY. Effects of pH adjustment and heat treatment on the stability and the formation of volatile compounds of garlic. *J Agric Food Chem* (1989) **37**:730–4. doi:10.1021/jf00087a033
54. Wong KC, Tie DY. Volatile constituents of durian (*Durio zibethinus* Murr.). *Flavour Fragr J* (1995) **10**:79–83. doi:10.1002/ffj.2730100205
55. Weenen H, Koolhaas WE, Apriyantono A. Sulfur-containing volatiles of durian fruits (*Durio zibethinus* Murr.). *J Agric Food Chem* (1996) **44**:3291–3. doi:10.1021/jf960191i
56. Jiang J, Choo SY, Omar N, Ahamad N. GC-MS analysis of volatile compounds in durian (*Durio zibethinus* Murr.). *Dev Food Sci* (1998) **40**:345–52. doi:10.1016/S0167-4501(98)80058-7
57. Voon YY, Abdul Hamid NS, Rusul G, Osman A, Quek SY. Characterisation of Malaysian durian (*Durio zibethinus* Murr.) cultivars: relationship of physico-chemical and flavour properties with sensory properties. *Food Chem* (2007) **103**:1217–27. doi:10.1016/j.foodchem.2006.10.038
58. Neti Y, Erlinda ID, Virgilio VG. The effect of spontaneous fermentation on the volatile flavor constituents of durian. *Int Food Res J* (2011) **18**:635–41.
59. Lee PR, Toh M, Yu B, Curran P, Liu SQ. Manipulation of volatile compound transformation in durian wine by nitrogen supplementation. *Int J Food Sci Technol* (2013) **48**:650–62. doi:10.1111/ijfs.12012
60. Block E, Naganathan S, Putman D, Zhao S-H. *Allium* chemistry: HPLC analysis of thiosulfonates from onion, garlic, wild garlic (ramsoms), leek, scallion, shallot, elephant (great-headed) garlic, chive, and Chinese chive. Uniquely high allyl to methyl ratios in some garlic samples. *J Agric Food Chem* (1992) **40**:2418–30. doi:10.1021/jf00024a017
61. Sinha NK, Guyer DE, Gage DE, Lira CT. Supercritical carbon dioxide extraction of onion. Flavors and their analysis by gas chromatography-mass spectrometry. *J Agric Food Chem* (1992) **40**(5):842–5. doi:10.1021/jf00017a027
62. Kuo MC, Ho CT. Volatile constituents of the solvent extracts of welsh onions (*Allium fistulosum* L. variety Maichuon) and scallions (*Allium fistulosum* L. variety Caespitosum). *J Agric Food Chem* (1992) **40**:1906–10. doi:10.1021/jf00022a036
63. Frérot E, Velluz A, Bagnoud A, Delort E. Analysis of the volatile constituents of cooked petal beans (*Parkia speciosa*) using high-resolution GC/ToF-MS. *Flavour Fragr J* (2008) **23**:434–40. doi:10.1002/ffj.1902
64. Miyazawa M, Osman F. Headspace constituents of *Parkia speciosa* seeds. *Nat Prod Lett* (2001) **15**:171–6. doi:10.1080/10575630108041277
65. Gmelin R, Susilo R, Fenwick GR. Cyclic polysulphides from *Parkia speciosa*. *Phytochemistry* (1981) **20**:2521–3. doi:10.1016/0031-9422(81)83085-3
66. Sakunpak A, Panichayupakaranant P. Antibacterial activity of Thai edible plants against gastrointestinal pathogenic bacteria and isolation of a new broad spectrum antibacterial polyisoprenylated benzophenone, chamuangone. *Food Chem* (2012) **130**:826–31. doi:10.1016/j.foodchem.2011.07.088
67. Gan CY, Latiff AA. Optimisation of the solvent extraction of bioactive compounds from *Parkia speciosa* pod using response surface methodology. *Food Chem* (2011) **124**:1277–83. doi:10.1016/j.foodchem.2010.07.074
68. Aisha AFA, Abu-Salah KM, Alrokayan SA, Ismail Z, Abdul Majid AMS. Evaluation of antiangiogenic and antioxidant properties of *Parkia speciosa* Hassk extracts. *Pak J Pharm Sci* (2012) **25**:7–14.
69. Siow HL, Gan CY. Extraction of antioxidative and antihypertensive bioactive peptides from *Parkia speciosa* seeds. *Food Chem* (2013) **141**:3435–42. doi:10.1016/j.foodchem.2013.06.030
70. Jamaluddin F, Mohamed S, Lajis MN. Hypoglycaemic effect of *Parkia speciosa* seeds due to the synergistic action of β -sitosterol and stigmasterol. *Food Chem* (1994) **49**:339–45. doi:10.1016/0308-8146(94)90002-7
71. Al Batran R, Al-Bayaty F, Jamil Al-Obaidi MM, Abdulkader AM, Hadi HA, Ali HM, et al. *In vivo* antioxidant and antiulcer activity of *Parkia speciosa* ethanolic leaf extract against ethanol-induced gastric ulcer in rats. *PLoS One* (2013) **8**:e64751. doi:10.1371/journal.pone.0064751
72. Näf R, Velluz A. Sulphur compounds and some uncommon esters in durian (*Durio zibethinus* Murr.). *Flavour Fragr J* (1996) **11**:295–303. doi:10.1002/(SICI)1099-1026(199609)11:5<295::AID-FFJ585>3.3.CO;2-W
73. Fenwick GR, Hanley AB. The genus *Allium* – Part 3. *Crit Rev Food Sci Nutr* (1985) **23**:1–73. doi:10.1080/10408398509527419
74. Eustache J, Bissere P, Van De Weghe P. 1.14 – One or more CC bond(s) by elimination of S, Se, Te, N, P, As, Sb, Bi, Si, Ge, B, or metal functions. In: Taylor ARKJK, editor. *Comprehensive Organic Functional Group Transformations II*. Oxford: Elsevier (2005). p. 601–68.
75. Pfister-Guillouzo G, Senio A, Gracian F, Khalid M, Ripoll J, Vallee Y. FVT-PES Study of reactivity of thionoacrylic compounds. *New J Chem* (1995) **19**:1071–80.
76. Block E, John Dane A, Cody RB. Crushing garlic and slicing onions: detection of sulfenic acids and other reactive organosulfur intermediates from garlic and other *Alliums* using direct analysis in real-time mass spectrometry (DART-MS). *Phosphorus Sulfur Silicon Relat Elem* (2011) **186**:1085–93. doi:10.1080/10426507.2010.507728
77. Shen C, Xiao H, Parkin KL. *In vitro* stability and chemical reactivity of thiosulfonates. *J Agric Food Chem* (2002) **50**:2644–51. doi:10.1021/jf011013e
78. Cavagnaro PF, Galmarini CR. Effect of processing and cooking conditions on onion (*Allium cepa* L.) induced antiplatelet activity and thiosulfinate content. *J Agric Food Chem* (2012) **60**:8731–7. doi:10.1021/jf301793b
79. Beato VM, Sánchez AH, De Castro A, Montaña A. Effect of processing and storage time on the contents of organosulfur compounds in pickled blanched garlic. *J Agric Food Chem* (2012) **60**:3485–91. doi:10.1021/jf3002075
80. Colina-Coca C, González-Peña D, Vega E, De Ancos B, Sánchez-Moreno C. Novel approach for the determination of volatile compounds in processed onion by headspace gas chromatography-mass spectrometry (HS GC-MS). *Talanta* (2013) **103**:137–44. doi:10.1016/j.talanta.2012.10.022
81. Rao PP, Nagender A, Rao LJ, Rao DG. Studies on the effects of microwave drying and cabinet tray drying on the chemical composition of volatile oils of garlic powders. *Eur Food Res Technol* (2007) **224**:791–5. doi:10.1007/s00217-006-0364-3
82. Jansen H, Muller B, Knobloch K. Characterization of an alliin lyase preparation from garlic (*Allium sativum*). *Planta Med* (1989) **55**:434–9. doi:10.1055/s-2006-962059
83. Cavagnaro PF, Camargo A, Galmarini CR, Simon PW. Effect of cooking on garlic (*Allium sativum* L.) antiplatelet activity and thiosulfonates content. *J Agric Food Chem* (2007) **55**:1280–8. doi:10.1021/jf062587s
84. Galmarini CR, Cavagnaro PF, Sance MM. Effect of heating on onion (*Allium cepa* L.) antiplatelet activity and pungency sensory perception. *Food Sci Technol Int* (2007) **13**:447–53. doi:10.1177/1082013207088108

85. Rejano L, Sanchez AH, De Castro A, Montano A. Chemical characteristics and storage stability of pickled garlic prepared using different processes. *J Food Sci* (1997) **62**:1120–3. doi:10.1111/j.1365-2621.1997.tb12226.x
86. De Castro A, Montaña A, Sánchez AH, Rejano L. Lactic acid fermentation and storage of blanched garlic. *Int J Food Microbiol* (1998) **39**:205–11. doi:10.1016/S0168-1605(98)00003-8
87. Li L, Rose P, Moore PK. Hydrogen sulfide and cell signaling. *Annu Rev Pharmacol Toxicol* (2011) **51**:169–87. doi:10.1146/annurev-pharmtox-010510-100505
88. Mancardi D, Penna C, Merlino A, Del Soldato P, Wink DA, Pagliaro P. Physiological and pharmacological features of the novel gasotransmitter: hydrogen sulfide. *Biochim Biophys Acta* (2009) **1787**:864–72. doi:10.1016/j.bbabi.2009.03.005
89. Gadalla MM, Snyder SH. Hydrogen sulfide as a gasotransmitter. *J Neurochem* (2010) **113**:14–26. doi:10.1111/j.1471-4159.2010.06580.x
90. Abe K, Kimura H. The possible role of hydrogen sulfide as an endogenous neuromodulator. *J Neurosci* (1996) **16**:1066–71.
91. Polhemus DJ, Lefer DJ. Emergence of hydrogen sulfide as an endogenous signaling molecule in cardiovascular disease. *Circ Res* (2014) **114**:730–7. doi:10.1161/CIRCRESAHA.114.300505
92. Hosoki R, Matsuki N, Kimura H. The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. *Biochem Biophys Res Commun* (1997) **237**:527–31. doi:10.1006/bbrc.1997.6878
93. Zhao W, Zhang J, Lu Y, Wang R. The vasorelaxant effect of H₂S as a novel endogenous gaseous KATP channel opener. *EMBO J* (2001) **20**:6008–16. doi:10.1093/emboj/20.21.6008
94. Liu YH, Lu M, Hu LF, Wong PT, Webb GD, Bian JS. Hydrogen sulfide in the mammalian cardiovascular system. *Antioxid Redox Signal* (2012) **17**:141–85. doi:10.1089/ars.2011.4005
95. Yang D, Wu L, Jiang B, Yang W, Qi J, Cao K, et al. H₂S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine γ -lyase. *Science* (2008) **322**:587–90. doi:10.1126/science.1162667
96. Johansen D, Ytrehus K, Baxter GF. Exogenous hydrogen sulfide (H₂S) protects against regional myocardial ischemia-reperfusion injury – Evidence for a role of K⁺ ATP channels. *Basic Res Cardiol* (2006) **101**:53–60. doi:10.1007/s00395-005-0569-9
97. Kondo K, Bhushan S, King AL, Prabhu SD, Hamid T, Koenig S, et al. H₂S protects against pressure overload-induced heart failure via upregulation of endothelial nitric oxide synthase. *Circulation* (2013) **127**:1116–27. doi:10.1161/CIRCULATIONAHA.112.000855
98. Mani S, Li H, Untereiner A, Wu L, Yang G, Austin RC, et al. Decreased endogenous production of hydrogen sulfide accelerates atherosclerosis. *Circulation* (2013) **127**:2523–34. doi:10.1161/CIRCULATIONAHA.113.002208
99. Chan MV, Wallace JL. Hydrogen sulfide-based therapeutics and gastrointestinal diseases: translating physiology to treatments. *Am J Physiol Gastrointest Liver Physiol* (2013) **305**:G467–73. doi:10.1152/ajpgi.00169.2013
100. Song ZJ, Ng MY, Lee Z-W, Dai W, Hagen T, Moore PK, et al. Hydrogen sulfide donors in research and drug development. *Med Chem Commun* (2014) **5**:557–70. doi:10.1039/c3md00362k
101. Myers AG, Cohen SB, Kwon BM. A study of the reaction of calicheamicin g1 with glutathione in the presence of double-stranded DNA. *J Am Chem Soc* (1994) **116**:1255–71. doi:10.1021/ja00083a012
102. Chatterji T, Gates KS. Reaction of thiols with 7-methylbenzopentathioepin. *Bioorg Med Chem Lett* (2003) **13**:1349–52. doi:10.1016/s0960-894x(03)00103-3
103. Benavides GA, Squadrito GL, Mills RW, Patel HD, Isbell TS, Patel RP, et al. Hydrogen sulfide mediates the vasoactivity of garlic. *Proc Natl Acad Sci U S A* (2007) **104**:17977–82. doi:10.1073/pnas.0705710104
104. Predmore BL, Kondo K, Bhushan S, Zlatopolsky MA, King AL, Aragon JP, et al. The polysulfide diallyl trisulfide protects the ischemic myocardium by preservation of endogenous hydrogen sulfide and increasing nitric oxide bioavailability. *Am J Physiol Heart Circ Physiol* (2012) **302**:H2410–8. doi:10.1152/ajpheart.00044.2012
105. Chuah SC, Moore PK, Zhu YZ. S-allylcysteine mediates cardioprotection in an acute myocardial infarction rat model via a hydrogen sulfide-mediated pathway. *Am J Physiol Heart Circ Physiol* (2007) **293**:H2693–701. doi:10.1152/ajpheart.00853.2007.-S-allylcysteine
106. Kan J, Guo W, Huang C, Bao G, Zhu Y, Zhu YZ. S-propargyl-cysteine, a novel water-soluble modulator of endogenous hydrogen sulfide, promotes angiogenesis through activation of signal transducer and activator of transcription 3. *Antioxid Redox Signal* (2014) **20**:2303–16. doi:10.1089/ars.2013.5449
107. Cai H, Griendling KK, Harrison DG. The vascular NADPH oxidases as therapeutic targets in cardiovascular diseases. *Trends Pharmacol Sci* (2003) **24**:471–8. doi:10.1016/S0165-6147(03)00233-5
108. Witztum JL, Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. *J Clin Invest* (1991) **88**:1785–92. doi:10.1172/JCI115499
109. Papaharalambus CA, Griendling KK. Basic mechanisms of oxidative stress and reactive oxygen species in cardiovascular injury. *Trends Cardiovasc Med* (2007) **17**:48–54. doi:10.1016/j.tcm.2006.11.005
110. Imai J, Ide N, Nagae S, Moriguchi T, Matsuura H, Itakura Y. Antioxidant and radical scavenging effects of aged garlic extract and its constituents. *Planta Med* (1994) **60**:417–20. doi:10.1055/s-2006-959522
111. Ide N, Lau B. Aged garlic extract attenuates intracellular oxidative stress. *Phytomedicine* (1999) **6**:125–31. doi:10.1016/S0944-7113(99)80047-6
112. Borek C. Antioxidant health effects of aged garlic extract. *J Nutr* (2001) **131**:1010S–5S.
113. Dillon SA, Burmi RS, Lowe GM, Billington D, Rahman K. Antioxidant properties of aged garlic extract: an *in vitro* study incorporating human low density lipoprotein. *Life Sci* (2003) **72**:1583–94. doi:10.1016/S0024-3205(02)02475-X
114. Kim J-M, Chang HJ, Kim W-K, Chang N, Chun HS. Structure-activity relationship of neuroprotective and reactive oxygen species scavenging activities for *Allium* organosulfur compounds. *J Agric Food Chem* (2006) **54**:6547–53. doi:10.1021/jf060412c
115. Medina-Campos ON, Barrera D, Segoviano-Murillo S, Rocha D, Maldonado PD, Mendoza-Patino N, et al. S-allylcysteine scavenges singlet oxygen and hypochlorous acid and protects LLC-PK(1) cells of potassium dichromate-induced toxicity. *Food Chem Toxicol* (2007) **45**:2030–9. doi:10.1016/j.fct.2007.05.002
116. Chung LY. The antioxidant properties of garlic compounds: allyl cysteine, alliin, allixin, and allyl disulfide. *J Med Food* (2006) **9**:205–13. doi:10.1089/jmf.2006.9.205
117. Arguello-García R, Medina-Campos ON, Perez-Hernandez N, Pedraza-Chaverri J, Ortega-Pierres G. Hypochlorous acid scavenging activities of thioallyl compounds from garlic. *J Agric Food Chem* (2010) **58**:11226–33. doi:10.1021/jf102423w
118. Wang H, Huang D. Dietary organosulfur compounds from garlic and cruciferous vegetables as potent hypochlorite scavengers. *J Funct Foods* (2014). doi:10.1016/j.jff.2014.07.001
119. Vaidya V, Ingold KU, Pratt DA. Garlic: source of the ultimate antioxidants – sulfenic acids. *Angew Chem Int Ed* (2009) **48**:157–60. doi:10.1002/anie.200804560
120. Ho S, Ide N, Lau B. S-allyl cysteine reduces oxidant load in cells involved in the atherogenic process. *Phytomedicine* (2001) **8**:39–46. doi:10.1078/0944-7113-00005
121. Ide N, Lau BH. Garlic compounds protect vascular endothelial cells from oxidized low density lipoprotein-induced injury. *J Pharm Pharmacol* (1997) **49**:908–11. doi:10.1111/j.2042-7158.1997.tb06134.x
122. Lei Y-P, Chen H-W, Sheen L-Y, Lii C-K. Diallyl disulfide and diallyl trisulfide suppress oxidized LDL-induced vascular cell adhesion molecule and E-selectin expression through protein kinase A- and B-dependent signaling pathways. *J Nutr* (2008) **138**:996–1003.
123. Kuo WW, Wang WJ, Tsai CY, Way CL, Hsu HH, Chen LM. Diallyl trisulfide (DATS) suppresses high glucose-induced cardiomyocyte apoptosis by inhibiting JNK/NF κ B signaling via attenuating ROS generation. *Int J Cardiol* (2013) **168**:270–80. doi:10.1016/j.ijcard.2012.09.080
124. Huang Y-T, Yao C-H, Way C-L, Lee K-W, Tsai C-Y, Ou H-C, et al. Diallyl trisulfide and diallyl disulfide ameliorate cardiac dysfunction by suppressing apoptotic and enhancing survival pathways in experimental diabetic rats. *J Appl Physiol* (2013) **114**:402–10. doi:10.1152/jappphysiol.00672.2012
125. Tsai CY, Wang CC, Lai TY, Tsu HN, Wang CH, Liang HY, et al. Antioxidant effects of diallyl trisulfide on high glucose-induced apoptosis are mediated by the PI3K/Akt-dependent activation of Nrf2 in cardiomyocytes. *Int J Cardiol* (2013) **168**:1286–97. doi:10.1016/j.ijcard.2012.12.004
126. Padiya R, Chowdhury D, Borkar R, Srinivas R, Pal Bhadra M, Banerjee SK. Garlic attenuates cardiac oxidative stress via activation of PI3K/AKT/Nrf2-Keap1 pathway in fructose-fed diabetic rat. *PLoS One* (2014) **9**:e94228. doi:10.1371/journal.pone.0094228

127. Liu LL, Yan L, Chen YH, Zeng GH, Zhou Y, Chen HP, et al. A role for diallyl trisulfide in mitochondrial antioxidative stress contributes to its protective effects against vascular endothelial impairment. *Eur J Pharmacol* (2014) **725**:23–31. doi:10.1016/j.ejphar.2014.01.010
128. Lei YP, Liu CT, Sheen LY, Chen HW, Lii CK. Diallyl disulfide and diallyl trisulfide protect endothelial nitric oxide synthase against damage by oxidized low density lipoprotein. *Mol Nutr Food Res* (2010) **54**:S42–52. doi:10.1002/mnfr.200900278
129. Khatua TN, Padiya R, Karnewar S, Kuncha M, Agawane SB, Kotamraju S, et al. Garlic provides protection to mice heart against isoproterenol-induced oxidative damage: role of nitric oxide. *Nitric Oxide* (2012) **27**:9–17. doi:10.1016/j.niox.2012.03.004
130. Liu DS, Gao W, Liang ES, Wang SL, Lin WW, Zhang WD, et al. Effects of allicin on hyperhomocysteinemia-induced experimental vascular endothelial dysfunction. *Eur J Pharmacol* (2013) **714**:163–9. doi:10.1016/j.ejphar.2013.05.038
131. Asdaq SM, Inamdar MN. Potential of garlic and its active constituent, S-allyl cysteine, as antihypertensive and cardioprotective in presence of captopril. *Phytomedicine* (2010) **17**:1016–26. doi:10.1016/j.phymed.2010.07.012
132. Calvert JW, Jha S, Gundewar S, Elrod JW, Ramachandran A, Pattillo CB, et al. Hydrogen sulfide mediates cardioprotection through nrf2 signaling. *Circ Res* (2009) **105**:365–74. doi:10.1161/CIRCRESAHA.109.199919
133. Kobayashi M, Yamamoto M. Molecular mechanisms activating the Nrf2-Keap1 pathway of antioxidant gene regulation. *Antioxid Redox Signal* (2005) **7**:385–94. doi:10.1089/ars.2005.7.385
134. Kobayashi M, Li L, Iwamoto N, Nakajima-Takagi Y, Kaneko H, Nakayama Y, et al. The antioxidant defense system Keap1-Nrf2 comprises a multiple sensing mechanism for responding to a wide range of chemical compounds. *Mol Cell Biol* (2009) **29**:493–502. doi:10.1128/MCB.01080-08
135. Zhu H, Jia Z, Misra BR, Zhang L, Cao Z, Yamamoto M, et al. Nuclear factor E2-related factor 2-dependent myocardial cytoprotection against oxidative and electrophilic stress. *Cardiovasc Toxicol* (2008) **8**:71–85. doi:10.1007/s12012-008-9016-0
136. Li J, Ichikawa T, Villacorta L, Janicki JS, Brower GL, Yamamoto M, et al. Nrf2 protects against maladaptive cardiac responses to hemodynamic stress. *Arterioscler Thromb Vasc Biol* (2009) **29**:1843–50. doi:10.1161/ATVBAHA.109.189480
137. Zhu H, Itoh K, Yamamoto M, Zweier JL, Li Y. Role of Nrf2 signaling in regulation of antioxidants and phase 2 enzymes in cardiac fibroblasts: protection against reactive oxygen and nitrogen species-induced cell injury. *FEBS Lett* (2005) **579**:3029–36. doi:10.1016/j.febslet.2005.04.058
138. Lee JS, Surh YJ. Nrf2 as a novel molecular target for chemoprevention. *Cancer Lett* (2005) **224**:171–84. doi:10.1016/j.canlet.2004.09.042
139. Kim S, Lee HG, Park SA, Kundu JK, Keum YS, Cha YN, et al. Keap1 cysteine 288 as a potential target for diallyl trisulfide-induced Nrf2 activation. *PLoS One* (2014) **9**:e85984. doi:10.1371/journal.pone.0085984
140. Blair A, Shaul PW, Yuhanna IS, Conrad PA, Smart EJ. Oxidized low density lipoprotein displaces endothelial nitric-oxide synthase (eNOS) from plasmalemmal caveolae and impairs eNOS activation. *J Biol Chem* (1999) **274**:32512–9. doi:10.1074/jbc.274.45.32512
141. Ji Y, Diao J, Han Y, Huang Y, Bai H, Chen Q, et al. Pyridoxine prevents dysfunction of endothelial cell nitric oxide production in response to low-density lipoprotein. *Atherosclerosis* (2006) **188**:84–94. doi:10.1016/j.atherosclerosis.2005.10.035
142. Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature* (1999) **399**:601–5. doi:10.1038/21224
143. Dikalov S. Cross talk between mitochondria and NADPH oxidases. *Free Radic Biol Med* (2011) **51**:1289–301. doi:10.1016/j.freeradbiomed.2011.06.033
144. Weiss N, Keller C, Hoffmann U, Loscalzo J. Endothelial dysfunction and atherothrombosis in mild hyperhomocysteinemia. *Vasc Med* (2002) **7**:227–39. doi:10.1191/1358863x02vm428ra
145. Weiss N, Heydrick SJ, Postea O, Keller C, Keaney JF Jr, Loscalzo J. Influence of hyperhomocysteinemia on the cellular redox state – impact on homocysteine-induced endothelial dysfunction. *Clin Chem Lab Med* (2003) **41**:1455–61. doi:10.1515/CCLM.2003.223
146. Tsai KH, Wang WJ, Lin CW, Pai P, Lai TY, Tsai CY, et al. NADPH oxidase-derived superoxide anion-induced apoptosis is mediated via the JNK-dependent activation of NF- κ B in cardiomyocytes exposed to high glucose. *J Cell Physiol* (2012) **227**:1347–57. doi:10.1002/jcp.22847
147. Huh JE, Kang KS, Chae C, Kim HM, Ahn KS, Kim SH. Roles of p38 and JNK mitogen-activated protein kinase pathways during cantharidin-induced apoptosis in U937 cells. *Biochem Pharmacol* (2004) **67**:1811–8. doi:10.1016/j.bcp.2003.12.025
148. Ogino T, Ozaki M, Hosako M, Omori M, Okada S, Matsukawa A. Activation of c-Jun N-terminal kinase is essential for oxidative stress-induced Jurkat cell apoptosis by monochloramine. *Leuk Res* (2009) **33**:151–8. doi:10.1016/j.leukres.2008.07.009
149. Shou Y, Li N, Li L, Borowitz JL, Isom GE. NF- κ B-mediated up-regulation of Bcl-Xs and Bax contributes to cytochrome c release in cyanide-induced apoptosis. *J Neurochem* (2002) **81**:842–52. doi:10.1046/j.1471-4159.2002.00880.x
150. Wang S, Kotamraju S, Konorev E, Kalivendi S, Joseph J, Kalyanaram B. Activation of nuclear factor- κ B during doxorubicin-induced apoptosis in endothelial cells and myocytes is pro-apoptotic: the role of hydrogen peroxide. *Biochem J* (2002) **367**:729–40. doi:10.1042/BJ20020752

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 15 October 2014; accepted: 05 January 2015; published online: 02 February 2015.

Citation: Tocmo R, Liang D, Lin Y and Huang D (2015) Chemical and biochemical mechanisms underlying the cardioprotective roles of dietary organopolysulfides. *Front. Nutr.* 2:1. doi: 10.3389/fnut.2015.00001

This article was submitted to Food Chemistry, a section of the journal *Frontiers in Nutrition*.

Copyright © 2015 Tocmo, Liang, Lin and Huang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.