Significance of Garlic and Its Constituents in Cancer and Cardiovascular Disease

Clarifying the Real Bioactive Constituents of Garlic[1]

Harunobu Amagase[2,3]

Department of Research and Development, Wakunaga of America Co., Mission Viejo, CA 92691

ABSTRACT   Compounds in garlic work synergistically to produce various effects, but, because of garlic's chemical complexity, processing methods yield preparations with differing efficacy and safety. Although thiosulfinates such as allicin have been long misunderstood to be active compounds due to their characteristic odor, it is not necessary for garlic preparations to contain such odorous compounds to be effective, and they decompose and disappear during any processing. Garlic exhibits hypolipidemic, antiplatelet, and procirculatory effects. It prevents cold and flu symptoms through immune enhancement and demonstrates anticancer and chemopreventive activities. In addition, aged garlic extract possesses hepatoprotective, neuroprotective, antioxidative activities, whereas other preparations may stimulate oxidation. Additional effects may be caused by S-allylcysteine, S-allyl mercaptocysteine), saponins, N^x-fructosyl arginine, and other substances formed during a long-term extraction process. Although not all of active ingredients of garlic are known, and allicin-like transient components are not directly active, ample research suggests that an allicin-free garlic preparation that is standardized with a bioavailable component such as S-allylcysteine, is active and various effects of garlic may be attributed to it. Furthermore, various chemical constituents in garlic products, including nonsulfur compounds such as saponins, may contribute to the essential biological activities of garlic. Further studies are needed to confirm their bioavailability and associated activities.   J. Nutr. 136: 716S–725S, 2006.

KEY WORDS: • *garlic* • *bioactive* • *aged garlic extract* • *organosulfur compounds*

Garlic (*Allium sativum*) has long been used both for flavoring and for the potential benefits of preventing and curing ailments in many cultures (1). Epidemiological, clinical, and preclinical studies have shown the close relation between dietary habits, including garlic intake, and the occurrence of disease. Garlic has been investigated extensively for health benefits, resulting in more than 1000 publications over the last decade alone, and it is considered one of the best disease-preventive foods, based on its potent and varied effects. However, some studies shed doubt on garlic's benefits, and careful examination of such research can help clarify the pros and cons of processing garlic by different methods. Although many garlic preparations are commercially available, confusion remains because of the inconsistency of clinical-study results and the lack of scientific studies on individual products. This article attempts to clarify the current ambiguity regarding the effects of garlic supplements and the differences among them in efficacy, chemistry (especially relating to standardization markers), and toxicity (including contraindication with medication).

Health benefits of garlic and current confusion

The chemistry of the *Allium* species has been dominated by many sulfur-containing compounds that give them a characteristic flavor. However, a variety of components, including nonsulfur compounds, work synergistically to provide various health benefits. Because of the complex chemistry in *Allium* plants, variations in processing yield quite different preparations (2). Highly reactive thiosulfinates such as allicin disappear during processing and are quickly transformed to other types of organosulfur compounds. Efficacy and safety are also contingent upon processing methods (2).

Garlic exhibits hypolipidemic, antiplatelet, and procirculatory effects. It prevents cold and flu symptoms through immune enhancement and exhibits anticancer and chemopreventive

activities. Many favorable experimental and clinical studies on the consumption of garlic preparations, especially of aged garlic extract (AGE)[4], demonstrate a wide variety of biological activities attributed to it. AGE also has hepatoprotective, neuroprotective, and antioxidative activities, whereas other preparations may stimulate oxidation (3). These additional biological effects may be due to conversion compounds that are formed during AGE's long-term extraction process, called the aging process.

It has long been known that the extraction process increases the potency and bioavailability of various crude herbs and eliminates undesirable harsh and toxic characteristics. The irritating, acidic, and oxidizing compounds in raw garlic, such as allicin, can be eliminated and modified by extracting it with alcohol, wine, milk, vinegar, or soy sauce before being used as a therapeutic, as is done in some cultures. Many adverse reactions to garlic can be attributed to allicin and its degraded compounds (2) and an appropriate extraction process can eliminate these undesirable compounds while retaining other, active ones. For example, the lipid-lowering effect attributed to oil-soluble sulfur compounds in hepatocytes may be due to their cytotoxicity, as revealed by cell damage (4). The elution of acetone from the breath of subjects consuming oil-soluble odorous compounds is also suggestive of their cytotoxicity (5). In contrast, water-soluble sulfur compounds effectively reduce cholesterol synthesis and are not cytotoxic (4). AGE, demonstrating the benefits of the extraction process, contains various nontoxic, active, and water-soluble constituents such as S-allylcysteine (SAC) and has a significantly reduced toxicity that has been confirmed by toxicological studies and a long history of human consumption (2). Extraction procedures have been commonly used in the preparation of many other herbal materials and for extracting the favorable components from them to use for health benefits, although extraction medium and time periods may differ. For example, commercially available ginkgo biloba extract is designed to eliminate ginkolic acid, which may cause allergic reactions.

Several clinical reports and meta-analyses have revealed the cholesterol-lowering effects of garlic supplementation in humans (6–9). These reports have affected public awareness of garlic's potential for lowering cholesterol. However, recent publications (7,10) report that neither garlic oil nor dehydrated garlic powder effect cholesterol levels. These publications have caused serious confusion in the public and in academia. Although one study concludes that the lack of effect is due to varied levels of allicin potential in the dehydrated garlic–powder supplements used in the clinical studies (11), it does not explain the cause of the inconsistency, because, as shown in the previous literature, allicin or allicin potential is not a correct marker for controlling the quality of garlic supplements (2). Standardization is the key to delivering consistent quality and efficacy of garlic products to consumers. As stated above, garlic changes its characteristics because of the complexity of its intrinsic chemistry, and processing procedures and standardization marker compounds are very important for ensuring consistent effects.

Chemistry of garlic

Nonvolatile sulfur-containing precursors in intact garlic. The major sulfur-containing compounds in intact garlic are γ-glutamyl-S-allyl-L-cysteines and S-allyl-L-cysteine sulfoxides (alliin). Both are abundant as sulfur compounds, and alliin is the primary odorless, sulfur-containing amino acid, a precursor of allicin (12), methiin, (+)-S-(trans-1-propenyl)-L-cysteine sulfoxide, and cycloalliin (13). These sulfoxides, except cyloalliin, are converted into thiosulfinates (such as allicin) through enzyme reactions when raw garlic is cut or crushed. Thus, no thiosulfinates are found in intact garlic.

γ-Glutamyl-S-allyl-L-cysteines are converted into S-allyl-cysteines (SAC) through an enzymatic transformation with γ-glutamyltranspeptidase when garlic is extracted with an aqueous solution (14). SAC, a major transformed product from γ-glutamyl-S-allyl-L-cysteine, is a sulfur amino acid detected in the blood that is verified as both biologically active and bioavailable. Determining the contents of these key precursor compounds is important for evaluating raw garlic.

Organosulfur compounds in the process of garlic-product preparation. Thiosulfinate formation. The disruption of garlic bulbs causes the formation of thiosulfinates such as allicin through the enzymatic reaction of sulfur-substituted cysteine sulfoxides, compartmentalized in the cytoplasma with alliinase in the vacuole, via sulfur-substituted sulfenic acids as a highly reactive intermediate (**Fig. 1**). The finding that allicin killed microrganisms in a Petri-dish (15) was a sensational discovery. However, hopes for a medicinal or antiseptic use of allicin based upon this Petri-dish study soon faded because of its extreme instability and toxicity. Other thiosulfinates, including allylmethyl-, methylallyl-, and trans-1-propenyl-thiosulfinate, were found in the garlic homogenates, and, like allicin, they are all unstable (16,17). When allicin itself was kept at 20°C for 20 h, it decomposed to diallyl disulfide (DADS) (66%), diallyl sulfide (DAS) (14%), diallyl trisulfide (9%), and sulfur dioxide (18). Allicin easily reacts with amino acids and proteins, creating an -SH group. Freeman found that allicin binds to protein and fatty acids in the plasma membrane, is thus trapped before absorption, and cannot circulate in the blood (19). In fact, no allicin was detected in the blood after the ingesting raw garlic or pure allicin (5,20).

Alliinase is the key enzyme that facilitates the transformation of cysteine sulfoxides to thiosulfinates. The purified enzyme possesses a pH optimum of 6.5 with S-methyl-L-cysteine as substrate (21). In addition, pyridoxal phosphate stimulates alliinase activity as a cofactor (22). A pH dependency of alliinase activity is indicated when allicin and other thiosulfinates are released during incubation of garlic powder in buffer solutions adjusted from pH 2 to 10. Thiosulfinates are not
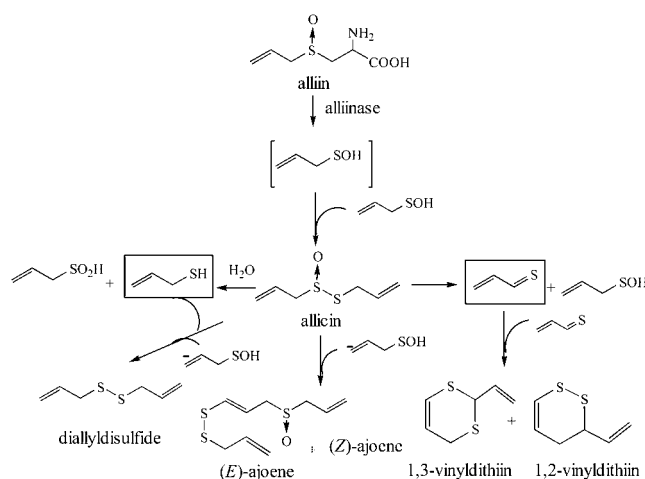

FIGURE 1 Enzymatic reaction of sulfur-substituted cysteine sulfoxides.

formed below pH 3.6, which is the usual pH range in the stomach (23). Furthermore, thiosulfinates are never generated through the neutralization of a mixture previously incubated below pH 3. Thus, alliinase is completely and irreversibly inhibited under the acidic conditions found in the stomach. Freeman et al. (19) also reported that no processed garlic preparations contain allicin, and furthermore, allicin is not generated in simulated gastric solution. Therefore, allicin-producing potential, which is defined as the allicin released from garlic preparations in water, should not be a meaningful chemical evaluation for garlic products. Findings clearly indicate that allicin itself does not contribute to any of garlic's beneficial effects inside the body. Allicin is thought to be a transient compound that is rapidly decomposed into other sulfur-containing compounds and is not a genuine active compound of garlic.

Organosulfur volatiles. Processed garlic contains a wider variety of organosulfur volatiles than the intact garlic clove. Typical volatiles that have been identified in crushed garlic and garlic essential oil include DAS, DADS, diallyl trisulfide, methylallyl disulfide, methylallyl trisulfide, 2-vinyl-4H-1, 3-dithiin, 3-vinyl-4H-1, 2-dithiin, and (E,Z)-ajoenes.

Over 20 sulfides have been identified in steam-distilled garlic oil and oil-soluble extract of garlic, and many of them, especially sulfides having an allyl group, are responsible for the characteristic smell and taste after ingesting garlic. The major sulfides in garlic oil include DAS (57%), allylmethyl (37%), and dimethyl (6%) mono- to hexasulfides, in some cases, together with a small amount of allyl 1-propenyl and methyl 1-propenyl di-, tri-, and tetrasulfides (17). Diallyl trisulfide is the most abundant in fresh garlic oil, but commercially available garlic-oil products have an increased amount of DADS (24,25). The level is speculated to be dependent upon the disproportionation of diallyl trisulfide in the oil. The component of these sulfides varies according to extraction temperature or time (26).

Vinyldithiins were first demonstrated to be thermal-degradation products derived from allicin during gas chromatographic analysis of allicin (18). These structures were elucidated to be 2-vinyl-4H-1, 3-dithiin and 3-vinyl-4H-1, 2-dithiin on the basis of spectroscopic analysis. The formation mechanism has been confirmed to be a type of Diels-Alder dimerization of thioacrolein derived from the β-elimination of allicin. A remarkable production of vinyldithiins from allicin is observed when less-polar solvents such as hexane are used. Vinyldithiins, especially 2-vinyl-4H-1, 3-dithiin, are rich in the oil macerate of raw garlic (27).

Apitz-Castro et al. (28) first isolated ajoene from the ether fraction of garlic extract as a potent antithrombotic agent. Block and Ahmad (29) determined ajoene structure was E and Z isomers of 4, 5, 9-trithiadodeca-1, 6, 11-triene-9-oxide. They also proposed that it is formed by S-thioallylation of allicin, followed by Cope-type elimination and readdition of 2-propenesulfenic acid. Iberl et al. (27) elaborated the influence of different media on allicin transformation including the E:Z ratio of ajoene. Another ajoene-type organosulfur compound, E-4,5,9-tritriadeca-1,7-diene-9-oxide, was isolated from oil-macerated garlic extract (30).

Water-soluble organosulfur compounds. Alcoholic and aqueous garlic extracts contain primarily S-allyl-L-cysteines derived from γ-glutamyl-S-allyl-L-cysteines (**Fig. 2**). S-Allyl-L-cysteine and *trans*-S-1-propenyl-L-cysteine, together with a small amount of S-methyl-L-cysteine, are found in garlic extract such as AGE. These cysteine derivatives are colorless crystals and are odorless and stable in the solid state or aqueous solution under neural or slight acidic conditions (31). SAC provides protection against oxidation, free radicals, cancer, and cardiovascular diseases. In
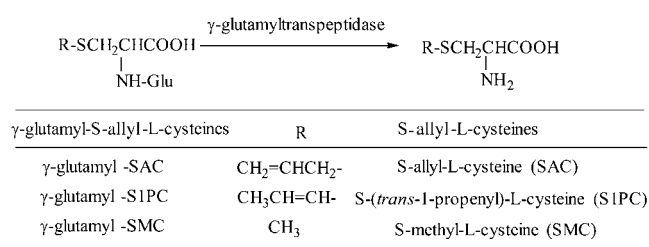

FIGURE 2 Variation of S-allylcysteines derived from γ-glutamyl-S-allyl-L-cysteines.

addition, S-allylmercapto-L-cysteine, which demonstrates an in vivo hepato-protective effect (32,33), an in vitro cancer-preventive effect in human prostate carcinoma cells (34), as well as antioxidant activity in vitro (3), is a characteristic compound present in AGE.

Bioavailability and metabolism of organosulfur compounds

Bioavailability of chemical components as active ingredients in the body is essential. Little data, however, is available from preclinical and clincal studies concerning the absorption, metabolism, and distribution of garlic-derived compounds.

Alliin. In a mouse murine study, 10 min after orally administering alliin (10 mg/mouse), alliin was observed in the stomach (7.2%), intestine (22.4%), and liver (2.5%)without the production of allicin and its degradation compounds such as DADS, vinyl dithiins, and allyl-SS conjugated compounds (35). In another experiment, alliin showed lower plasma concentration with a bioavailability of 16.5% within 4 h after oral ingestion of 60 mg/kg alliin in rats (35). Lachmann et al. (36) reported that in pharmacokinetic studies using synthesized ^{35}S-labeled alliin, 60–70% was absorbed in rats . It was found that alliin along with DADS could be detected in the perfusate after the isolated rat liver passage, but no allicin was found (37). These findings indicate that alliin itself is never converted to allicin in the body and metabolized to various organosulfur compounds such as DADS by liver enzymes.

Allicin. Definitive investigations have not been made concerning the absorption of allicin from the digestive tract. Freeman et al. (19) reported that ingestion of allicin causes instability and metabolites in the blood. They found that allicin quickly disappeared from whole blood within a few minutes while DAS and allylmercaptan were formed. They also revealed the maximum band of allicin at 630 nm in the visible spectrum of the blood after ingestion. The appearance of allicin in the visible spectrum of the blood depends on the formation of methemoglobin, which is produced by allicin's oxidation of iron in hemoglobin. It is notable that allicin acts as an oxidant in the blood. When allicin is mixed with blood in vitro, almost all allicin disappears within a few minutes, because allicin binds to the protein of red blood cells and oxidizes them immediately (19). It is assumed that if allicin is ingested through the mouth, it immediately binds to lumen and is trapped instantly as we experience a harsh sensation in the mouth after chewing the garlic clove. Therefore, it will not pass through the digestive tract membrane to get through the serosa into the bloodstream. Egen-Schwind et al. (37) reported a remarkable first-pass effect of allicin in the isolated perfused rat liver. DADS quickly forms after infusion of allicin in a low concentration. Later, the formation of allylmercaptan was observed in the collected bile as well as the liver tissue. No allicin could be detected in liver

passage. Therefore, we can conclude that allicin is not a biologically active component of garlic.

Although allicin is reported to be metabolized into allyl methyl sulfoxide (AMS) and released into the breath (38), blood concentration of AMS and its bioavailability have not been studied, and the actual rate of allicin conversion to AMS has not been clearly evaluated or calculated. Therefore, AMS has not been well-established as a metabolite of allicin, and furthermore, because AMS has not been reported as active compound of garlic in any clinical studies, it is not clear if allicin and AMS are in fact active compounds or represent biologically full activities of garlic.

Organosulfur volatiles. DASs and vinyldithiins are the major components of garlic oil and oil-macerate preparations. Vinyldithiins, 2-vinyl-4H-1,3-dithiin and 3-vinyl-4H-1,2-dithiin, have been detected in the serum, kidney, and fat tissue >24 h after oral ingestion, while only 1,3-vinyldithiin was found in the liver. No metabolites of vinyldithiins in the isolated perfused rat liver were identified in perfusate, bile, or the liver (39). Pushpendran et al. (40) reported the metabolic fate of $[^{35}S]$-labeled DADS in rats after intraperitoneal injection. The maximum concentration of $[^{35}S]$-labeled DADS by mice livers occurred 90 min after treatment. Seventy percent of the radioactivity was distributed in the liver cytosol, of which 80% was metabolized to sulfate. Egen-Schwind et al. (37) revealed the identification of allylmercaptan as a metabolite of DADS in the perfusion medium after isolated rat liver passage. Incubation with whole blood at 37°C demonstrated the rapid decrease of ajoene (half-life, 1 min) and diallyl trisulfide (half-life, 4 min), while 1,2-vinyldithiin (half-life, 15 min) and DADS (half-life, 60 min) decreased more slowly. No change was observed in 2-h incubation of 1,3-vinyldithiin and DAS.

S-Allyl-L-cysteine. SAC is one of the water-soluble organosulfur compounds in garlic and its concentration increases through a long-term extraction in an aqueous medium. The pharmacokinetics of SAC are well-established in vivo (41). SAC is detected in the blood, and its blood concentration and other pharmacokinetic parameters are well-associated with doses of orally administered SAC in animal studies. Significant concentration of N-acetyl-S-allyl-L-cysteine is also identified as a metabolite of SAC in the urine. This indicates that SAC could be transformed into N-acetylated metabolite by N-acetyltransferase in the body. The bioavailability of SAC is 103.0% in mice, 98.2% in rats, and 87.2% in dogs. Because SAC is present in garlic preparations and has many biological effects in addition to its bioavailability, it must be one of the active substances in garlic preparations and account for at least a portion of garlic's biological activities. Thus, the standardization of garlic preparations using SAC as a chemical marker is scientifically reasonable and well justified.

Metabolites after the human consumption of garlic and garlic preparations. Although there are many chemical and biological studies of garlic and its characteristic organosulfur compounds, there has been little research on the metabolites in humans after garlic consumption. Minami et al. (42) reported that after ingesting grated garlic, 2 major peaks, which were identical to allylmercaptan and DADS by GC-MS analysis, could be detected in human breath without other organosulfur volatiles. No allicin was detected in either the serum or urine from 1 to 24 h, even after ingesting 25 g of raw garlic containing a significant amount of allicin (20). Rosen et al. (38) indicated that allicin decomposes in stomach acid to release DAS, DADS, and other volatiles that are postulated to be metabolized by glutathione or S-adenosylmethionine to form AMS from human breath after consumption of raw garlic. The breath analysis, however, may not reflect the real bioavailability of

garlic constituents because it analyzes a mixture of breath from the lungs and a burp from the stomach, which are not absorbed by the body (5). The quantitative analysis of AMS's bioavailability in the blood has not yet been presented. Therefore, breath analysis is not an accurate bioavailability test. Other metabolites of garlic constituents, such as N-acetyl-S-(2-carboxypropyl)-cysteine and N-acetyl-S-allylcysteine, have been detected in human urine after ingesting garlic (43). Recently, SAC was found in human blood in a dose-dependent manner after ingesting a preparation containing AGE (38,44). Based on the above evidence, water-soluble organosulfur compounds such as SAC or N-acetyl-S-allylcysteine should be considered reliable compliance makers for human clinical studies involving garlic intake because they are among the active compounds of garlic, are stable, and they are easy samples to handle for analysis.

Nonsulfur compounds, steroid saponins. Saponins have characteristic properties, including the production of a stable foam when shaken with water, hemolytic activity, and a bitter taste. They are generally classified into two groups, triterpenoid saponins and steroid saponins, based on the molecular structure of aglycone (45). There are many examples of triterpenoid saponins among biologically active compounds in herbal medicines, for example, ginsenosides for ginseng and glycyrrhizin for licorice. Steroid saponins are further divided into furostanol saponins and spirostanol saponins. Furostanol saponins have a β-glucosyl unit at the 26th position of the aglycone moiety and are easily transformed into spirostanol saponins by an enzymatic reaction to close a ring with β-glucosidase. Furostanol saponins are reported to be usually contained in fresh plants as original saponins and are gradually converted into spirostanol saponins during drying. Many steroid saponins have been reported in plants and animals, especially in the *Liliaceae* family, which includes garlic.

The presence of steroid saponins has been previously detected in garlic extract by thin-layer chromatography (TLC) (46). In 1988, a furostanol saponin named proto-eruboside-B was isoloated from a crude glycoside fraction prepared from a methanolic extract of frozen garlic bulbs by a reversed-phase porous polymer (47). This study found that freezing depresses β-glucosidase activity during extraction to isolate the original saponins from raw garlic. Further studies on steroid saponins from garlic led to the isolation and the structure determination of a furostanol saponin named sativoside-B1, and to the discovery of a known furostanol saponin, proto-desgalactotigonin (48). No spirostanol saponins have been isolated from frozen garlic bulbs. On the other hand, eruboside-B, a spirostanol saponin corresponding to proto-eruboside-B, was isolated from garlic bulbs that were crushed at room temperature and then extracted with methanol. These results show that processing garlic leads to steroid saponins in addition to various organosulfur compounds. Further studies on the distribution of steroid saponins have yielded the isolation of two new steroid saponins, named sativoside-R1 and sativoside-R2, from the roots. Their structures have been established to be gluco-proto-desgalacto-tigonin and its corresponding spirostanol saponin. In addition, 3 known steroid saponins have been isolated and identified; however no glycosides of β-chlorogenin, which is the aglycone of eruboside-B, have been isolated from the roots. No steroid saponins and aglycones have been detected through the analysis of the crude glycoside fraction and its hydrolyzate from aerial parts of garlic.

Peng et al. (49) recently reported the isolation and structure determination of new steroid saponins named proto-isoeruboside-B and isoeruboside-B, which are elucidated to be the C-25 epimers of proto-eruboside-B and eruboside-B, respectively.

Steroid saponins in the crude glycoside fraction, which we prepared from a methanolic extract of crushed raw garlic at room temperature, were also reinvestigated under the inspiration of this report. New spirostanol saponins, named sativoside-B2, -B3, -B4, and -B5, were isolated along with eruboside-B (50). Sativoside-B4 and -B5 were determined to be spirostanol saponins having a new aglycone, 27-hydroxy-β-chlorogenin.

Ten furostanol saponins and seven spirostanol saponins were isolated from AGE, and their structures were determined by spectroscopic analysis, including 2D-NMR and FAB-MS. Spirostanol saponins isolated from AGE should be obtained from the corresponding furostanol saponins through the reaction with β-glucosidase originally contained in raw garlic. It has also been suggested that the isolation of three 26-O-monoglucosides of furostanol saponins indicates the presence of the enzymes, which can completely hydrolyze the sugar moiety attached at the C-3 position.

Steroid saponins and sapogenins could be considered reliable chemical markers for the identification of garlic and garlic preparations, except for garlic oil. Itakura et al. (51) discriminated between garlic and other *Allium* plants in the TLC analysis of the steroid sapogenins after a hydrolysis of the crude glycoside fraction from *Allium* plants. His group tried to distinguish garlic from other *Allium* species by using alliin as a chemical marker on TLC, but it was not available for *Allium* plants such as elephant garlic. Furthermore, alliin was not suitable for some of the garlic preparations in which alliin was enzymatically converted into other organosulfur compounds by the enzymatic reaction with alliinase. β-Chlorogenin is a characteristic steroid sapogenin of garlic. The spot corresponding to β-chlorogenin on TLC was not detected in 26 kinds of common *Allium* plants, except for elephant garlic. A slight spot of β-chlorogenin in elephant garlic was observed on TLC; however, the additional observation of an intense TLC spot corresponding to agigenin, which has been reported as a major sapogenin in elephant garlic (52) and different from β-chlorogenin, was specific to the identification of elephant garlic. Chemical identification using TLC analysis of steroid sapogenin has to account for raw garlic, heated garlic, and garlic preparations such as AGE. In addition, a methodology for specific species discrimination, based on the steroid saponin profile by liquid chromatography-mass spectrometer (LC-MS), has been also developed (53). High performance liquid chromatography (HPLC) determination of furostanol saponins of garlic by ultraviolet derivatization with p-nitrobenzoate was reported and applied to the analysis of garlic and garlic preparations (54).

Among the biological activities of steroid saponins isolated from the garlic bulb, eruboside-B exhibited antifungal activity for *Candida albicans* (47), antitumor activity (14), and cytotoxic activities in vitro (55). In contrast, proto-eruboside-B, which is an original furostanol saponin, did not show any biological activities. Koch (56) indicated that the cholesterol-lowering effect of garlic was probably due to the saponin content. Other studies report that the crude glycoside fraction (55,57) from methanolic raw-garlic extracts, which mainly contains spirostanol saponins produced by the conversion of furostanol saponins via β-glucosidase, lowered total plasma cholesterol and LDL cholesterol without changing HDL cholesterol levels in hypercholesterolemic animal models. Plant saponins have been shown to inhibit cholesterol absorption from intestinal lumen in experimental animals, and consequently, to reduce the concentration of plasma cholesterol. This may be the result of a complex formation with cholesterol in the digestive tract that has a direct effect on cholesterol metabolism. Furthermore, β-chlorogenin has been shown to inhibit platelet aggregation (58). Since β-chlorogenin is bioavailable in vivo and detected in blood, this indicates that β-chlorogenin may, in addition to the sulfur compounds, be an active compound in garlic.

Various other characteristic chemical constituents of garlic include allixin and organo-selenium compounds. These chemical compounds are reported to exhibit various biological effects, including cholesterol reduction and others, and probably work synergistically with organosulfur compounds.

Commercially available garlic products

Garlic supplement products have experienced increasing popularity in the last decade. The top herbal supplements used by U.S. households in 2004 are shown in **Table 1** (59). Market research indicates that garlic products were the most popular herbal supplement in the single herb category. There are dozens of brands of garlic products on store shelves that provide a convenient way to obtain the health benefits of garlic. They can be classified into four groups: garlic essential oil, garlic oil macerate, garlic powder, and garlic extract (see **Table 2**). The manufacturing process is an important consideration when choosing a garlic supplement. As described earlier, the chemistry of garlic is quite complicated, and different types of processing produce products that are more than just preparations in different forms. The various forms also differ in their ingredients, effects, and toxicities. Garlic products that contain the most safe, effective, stable, and odorless components are the most valuable as dietary supplements.

Because the structure of chemical constituents in garlic is so complicated, their final concentration in each garlic preparation varies significantly and depends heavily upon the processing method. Manufacturing and handling processes of garlic modify the chemical characteristics, efficacy, and safety of the final garlic preparations. It is well known that extraction generally increases potency and bioavailability of various crude botanicals including garlic, and eliminates harsh and toxic characteristics. According to many studies of AGE, garlic extraction results in greater and more consistent efficacy and safety compared with raw garlic, dehydrated garlic powder, or other preparations.

Documenting safety and effectiveness are crucial in evaluating drugs and dietary supplements used for health purposes. Because different garlic preparations consist of different constituents, the safety and effectiveness of each product must be examined through toxicological and pharmacological tests.

The daily dosage in most of the clinical studies using dehydrated garlic powder is 900 mg, but a dose-response relation has not yet been clearly demonstrated. AGE has a wide range of effectiveness based upon clinical studies. Within a dosage range of ~1–7.2 g/d, AGE has been shown to lower plasma cho-

TABLE 1

Top herbal supplements used by U.S. households[1]

	Herb	Sales ($1000)	Sales % change	Share (%)
1	Multi-herbs	52,116	28.7 (45.2)	14.2
2	Garlic	27,038	−13.8 (−9.6)	10.5
3	Echinacea	23,785	−17.9 (−11.5)	9.2
4	Saw palmetto	20,336	−14.2 (1.8)	7.9
5	Ginkgo biloba	19,336	−16.3 (5.7)	7.5
6	Soy	17,437	−24.8 (−30.4)	6.8
7	Cranberry	13,490	3.7 (26.2)	5.2
8	Ginseng	12,166	−12.8	4.7
	Total	257,747	−9.7 (−4.2)	100.0

[1] Sales by supermarkets and mass merchandisers, except Wal-Mart, in 2004. Adapted from *The Natural Foods Merchandiser*, June 2005 (59).

TABLE 2

Garlic products on the market

Type of Product	Main compounds and characteristics
Garlic Essential Oil	Only 1% of Oil-soluble sulfur compounds (DAS, DADS, etc.) in 99% vegetable oil No water-soluble fraction No allicin* Not well-standardized No safety data
Garlic oil macerate Oil	soluble sulfur compounds and alliin No allicin* Not well-standardized No safety data
Garlic powder	Alliin and a small amount of oil-soluble sulfur compounds No allicin* Not well-standardized Results on cholesterol is not consistent. No safety data
Aged garlic extract (AGE)	Mainly water-soluble compounds (SAC, SAMC, saponins, etc.) Standardized with SAC Small amount of oil-soluble sulfur compounds Various beneficial effects Well-established safety Heavily researched (400+ papers)

* Allicin is a highly unstable and reactive compound that rapidly decomposes to other compounds. For this reason no garlic product on the market contains a detectable amount of allicin (<1 μg/g) (19).

lesterol in humans (60). Studies show that as little as 1.8 g to as much as 10 g/d of AGE is effective in enhancing human immune responses (61,62). Interestingly, no severe toxic side effects were reported in these clinical studies even at high dosages. Other garlic supplements have not been studied for toxicity or safety, and few have any clinical studies to confirm their efficacy. In addition, contraindication studies have been not done on garlic supplements except AGE. AGE has been tested in several clinical trials and show no contraindications with several medications, including warfarin (63,64), aspirin (65), statins (cholesterol-lowering drugs) (65), adriamycin/doxysorubicin (66), 5-fluorouracil/methotrexate (67,68), and others. The way in which AGE is prepared, that is, to eliminate toxicity or other unfavorable characteristics of garlic, allows it to be combined with complementary medications without undesirable side effects.

Safety, drug interaction, and quality control of garlic preparations

Garlic may be more effective in preventing health problems and in use as a complementary medicine than as a therapeutic medication. Long-term supplementation is required to obtain the preventative benefits of garlic, which makes it necessary to consider toxicity. Toxicological testing is needed to ensure the safety of each product, and safety is a major factor in the quality control of garlic preparations. Scientifically reasonable quality control standards are essential for high-quality products.

Although garlic has been safely used in cooking as a popular condiment or flavoring and has been used traditionally for medicinal purposes, it is commonly known that excessive consumption of garlic can cause burning sensations and diarrhea. Garlic odor on the breath and skin (69) and occasional allergic reactions (70) may also occur. Raw-garlic preparations containing allicin can cause chemical burns on the skin, contact

dermatitis, and bronchial asthma (71,72). Oil-soluble sulfur compounds are irritants and allergens, and topically applied DAS is the most allergenic (105). When administered orally to laboratory animals, garlic causes stomach ulcers, anemia, decrease in serum protein, inhibition of spermatogenesis, and a decrease in intestinal flora (2,73–75). Many serious concerns over surgery or contraindications with anticlotting medications such as warfarin are expressed in the medical arena regarding garlic.

However, processing methods greatly affect chemical structure of the garlic preparations, and adverse effects can be eliminated by proper extraction and preparation methods. Among the various garlic preparations, AGE has been proven to be safe in toxicological studies such as acute and chronic toxicity tests (2). Recent clinical trials report AGE to be safe as a complementary medicine with warfarin (63,64). Such characteristics may come from the AGE processing method and clearly differentiate the extract from other preparations.

One of the active ingredients in garlic preparations including AGE is SAC (2). SAC is a safe compound and its biological effects are well researched. The U.S. National Cancer Institute tested the toxicity of SAC compared with other typical garlic compounds and found that SAC has less toxicity than allicin and DADS (104). The oral 50% lethal dose in mice (mg/kg body wt) is as follows for allicin: 309 in males and 363 in females; for DADS: 145 in males and 130 in females; and for SAC: 8890 in males and 9390 in females. Thus, SAC has no more than ~4% of the toxicity of allicin and DADS.

The different constituents in various garlic preparations, in addition to having different safety characteristics, also means that the biological and pharmacological activities of the preparations vary. Typical biological and pharmacological activities that describe the differences among the garlic preparations are discussed below.

Cholesterol reduction in clinical studies

Meta-analysis has been done on studies of cholesterol reduction and concludes that dehydrated garlic powder is ineffective in lowering blood-cholesterol levels (10). There is no reasonable explanation for this inconsistency with research results that demonstrate the cholesterol-lowering effects of garlic. However, it is wrong to use allicin as the standardization marker for potential or yield, because allicin's lack of bioavailability means that it is not a genuinely active compound of garlic. The media and lay publications that report such negative studies and meta-analyses have a strong impact on the public (76). They create confusion and skepticism, thereby reducing the intake of garlic supplements that can have health-promoting effects, especially among populations at high risk for disease.

However, the above meta-analysis excluded the results of several clinical studies of the effects of AGE on cholesterol. AGE has consistent effects on risk factors for cardiovascular disease, including cholesterol and others (60,65,77–89). In some of these studies, blood SAC level was measured in the subjects as a compliance marker. The blood SAC level in the group taking supplements was significantly higher than that of the placebo group (65,89). It is clear that SAC is bioavailable because it was absorbed into the blood and is therefore active in the human body. The bioavailability of a chemical compound such as SAC makes it possible to obtain consistent measured effects for the standardization of garlic products.

Anti-oxidation

Reactive oxygen species (ROS), or free radicals, have been implicated in mediating various pathological processes such as cancer, ischemia, inflammatory diseases, diabetes, and athero-

sclerosis. Garlic has been reported to be effective against diseases of which ROS are considered a main cause. The studies suggest that garlic may work by reducing ROS or interacting with them to minimize the negative impact on the body. However, the degree of antioxidative efficacy of various garlic preparations differs according to variations in chemical structures and standardization procedures.

Since antioxidative activity is caused by the relative electron status of the materials, in vivo reaction in the whole body should be taken into account when considering the active compounds of garlic. LDL oxidation has been recognized as playing an important role in the initiation and progression of atherosclerosis. Popov et al. (90) observed the antioxidant effect of the aqueous extract from a dehydrated garlic–powder preparation by using photochemiluminescence on the Cu^{2+}-initiated oxidation of LDL. The formation of conjugated diene, which accompanies the lipid peroxidation process, was detected photometrically. Allicin-free AGE and its constituent SAC have a similar preventative effect against Cu^{2+}-initiated oxidation of LDL taken from the human subjects who consume AGE (77). Ide et al. (91) investigated and found clear supportive data that AGE and SAC significantly prevent membrane damage, loss of cell viability, and lipid peroxidation in bovine pulmonary artery endothelial cells (PAECs) exposed to oxidized LDL. Wei et al. (92) and Yamasaki et al. (93), using PAECs, also observed that AGE suppresses hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) generation, and thus protects vascular endothelial cells from oxidant injury. It also significantly increases the activities of superoxide dismutase (SOD), catalase, and glutathione peroxidase in PAECs. AGE pretreatment significantly reduced the loss of cell viability induced by H_2O_2. AGE and SAC inhibited both lactate-dehydrogenase release and lipid peroxidation induced by H_2O_2. These data indicate that the antioxidative capabilities of AGE and SAC may be useful in preventing of atherosclerosis. Furthermore, Geng et al. (94) showed that AGE increases intracellular glutathione levels, glutathione disulfide reductase, and SOD activity in PAECs, whereas the level of glutathione disulfide decreased. These results suggest that the antioxidant effect of AGE may be due to its modulation of the glutathione redox cycle and SOD activity in vascular endothelial cells.

ROS are involved in signal transduction pathways leading to nuclear factor kappa B (NF-κB) activation that has been implicated in the regulation of gene transcription. Geng et al. (95) determined the effects of SAC on NF-κB cultivation in human T lymphocytes (Jurkat cells) induced by tumor necrosis factor alpha and H_2O_2. SAC consistently inhibited NF-κB activation induced by both tumor necrosis factor alpha and H_2O_2 in nuclear extracts. The results suggest that SAC might act through antioxidant mechanisms to block NF-κB activation in Jurkat cells. These studies are meaningful because SAC is bioavailable and can be delivered to such cells in vivo. If SAC could not reach the target cells in vivo after consumption of garlic, it would not act like an active compound. Therefore, analysis of the bioavailability of such compounds is important, especially for in vitro studies and designing isolated systems.

Horie et al. (96) demonstrated that AGE prevents the formation of thiobarbituric acid–reactive substances and fluorescent substances during lipid peroxidation of rat liver microsomes. AGE protects the membranes from lipid peroxidation and serve to maintain membrane fluidity. Imai et al. (3) compared the antioxidant properties of 3 garlic preparations and organosulfur compounds in garlic. AGE inhibited the emission of low-level chemiluminescence and the early formation of thiobarbituric acid–reactive substances in a liver microsomal fraction initiated by t-butyl hydroperoxide. However,

the water extracts of raw and heat-treated garlic enhanced the emission of low-level chemiluminescence. Among a variety of organosulfur compounds, SAC and S-allylmercaptocysteine (SAMC), major organosulfur compounds found in AGE, showed radical scavenging activity in both chemiluminescence and 1,1-diphenyl-2-picrylhydrazyl assays, indicating that these compounds may play an important role in the antioxidative activity of AGE. Numagami et al. (97) examined effects of AGE and its thioallyl components on rat brain ischemia using a middle cerebral artery occlusion model and a transient global ischemia model. SAC significantly prevented the elevation of water content in ischemic brains and reduced infarct volume. On the other hand, neither allyl sulfide nor allyl disulfide was effective.

The direction of in vitro research must be considered and designed based upon the information from both in vivo and pharmacokinetic analysis of candidates for the active compounds of herbs and botanicals.

Drug-garlic interaction and influence on metabolizing enzymes

Herbal and botanical preparations used as complementary medicines with drugs are heavily scrutinized due to their capability to influence P450 enzymes in the liver, which are responsible for metabolizing exogeneous chemical compounds. Several studies demonstrated the stimulating effect of garlic on P450 enzymes, indicating it has an influence on medications and their levels in the blood. Many herbal supplements are now being closely studied for their potential interaction with medication, especially ones that have an influence on P450 isozymes. Many herbal supplements are consumed by people also taking medications, and these medications may interact with the supplements through the metabolizing systems in the body. The issue is therefore of great interest to the medical, academic, and public communities. Further research in this area must be undertaken and reflected through the development of herbal extract preparations that are less interactive with traditional synthesized drugs.

Piscatelli (98) reported that cytochrome enzyme P450 isozymes were significantly influenced by the intake of a dehydrated garlic–powder supplement, and blood concentration of the AIDS medication Saquinavir (Forlovase, Roche Laboratories) was drastically reduced due to the stimulation of P450 isozymes responsible for metabolizing the drug. Because the study was small and the research protocol was criticized, the National Canter for Complementary and Alternative Medicine supports both basic-mechanism and clinical studies to confirm and compare the effects of the two different garlic preparations, that is, dehydrated garlic powder and AGE, on saquinavir metabolism in humans.

Dehydrated garlic–powder products contain oil-soluble sulfur compounds derived from allicin, and AGE mainly contains water-soluble sulfur compounds such as SAC. Because previous reports indicate that oil-soluble, but not water-soluble, sulfur compounds stimulate P450s, it may be interesting to learn whether these products have different results on saquinavir metabolism.

Hu et al. (99) observed the effects of DAS on oxidative metabolism and hepatotoxicity induced by acetaminophen in rats. Treatment with DAS significantly protected rats from acetaminophen-related mortality and elevation of serum lactate dehydrogenase. DAS was also found to induce cytochrome P450 2B1 in rat livers but to inhibit and inactivate P450 2E1 (100). It is also reported that DAS induced liver microsomal pentoxyresorufin dealkylase activity, a representative activity of P450 2B1. Correspondingly, the levels of P450 2B1/2 protein

and P450 2B1/2 mRNA were markedly increased by DAS treatment. In contrast, the level of P450 2E1 mRNA in the liver was not changed. Nakagawa et al. (101) demonstrated the hepato-protective effects of SAC and SAMC using mice with acute hepatitis induced by hepatotoxins. SAC and SAMC reduced the rise of serum enzyme levels and liver necrosis caused by acetaminophen. By studying the mechanism of SAMC's hepatoprotective effect, Sumioka et al. (102) observed that SAMC pretreatment significantly suppressed declines in hepatic-reduced glutathione levels that were induced by administering acetaminophen. SAMC pretreatment also suppressed the increase in hepatic lipid peroxidation and the decrease in levels of hepatic-reduced coenzyme CoQ9H2 that were induced by administering acetaminophen. Dion et al. (103) found that water extracts of AGE significantly reduced the in vitro formation of N-nitrosomorpholine, a mutagen and liver carcinogen. Water-soluble sulfur compounds reduce cancer risk or prevent carcinogenesis without modifying the P450 system. SAC and its nonallyl analog, S-propyl cysteine, effectively blocked the formation of N-nitrosomorpholine. Because water-soluble sulfur compounds like SAC or SAMC protect the liver through P450-independent pathways, this suggests that another hepatoprotective mechanism of SAMC may be attributable to its antioxidant activity.

Dehydrated garlic powder, which contains oil-soluble sulfur compounds such as DAS, DADS, and others, decreased p-Nitrophenol hydroxylase activity and the level of cytochrome P450 2E1 protein in the hepatic microsomes and induced cytochrome P450 1A1/2 protein. DAS suppressed vitamin C-induced mutagenesis in *Salmonella typhimurium* TA100, correlated with an inhibition of cytochrome P-450 2E1-mediated p-nitrophenol hydroxylation. These results suggested that DAS suppresses vitamin C-induced mutagenesis or tumorigenesis, in part, through inhibition of the cytochrome P-450 2E1 isoform responsible for activation of this carcinogen.

According to the above investigations, oil-soluble sulfur compounds induce various P450 isozymes, but water-soluble sulfur compounds in garlic may not. Therefore, water-extracted garlic materials will not cause P450-induced contraindications with drugs. Traditional extraction procedures may be a reasonable way to minimize side effects of the herbal-supplement preparations.

SUMMARY

Many clinical, preclinical, and in vitro studies have shown that allicin-free garlic products, such as AGE, have clear and significant biological effects in cardiovascular, immunological, cancer, hepatoprotective, and other areas. Various chemical constituents have also been identified in this allicin-free preparation, such as nonsulfur compounds, saponins, Maillard-reaction compounds, protein fractions, and others. Each compound is closely related to and responsible for the various biological effects, and it is unnecessary to retain allicin or its degraded odorous oil-soluble sulfur compounds in the garlic product. This clearly indicates that while garlic preparations are traditionally recognized as a source of sulfur compounds, much more interesting compounds than allicin may be actually responsible for the various activities, such as cardioprotective, immune-enhancing, and many others. Those compounds can be studied from different perspectives in connection with biological activities and action mechanisms, combined with a bioavailability analysis through blood concentration of the compounds. Although these directions of herbal research are not easy to pursue, it is essential that we pay attention to the totality of the materials while taking steps toward establishing unique and scientifically reasonable herbal preparations that are grounded in solid scientific evidence.

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