

CHAPTER 9

Nutraceutical Use of Garlic Sulfur-Containing Compounds

Eleftherios Touloupakis* and Demetrios F. Ghanotakis

Abstract

Garlic is one of world's oldest medicines that have been employed not only for flavouring but also as a medical herb for its prophylactic and therapeutic actions. Most of garlic beneficial effects are due to the presence of the organosulphate molecule allicin. Allicin is a highly unstable molecule and, during processing, is rapidly transformed into a variety of organosulfur components. The enzyme alliinase, which is responsible for the conversion of alliin to allicin, is irreversibly destroyed at the acidic environment of stomach. This is the reason why most garlic supplements contain garlic powder or granules, but do not contain allicin itself. Garlic alliinase could be encapsulated and coated with materials which would protect it in the harsh conditions of the stomach. The objective of this chapter is to summarize the most important garlic health benefits and to discuss promising encapsulation/stabilization approaches.

Introduction

Garlic (*Allium sativum* L.) is a perennial, erect bulbous herb, 30-60 cm tall which is native to the Mediterranean regions of Africa and Europe. The underground portion consists of a bulb with numerous fibrous rootlets. The bulb contains numerous cloves and gives rise above ground to a number of narrow, grass-like leaves (Fig. 1). Although formally classified in the family *Liliaceae*, represented by 280 separate genera and 4000 species, recent taxonomic revisions have seen members of the genus *Allium* placed in the family *Alliaceae*. Originated from Central Asia, garlic has been used as a spice, food and folklore medicine for over 5000 years and is the most widely researched medicinal plant. Through trade, garlic spread its popularity throughout Asia and eventually to Egypt and Europe. There are various ancient medical texts from Greece, Egypt, Rome, China and India each prescribing medical applications for garlic. Excavations of ancient Greek temples have unearthed garlic and the palace of Knossos in Crete, dating to 1400-1800 BC, contained well-preserved garlic when it was excavated.¹ The *Codex Ebers*, an Egyptian medical papyrus translated in 1937, contained over 800 medical formulations, 22 of which contained garlic. The Romans also extolled the virtues of garlic. Pliny the elder, a roman naturalist, described in his *Historia Naturalis* how garlic could be used for gastrointestinal disorders, dog and snake bites, scorpion stings, asthma, madness, convulsions and tumours. Dioscorides, physician to the Roman army in first century A.D., also recommended garlic to be used as a vermifuge. Use of garlic has been recorded by Hippocrates, the father of modern medicine, as a laxative and a diuretic and also by Aristophanes and Galen for the treatment of uterine tumours.

*Corresponding Author: Eleftherios Touloupakis—Department of Chemistry, University of Crete, PO Box 2208, 71003 Voutes-Heraklion, Greece. Email: toulou_e@chemistry.uoc.gr



Figure 1. *Allium sativum* bulbs.

The majority of garlic supplements sold today contain either a garlic powder or granules. Garlic powder can serve as an important nutritional supplement. However if garlic powder is stored for long periods, active ingredients present in freshly ground garlic are often eliminated or otherwise rendered inactive.

Most of garlic beneficial effects are due to allicin. Allicin is produced during the crushing of garlic cloves by the enzyme alliinase. Once formed, allicin begins reacting with itself, resulting in more stable organosulfur compounds. This is the reason that no garlic supplement actually contains allicin itself.

In this mini review we summarize the most important garlic health benefits and we discuss promising encapsulation/stabilization approaches.

Garlic Chemical Compounds

Numerous chemical substances are present in fresh garlic. Some of them are: ajoene, allicin, alliin, allyl disulfides, allyl sulfides, allyl trisulfides, cycloalliin, cysteine, cysteine sulfoxides, cystine, diallyl sulfides, dimethyl sulfides, disulfides, glutathione, methionine, methyl sulfides, pseudoscordinine, scordinine, sulfanes, tetrathiol, thiosulfonates and trisulfides.^{2,3} Garlic is known to contain high levels of phosphorous, calcium and iron. Vitamins like riboflavin, thiamine, nicotinic acid and vitamin-C are also present. In addition, garlic contains the minerals selenium and germanium. Garlic also contains linalool, citral, α -phellandrene, geraniol, propionic aldehyde, valeraldehyde.⁴ These phytochemicals can be useful for the treatment or prevention of a number of diseases, including cancer, coronary heart disease, obesity, hypercholesterolemia, diabetes type 2, hypertension, cataract and disturbances of the gastrointestinal tract (e.g., colic pain, flatulent colic and dyspepsia).

The health benefits of garlic are derived from a wide variety of components and from the different ways it is administered. It is possible that such a variety of effects partly depends on the processing of garlic extract preparations.⁵ There are many garlic product processes; those used more frequently are shown in (Table 1).⁶ The oil obtained by the maceration of garlic contains 2-vinyl-[4H]-1,3-dithiin and 3-vinyl-[4H]-1,2-dithiin, allyl sulfides and ajoene. Steam-distilled garlic oil contains diallyl disulfide and diallyl trisulfide. The lipid-lowering effects of some oil-soluble sulfur compounds observed in vitro are associated with cytotoxicity.⁷ Water-soluble garlic extracts are not cytotoxic.⁸ Moreover, the oil-soluble organosulfur compounds of garlic, such as allicin, ajoene and their intermediate products, are not found in urine or blood even after consumption of a large amount of garlic because alliinase, the enzyme that transforms alliin in

Table 1. The most used garlic product processes

Method	Characteristics
Freeze-drying	No change in chemical composition, the resulting product is often used for culinary purposes.
Low Temperature Drying	This process involves drying sliced fresh cloves at 50°C for 3-4 days. Some allicin is formed due to the slicing process.
Distillation	Steam-distilled garlic contains mainly allyl sulfides. Allicin is probably lost or converted to the allyl sulfide degradation compounds.
Maceration in Oil	Chopped garlic is homogenized and slowly extracted in soybean or another vegetable oil. Such products contain vinylidithins, allyl sulfides and ajoene.
Hydroalcoholic Short Extraction	The stability of the constituents in fluid and dry extracts and their final chemical content could be variable.
Hydroalcoholic Long Maceration	Sliced garlic is placed in 20% ethanol and macerated for a long period, filtered and subsequently concentrated. Allicin is completely converted to allyl sulfides, which are largely all volatilized or converted to other compounds.

allicin, is inactivated by the low pH in the stomach.⁹ The water-soluble compounds, besides being more stable, appear to have a higher bioavailability and appear to be able to enter the blood and reach target organs. In fact, after garlic consumption, a water-soluble compound, such as N-acetyl-S-allyl-cysteine, was found in human blood and urine.¹⁰ Raghavan et al, reported that drying the garlic and converting it to powder results in 30-35% loss of volatile flavour.¹¹ Garlic powder could be stabilized with respect to flavour and colour by mixing it with encapsulated garlic oil.^{12,13}

Garlic Biological Activities

A variety of biological activities have been reported for garlic extracts.^{14,15} The most important ones are summarized below.

Antimicrobial. Garlic is regarded as a natural antibiotic, effective against a wide number of microorganisms.^{16,17}

Antibacterial. Garlic extracts have been demonstrated to inhibit bacterial growth. Sulfur compounds in garlic are known to destroy thiol groups in bacterial enzymes. Garlic preparations have been shown to exhibit antibacterial activity against *Helicobacter pylori*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei* and *Escherichia coli*.¹⁸ Garlic has been found to be effective against clinical strains of *Staphylococcus*, *Escherichia*, *Proteus*, *Pseudomonas* and *Klebsiella* bacteria.¹⁹

Antiparasitic. Garlic has been found to be effective against gastrointestinal parasites of humans and animals.²⁰ In addition, it is effective against *Entamoeba histolytica*, *Hymenolepis nana* and *Giardia lamblia*.²¹ Garlic can be used as an alternative treatment against nematode parasites in humans.²² Nok et al, also investigated the garlic induced death of protozoans.²³

Antifungal. Garlic extracts have a strong antifungal effect and inhibit the formation of mycotoxins like the aflatoxin of *Aspergillus parasiticus*.²⁴ Davis et al, reported that garlic extract displayed significant in vitro fungicidal and fungistatic activity against 3 different isolates of *Cryptococcus neoformans*.²⁵

Antiviral. Garlic extracts have an antifungal effect against human cytomegalovirus, influenza B, herpes simplex virus type 1, herpes simplex virus type 2, parainfluenza virus type 3, vaccinia virus, vesicular stomatitis virus and human rhinovirus type 2.²⁶ Weber et al, reported the efficacy of allicin and its various transformation products against Herpes simplex virus 1 and 2, Vesicular stomatitis virus, Vaccinia virus and Parainfluenza virus.²⁷

Anti-carcinogenic. Garlic can be used as a preventive against stomach, colon and other types of cancers, due to possible immune enhancing and antitumorogenic effects of its sulfur containing compounds.²⁸⁻³⁰ Allyl sulfur and other garlic compounds can slow or prevent the growth of tumor cells.^{31,32} Aqueous extracts of garlic are reported to cause significant improvement in prostate cancer patients.³³ Some researchers have stated that garlic's potential anticancer effects may be due to its content of the trace mineral selenium.³⁴

Anti-inflammatory. Garlic promotes an anti-inflammatory environment by cytokine modulation as well as bringing in modulation in human blood that leads to an overall inhibition of NF- κ B activity in surrounding tissue.^{35,36}

Anti-thrombotic. Several studies have indicated that whole garlic and garlic aqueous extract have an antiplatelet action.³⁷⁻³⁹ This property is due in part to the compounds alliin and ajoene, which have fibrinolytic activity. Ajoene inhibits thromboxane synthesis through the inhibition of the cyclo-oxygenase and lipoxygenase enzymes.⁴⁰ Garlic extract can be used as a potential treatment against atherosclerosis.⁴¹

Cholesterol lowering. Garlic and its constituents inhibit key enzymes involved in cholesterol and fatty acid synthesis in cultured rat hepatocytes and human HepG2 cells.^{7,42} Garlic treatment reduces circulating triglycerides and cholesterol.⁴³⁻⁴⁵ Anim-Nyame et al, have shown that garlic will increase peripheral blood flow mediated by interleukin-6, an important property attributed to be beneficial for persons with cardiovascular diseases.⁴⁶

Garlic and diabetes. Garlic was effective in reducing blood glucose in streptocin-induced as well as alloxan-induced diabetes mellitus in rats and mice models.⁴⁷⁻⁴⁹ Kasuga et al, have reported that treatment with aged garlic extract is effective against adrenal hypertrophy, hyperglycemia and production of elevated levels of corticosterones in stress induced hyperglycemic mice.⁵⁰ Due to its hypoglycemic effect garlic may be useful as coadjuvant therapy in the treatment of type 2 diabetes and some of its physiological complications.^{51,52}

Antioxidant. The antioxidant properties of garlic and different garlic preparations are well documented.⁵³⁻⁵⁷ The high antioxidant properties of garlic presents evidence that it may either prevent or delay chronic diseases associated with aging. Among garlic-derived products, aged garlic extract is the preparation with higher antioxidant activity. Aged garlic extract is obtained by storage at room temperature of sliced and soaked, in a water/ethanol mixture, garlic for longer than 10 months.⁵⁸ The potent antioxidant activity is due to the extraction procedure which increases stable and highly bioavailable water-soluble organo-sulphur compounds content, such as SAC and S-allylmercaptocysteine.⁵⁹ This garlic preparation has other compounds with antioxidant effect, such as stable lipid-soluble allyl sulphides, as diallyl polysulphides, tetrahydro-b-carboline derivatives, which are formed during the natural aging process, flavonoids; saponins; and essential micronutrients (selenium, Se) and macronutrients, as lectins, whose antiperoxide effect has been demonstrated in the liver, kidney and heart of rats.^{10,60} Another recently identified antioxidant compounds of aged garlic extract are N-fructosyl glutamate, N-fructosyl arginine.⁶¹⁻⁶² Garlic extract can be used as a prophylactic treatment against the common cold and in the treatment of sickle-cell anemia,^{63,64} to treat hypertension and reduce the risk of other cardiovascular diseases^{62,65,66} and against heavy metal poisoning.⁶⁷ Garlic compounds may also have neuroprotective effects.^{68,69} Garlic possesses a mild diuretic action^{70,71} and can be used as a renoprotective agent. It has been also found that the aged garlic extract can be a potential protective regimen for arsenic mediated toxicity.⁷²

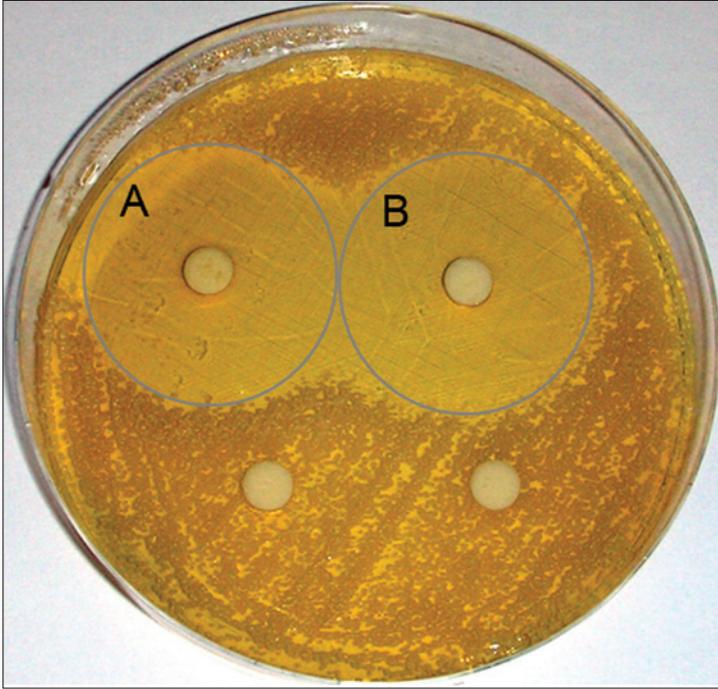


Figure 2. Effect of garlic crude extract on *Saccharomyces cerevisiae* culture. Inhibition zone of A) 50 μ l of garlic extract and B) 10 μ g of miconazole.

In our laboratory we have demonstrated the inhibitory action of garlic extract against *Saccharomyces cerevisiae*. The antibiogram method was used for measuring the diameter of the garlic extract zone of inhibition (Fig. 2).

Alliin and Allicin

Alliin ((+)-S-allyl-L-cysteine sulfoxide) and allicin (S-(+)-2-propenyl-L-cysteine sulfoxide) are two important sulfur-containing compounds found in garlic (Fig. 3). Both alliin and allicin are known as “organosulfur” compounds. Many medicinal organosulfur compounds are found in the *Alliums*, but garlic contains the highest concentration of them. Alliin is further classified as a “cysteine sulfoxide”. Garlic contains other cysteine sulfoxides, but alliin is the most abundant of these, as well as the most abundant sulfur compound in garlic overall. The amounts of alliin present in different strains of garlic were studied by numerous investigators. Considerable variations have been reported, ranging from 2.8 to 7.7 mg g^{-1} .

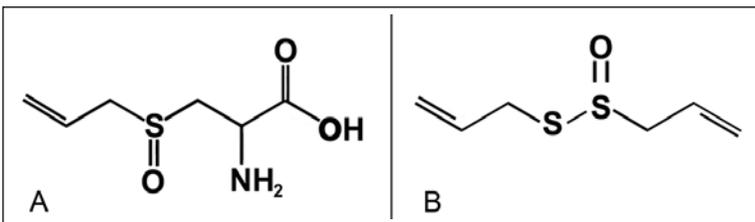


Figure 3. Molecular formulas of: A) alliin and B) allicin.

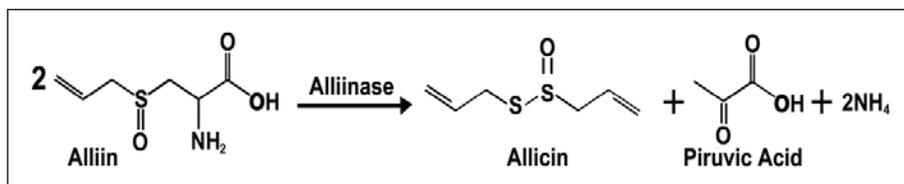


Figure 4. Enzymatic reaction of garlic alliinase.

Alliin demonstrates a variety of important activities. It reduces serum glucose and serum enzyme levels.⁷³ Alliin at levels of 200 mg kg⁻¹ body weight can significantly reduce the blood glucose level in diabetic mice, rats and rabbits.⁵⁰ Alliin has an inhibitory effect upon key enzymes involved in cholesterol biosynthesis; it also possesses an antioxidant activity.^{59,74} Kim and Kyung demonstrated that alliin has an antifungal activity.⁷⁵ Alliin was found to be the stable precursor. When garlic is cut, chopped or crushed, the clove's membrane disrupts and alliin is transformed enzymatically into allicin by allinase (Fig. 4).⁷⁶ The transformation of alliin into the biologically active allicin molecule upon crushing of a garlic clove is very rapid and is complete in seconds. Garlic cloves are odour-free until crushed or processed when garlic supplements are manufactured and cross-section studies have indicated that the substrate alliin and the enzyme alliinase are located in different compartments. This organization suggests that it is designed as a potential defence mechanism against microbial pathogens in the soil. Invasion of the cloves by fungi and other soil pathogens causes the interaction between alliin and alliinase that rapidly produces allicin, which in turn inactivates the invader. The produced reactive allicin molecules have a very short half-life, as they react with many of the surrounding proteins, including alliinase. Allicin is often thought of as the "active ingredient" in garlic and has been the subject of numerous scientific studies, but many of its transformation products have medicinal properties as well.

Allicin, which is classified as a "thiosulfinate", is responsible for the typical odor of garlic. Thiosulfinates contain two sulfur atoms bonded together, one of which is also bonded to an oxygen atom. Allicin accounts for about 70-80% of total thiosulfinates in garlic. The total allicin yield has been determined as 2.5 mg g⁻¹ of fresh crushed garlic or about 5-20 mg per clove. Allicin is unstable and converts readily into mono-, di- and trisulfides and other compounds such as ajoene.⁹ Further transformation of organosulfur compounds can occur after interaction with free sulfhydryl groups, including those present in cysteine, glutathione or proteins.

Allicin possesses a variety of important activities. It has a high antioxidant activity and has been reported to induce caspase mediated apoptosis in cancer cells.⁷⁷⁻⁷⁹ The antibacterial action of garlic is mainly due to allicin.¹⁶ Yamada and Azuma demonstrated that pure allicin is effective in vitro against species of *Candida*, *Cryptococcus*, *Trichophyton*, *Epidermophyton* and *Microsporum* at low concentration.⁸⁰ Allicin may be an effective therapeutic candidate to control the pain, promote ulcer healing and prevent the recurrence of recurrent aphthous ulcer.⁸¹ Garlic antiatherosclerotic properties are mainly attributed to allicin.⁸² Many of allicin transformation products have medicinal properties as well.⁸³

Garlic Alliinase

Alliinase (Aliin lyase) (EC 4.4.1.4) is a homodimeric glycoprotein found in many plants of the genus *Allium* such as garlic (*Allium sativum*), onion (*Allium cepa*) and Chinese chives (*Allium tuberosum*). The three-dimensional structure of the garlic alliinase has been obtained at 1.5 Å resolution.⁸⁴ The active enzyme is a pyridoxal-5'-phosphate-dependent protein and belongs to the class I family of pyridoxal-5'-phosphate-dependent enzymes. In garlic, each alliinase subunit consists of 448 aminoacids accounting for a molecular weight of 51,500 Da, four putative N-glycosylation sites and one pyridoxal 5-phosphate (PLP) molecule as a cofactor.⁸⁵ Alliinase belongs to the family of mannose-rich glycoproteins with an estimated carbohydrate content of about 5.5-6.0% and can form a stable complex with garlic lectins.⁸⁶ The lectin-alliinase complex offers a high stability to the

enzyme and its enzymatic activity. Alliinase catalyses the production of allicin from alliin (Fig. 4). The reaction catalyzed by alliinase is categorized as α,β -elimination reaction and involves an aminoacryl intermediate bound to PLP. Alliinase converts the substrate alliin to allylsulfenic acid and aminoacrylate. Two molecules of allylsulfenic acid condense spontaneously to form the sulfur containing volatile, allicin. The aminoacrylate is lysed from the PLP and spontaneously decompose to pyruvate and ammonia. The K_m of alliinase is 1.1 mM, its pH optimum 6.5 and its isoelectric point is 6.35. Alliinase is present in large amounts in garlic cloves: at least 10% of the total protein content (10 mg g^{-1} fresh weight). With its C-S lyase activity, alliinase is able to cleave the Cb-Sc bond of sulfoxide derivatives of the amino acid cysteine thus giving rise to all the garlic sulfur compounds.

Garlic Supplements

Garlic supplements vary widely in their chemical composition, depending on the age of the garlic and how it is processed. Significant amounts of alliin and alliinase can survive the drying and processing necessary to manufacture garlic supplements. Freeze-drying, for example, preserves higher levels of these phytochemicals compared to other types of drying, but it is an expensive process. When dried garlic powder is added to water, alliin and alliinase quickly react and allicin is produced. Most of garlic supplements found in the market do not contain allicin. Some products are standardized to contain a certain amount of alliin and to have an uncertain allicin yield (amount of allicin generated inside the human body). No allicin was detected in human blood after oral intake of commercially available products standardized for allicin potential or allicin yield.^{10,87} Even if the supplements contain plenty of alliin and active alliinase, they must be enteric-coated to protect alliinase from the low pH in the stomach, to reach small intestine, where it can act on the alliin and convert it to allicin.⁸⁷ Enteric-coated garlic products resist human stomach conditions in order to prolong the shelf life and protect alliinase activity and deliver allicin directly into the intestinal tract. Enteric coatings are cellulose or poly(acrylic acid) esters that are acid-resistant but readily dissolve at neutral pH. Garlic extract can be also encapsulated.^{88,89} In a similar manner alliinase and/or alliin can be encapsulated. Encapsulation enables alliinase and/or alliin to maintain their viability for extended periods of time protecting, for example, from ions or free radicals. Capsule structure is divided into the core and the coating material. The coating material is the external layer or layers that cover the core material. Coating substances are basically film forming materials that can be selected from a wide variety of natural or synthetic polymers, depending on the material to be coated and the characteristics desired to the final capsules.⁹⁰ An effective coating material should have good rheological properties at high concentration and easy manipulation during the process of encapsulation. It should be selected so that it produces a stable emulsion with the active ingredient and does not react or degrade the active material during process and storage. The interior content of the capsules can be released under different mechanisms, like diffusion, dissolution, fracturation and biodegradation.

Two of the most promising encapsulation materials are layered double hydroxides and alginate gels. These materials are useful because they are biocompatible inorganic matrices that are cheap and easy to work with.⁹¹⁻⁹⁴

Layered Double Hydroxides

Layered double hydroxides (LDH), also known as hydrotalcite-like compounds, belong to a class of lamellar compounds that enables the incorporation of several species by guest-host chemistry.⁹⁵ LDH structure can be described by considering the brucite-like structure, $Mg(OH)_2$, in which the Mg(II) cations are in the center of edge-sharing octahedra, with hydroxyl groups in their vertices, resulting in a planar structure (Fig. 5). The chemical composition of this class of intercalation compounds can be expressed by the general formula $[M^{2+}_{1-x}M^{3+}_x(OH)_2]^{x+}A^{m-}_{x/m}nH_2O$: where M^{2+} and M^{3+} are respectively the divalent and trivalent cations in the brucite-like layers and A^{m-} is the charge balancing interlayer anion. The specific charge of the layer is directly related to the exchange ratio [x in the general formula, $x = M3/(M^{2+} + M^{3+})$]. Thus, a wide variety of LDHs can be obtained by varying the M^{2+} and M^{3+} cations species and their ratio, as well as the intercalated anion A^{m-} , which can be exchanged by an ion-exchange process.⁹⁵ Due to their properties, several new LDHs

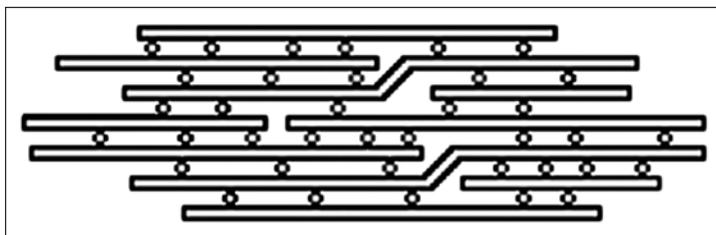


Figure 5. Schematic planar structure of layered double hydroxides.

compounds have been prepared with potential technological applications, such as heterogeneous catalysis, host structures for photoactivation and photocatalysis and ion exchange.^{96,97} Inorganic layered double hydroxides can be also used as nonviral vectors.⁹⁸ Enteric-coated layered double hydroxides can be used as a controlled release drug delivery system.⁹³

Alginates

Alginate is the term usually used for the salts of alginic acid. Alginate is present in the cell walls of brown algae as the calcium, magnesium and sodium salts of alginic acid. Alginates constitute a family of unbranched binary copolymers of 1-4-linked β -D-mannuronic acid (M) and α -L-guluronic acid (G). The monomers are arranged in a pattern of blocks along the chain, with homopolymeric regions (termed M and G blocks) (Fig. 6). The way in which these M and G units are arranged in the chain and the overall ratio, M/G, of the two units in a chain can vary from one species of seaweed to another.

The uses of alginates are based on three main properties. The first is their ability, when dissolved in water, to thicken the resulting solution. The second is their ability to form gels; gels form when a calcium salt is added to a solution of sodium alginate in water. The gel forms by chemical reaction, the calcium displaces the sodium from the alginate, holds the long alginate molecules together and a gel is the result. No heat is required and the gels do not melt when heated. The third property of alginates is the ability to form films of sodium or calcium alginate and fibres of calcium alginates. Alginates are used in a wide variety of foodstuff such as pet food chunks, onion rings, stuffed olives, low fat spreads, sauces and pie fillings. Alginates are used as food additives to improve, modify and stabilize the texture of foods. The fact that alginates belongs to EU-approved additives makes it an ideal support material. Alginates are widely used to immobilize cells or enzymes.^{99,100}

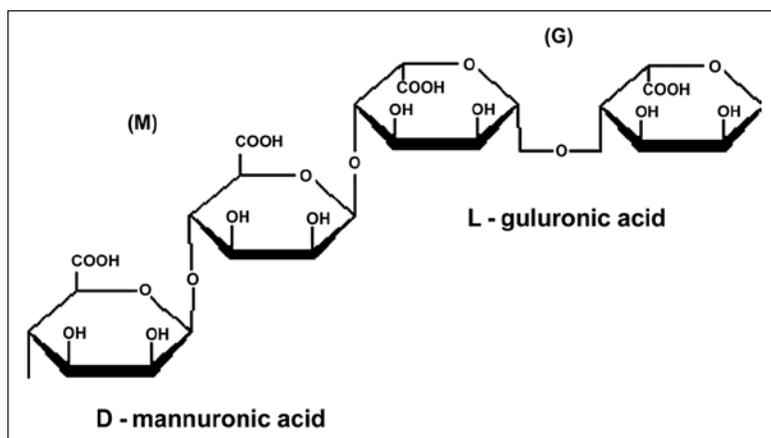


Figure 6. Chemical structures of alginic acid.

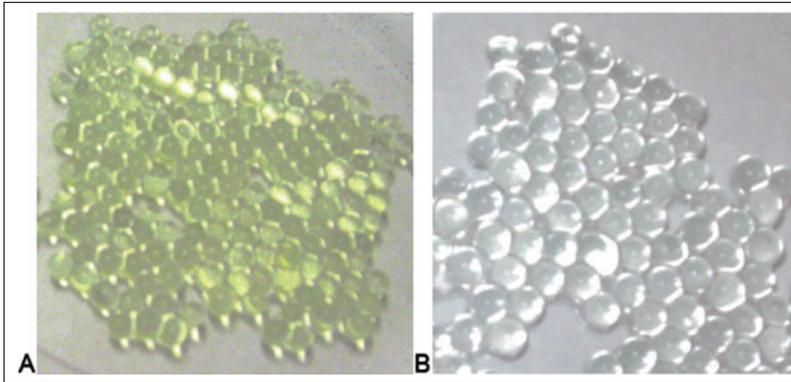


Figure 7. Alginate bead with (A) and without (B) entrapped alliinase. The yellow color is due to the presence of the alliinase cofactor PLP. A color version of this image is available at www.landesbioscience.com/curie.

In a series of immobilization experiments we managed to stabilize purified alliinase from garlic by entrapping it in calcium alginate beads (3 mm diameter, 1.5 mg protein per 1g beads) (Fig. 7). The entrapped enzyme was highly active and efficiently converted alliin to alliin (manuscript in preparation).

Conclusion

Garlic contains a lot of volatile organosulfur compounds with a vast variety of biological functions. Most of garlic beneficial effects are due to the presence of alliin and alliin. Alliin is a very unstable molecule and this is the reason most of garlic supplements in the market do not contain this important molecule.

Alliinase and alliin are present in high quantities in garlic and are easily isolated in pure and active forms. The garlic enzyme alliinase can be stabilized in its active form into biocompatible materials, like layered double hydroxides or alginate beads. The immobilized enzyme along with purified alliin can be used in garlic nutraceutical products. An enteric-coating of the immobilized molecules, would allow them to bypass the acidic environment of the stomach and thus result to the production of alliin in the human intestine.

References

1. Moyers S. Garlic in health, history and world cuisine. St. Petersburg, FL. Suncoast Press, 1996;1-36.
2. Rose P, Whiteman M, Moore PK et al. Bioactive S-alk(en)yl cysteine sulfoxide metabolites in the genus Allium: the chemistry of potential therapeutic agents. *Nat Prod Rep* 2005; 22:351-368.
3. Lanzotti V. The analysis of onion and garlic. *J Chromatogr A* 2006; 1112;3-22.
4. Milner JA. Garlic: its anticarcinogenic and antitumorogenic properties. *Nutr Rev* 1996; 54:S82-S86.
5. Gorinstein S, Jastrzebski Z, Leontowicz H et al. Comparative control of the bioactivity of some frequently consumed vegetables subjected to different processing conditions. *Food Control* 2009; 20:407-413.
6. Staba EJ, Lash L, Staba JE. A Commentary on the effects of garlic extraction and formulation on product composition. *J Nutr* 2001; 131:1118S-1119S.
7. Liu L, Yeh Y. Water-soluble organosulfur compounds of garlic inhibit fatty acid biosynthesis in cultured rat hepatocytes. *Lipids* 2001; 36(4):395-400.
8. De Martino A, Filomeni G, Aquilano K et al. Effects of water garlic extracts on cell cycle and viability of HepG2 hepatoma cells. *J Nutr Biochem* 2006; 17:742-749.
9. Lawson LD, Hughes BG. Characterization of the formation of alliin and other thiosulfonates from garlic. *Planta Med* 1992; 58:345-350.
10. Amagase H, Petesch BL, Matsuura H. Intake of garlic and its bioactive components. *J Nutr* 2001; 131:955S-926S.
11. Kaymak-Ertekin F, Gedik A. Kinetic modelling of quality deterioration in onions during drying and storage. *J Food Eng* 2005; 68(4):443-453.

12. Ramalakshmi K, Prabhakar Rao PG, Raghavan B. Effect of storage on the quality of stabilized garlic powder. *Indian Spices* 1999; 34(4):5-7.
13. Yu LI, Shi-ying XU. Preparation of garlic powder with high allicin content. *Agric Sci China* 2007; 6(7):890-898.
14. Tattelman EMD. Health effects of garlic. *Am Fam Phys* 2005; 72:103-106.
15. Agarwal KC. Therapeutic actions of garlic constituents. *Med Res Rev* 1996; 16:111-124.
16. Aukris S. Antimicrobial properties of Allicin from garlic. *Microbes infect* 1999; 11:125-129.
17. Davis SR, Perrie R, Apitz-Castro R. The in vitro susceptibility of *Scedosporium prolificans* to ajoene, allitridium and a raw extract of garlic (*Allium sativum*). *J Antimicrob Chemother* 2003; 51(3):593-597.
18. Chowdhury AKZ, Ahsan M, Islam SN et al. Efficacy of aqueous extract of garlic and allicin in experimental shigellosis in rabbits. *Indian J Medic Res* 1991; 93:33-36.
19. Sivam GP. Recent protection against *Helicobacter pylori* and other bacterial infections by garlic. *J Nutr* 2001; 131:1106S-1108S.
20. Zenner L, Callait MP, Granier C et al. In vitro effect of essential oils from *Cinnamomum aromaticum*, *Citrus limon* and *Allium sativum* on two intestinal flagellates of poultry, *Tetratrichomonas gallinarum* and *Histomonas meleagridis*. *Parasite* 2003; 10(2):153-157.
21. Soffar SA, Mokhtar GM. Evaluation of the antiparasitic effect of aqueous garlic (*Allium sativum*) extract in *hymenolepiasis nana* and giardiasis. *J Egypt Soc Parasitol* 1991; 21:497-502.
22. Ayaz E, Turel I, Gul A et al. Evaluation of the anthelmintic activity of garlic (*Allium sativum*) in mice naturally infected with *Aspiculuris tetraptera*. *Recent Pat Anti-Infect Drug Discov* 2008; 3(2):149-152.
23. Nok AJ, Williams S, Onyenekwe PC. *Allium sativum*-induced death of African trypanosomes. *Parasitol Res* 1996; 82:634-637.
24. Lawson LD. The composition and chemistry of garlic cloves and processed garlic. In: Koch HP, Lawson LD, eds. *Garlic: The Science And Therapeutic Application Of Allium Sativum L*. Baltimore: Williams and Wilkins, 1996:37-108.
25. Davis LE, Shen J, Royer RE. In vitro synergism of concentrated *Allium sativum* extract and amphotericinB against *Cryptococcus neoformans*. *Planta Med* 1994; 60:546-549.
26. Harris JC, Cottrell SL, Plummer S et al. Antimicrobial properties of *Allium sativum* (garlic). *Appl Microbiol Biotechnol* 2001; 57:282-286.
27. Weber ND, Andersen DO, North JA et al. In vitro virucidal effects of *Allium sativum* (Garlic) Extract and Compounds. *Planta Med* 1992; 58:417-423.
28. Thomson M, Ali M. Garlic [*Allium sativum*]: a review of its potential use as an anti-cancer agent. *Curr Cancer Drug Targets* 2003; 3(1):67-81.
29. Li H, Li HQ, Wang Y et al. An intervention study to prevent gastric cancer by micro selenium and large dose of allitridium. *Chin Med J* 2004; 117(8):1155-1160.
30. Milner JA. Preclinical perspectives on garlic and cancer. *J Nutr* 2006; 136:827S-831S.
31. Lea MA, Ayyala US. Differentiating and growth inhibitory effects of diallyl disulfide on cancer cells. *Int J Oncol* 1997; 11:181-185.
32. Sigounas G, Hooker J, Anagnostou A et al. S-Allylmercaptocysteine inhibits cell proliferation and reduces the viability of erythro leukemia, breast and prostate cancer cell lines. *Nutr Cancer* 1997; 27:186-191.
33. Durak I, Yilmaz E, Devrim E et al. Consumption of aqueous garlic extract leads to significant improvement in patients with benign prostate hyperplasia and prostate cancer. *Nutrit Res* 2003; 23:199-204.
34. Dong Y, Lisk D, Block E et al. Characterization of the biological activity of gamma glutamyl-S-methylselenocysteine: a novel, naturally occurring anticancer agent from garlic. *Cancer Res* 2001; 61(7):2923-2928.
35. Hodge G, Hodge S, Han P. *Allium sativum* (garlic) suppresses leukocyte inflammatory cytokine production in vitro: Potential therapeutic use in the treatment of inflammatory bowel disease. *Cytometry* 2002; 48:209-215.
36. Keiss HP, Dirsch VM, Hartung T et al. Garlic (*Allium sativum L.*) modulates cytokine expression in lipopolysaccharide activated human blood thereby inhibiting NF- κ B activity. *J Nutr* 2003; 133:2171-2175.
37. Allison GL, Lowe GM, Rahman K. Aged garlic extract and its constituents inhibit platelet aggregation through multiple mechanisms. *J Nutr* 2006; 136:782S-788S.
38. Ohaeri OC, Adoga GI. Anticoagulant modulation of blood cells and platelet reactivity by garlic oil in experimental diabetes mellitus. *Biosci Rep* 2006; 26(1):1-6.
39. Chan K, Yin M, Chao W. Effect of diallyl trisulfide-rich garlic oil on blood coagulation and plasma activity of anticoagulation factors in rats. *Food Chem Toxicol* 2007; 45:502-507.
40. Srivastava KC, Tyagi OD. Effects of a garlic-derived principle (ajoene) on aggregation and arachidonic acid metabolism in human blood platelets. *Prostaglandins Leukot Essent Fatty Acids* 1993; 49:587-595.
41. Koscielny J, Klussendorf D, Latza R et al. The antiatherosclerotic effect of *Allium sativum*. *Atherosclerosis*, 1999; 144(1):237-249.

42. Yeh YY, Liu L. Cholesterol-lowering effects of garlic extracts and organosulfur compounds: human and animal studies. *J Nutr* 2001; 131:989S-993S.
43. Chetty KN, Calahan L, Harris KC et al. Garlic attenuates hypercholesterolemic risk factors in olive oil fed rats and high cholesterol fed rats. *Pathophysiology* 2003; 9(3):127-132.
44. Kannar D, Wattanapenpaiboon N, Savage GS et al. Hypocholesterolemic effect of an enteric-coated garlic supplement. *J Am Coll Nutr* 2001; 20(3):225-231.
45. Qidwai W, Qureshi R, Hasan SN et al. Effect of dietary garlic (*Allium sativum*) on the blood pressure in humans—a pilot study. *J Pak Med Assoc* 2000; 50(6):204-207.
46. Anim-Nyame N, Sooranna SR, Johnson MR et al. Garlic supplementation increases peripheral blood flow; a role for interleukin-6? *J Nutr Biochem* 2004; 15:30-36.
47. Ohaeri OC. Effect of garlic oil on the levels of various enzymes in the serum and tissue of streptozotocin diabetic rats. *Biosci Rep* 2001; 21:19-24.
48. Bhagyalakshmi N, Thimmaraju R, Venkatchalam L et al. Nutraceutical applications of garlic and the intervention of biotechnology. *Crit Rev Food Sci Nutr* 2005; 45(7):607-621.
49. Eidi A, Eidi M, Esmaili E. Antidiabetic effect of garlic (*Allium sativum* L.) in normal and streptozotocin-induced diabetic rats. *Phytomedicine* 2005; 13:624-629.
50. Kasuga S, Ushijima M, Morihara N et al. Effect of aged garlic extract (AGE) on hyperglycemia induced immobilization stress in mice. *Nippon yakurigaku Zasshi* 1999; 114:192-197.
51. Ryan EA, Pick Mea, Marceau C. Use of alternative medicines in diabetes mellitus. *Diabet Med* 2001; 18(3):242-245.
52. Baluchnejadmojarad T, Roghani M. Garlic extract attenuates time-dependent changes in the reactivity of isolated aorta in streptozotocin-diabetic rats. *Life Sci* 2003; 73(18):2281-2289.
53. Borek C. Antioxidant health effects of aged garlic extract. *J Nutr* 2001; 131:1010S-1015S.
54. Banerjee SK, Mukherjee PK, Maulik SK. Garlic as an antioxidant: the good, the bad and the ugly. *Phytother Res* 2003; 17(2):97-106.
55. Benkeblia N. Free-radical scavenging capacity and antioxidant properties of some selected onions (*A. cepa* L.) and garlic (*A. sativum* L.) extracts. *Braz Arch Biol Technol* 2005; 48(5):753-759.
56. Bozin B, Mimica-Dukic N, Samojlik I et al. Phenolics as antioxidants in garlic (*A. sativum* L., Alliaceae). *Food Chem* 2008; 111:925-929.
57. Queiroz YS, Ishimoto EY, Bastos DHM et al. Garlic (*Allium sativum* L.) and ready-to-eat garlic products: In vitro antioxidant activity. *Anal Meth* 2009; 115(1):371-374.
58. Amagase H. Clarifying the real bioactive constituents of garlic. *J Nutr* 2006; 136:716S-725S.
59. Chung LY. The antioxidant properties of garlic compounds: Allyl cysteine, alliin, allicin and allyl disulfide. *J Med Food* 2006; 9:205-213.
60. Ichikawa M, Yoshida J, Ide N et al. Tetrahydro-b-carboline derivatives in aged garlic extract show antioxidant properties. *J Nutr* 2006; 136:726S-731S.
61. Ryu K, Ide N, Matsuura H et al. N Alpha-(1-deoxy-D-fructosyl)-L-arginine, an antioxidant compound identified in aged garlic extract. *J Nutr* 2001; 131:972S-976S.
62. Corzo-Martinez M, Corzo N, Villamiel M. Biological properties of onions and garlic. *Trends Food Sci Tech* 2007; 18:609-625.
63. Josling P. Preventing the common cold with a garlic supplement: a double-blind, placebo controlled survey. *Adv Ther* 2001; 18(4):189-193.
64. Takasu J, Uykipang R, Sunga M et al. Aged garlic extract therapy for sickle cell anemia patients. *BMC Blood Disord* 2002; 2(1):3.
65. Gardner CD, Messina M, Lawson LD et al. Soy, garlic and ginkgo biloba: their potential role in cardiovascular disease prevention and treatment. *Curr Atheroscler Rep* 2003; 5(6):468-475.
66. Rahman K, Lowe GM. Garlic and cardiovascular disease: A critical review. *J Nutr* 2006; 136:736S-740S.
67. Schulz V, Hansel R, Tyler V et al. Rational phytotherapy: A physician's guide, 5th ed. Berlin: Springer-Verlag, 2004;128-138.
68. Ito Y, Ito M, Takagi N et al. Neurotoxicity induced by amyloid beta-peptide and ibotenic acid in organotypic hippocampal cultures: protection by S-allyl-L-cysteine, a garlic compound. *Brain Res* 2003; 985(1):98-107.
69. Peng Q, Buzzard AR, Lau BH. Neuroprotective effect of garlic compounds in amyloidbeta peptide-induced apoptosis in vitro. *Med Sci Monit* 2002; 8:328-337.
70. Pedraza-Chaverri J, Yam-Canul P, Chirino YI et al. Protective effects of garlic powder against potassium dichromate-induced oxidative stress and nephrotoxicity. *Food Chem Toxicol* 2008; 46:619-627.
71. Kabasakal L, Sehrlir O, Cetinel S et al. Protective effect of aqueous garlic extract against renal ischemia/reperfusion injury in rats. *J Med Food* 2005; 8:319-326.
72. Chowdhury R, Dutta A, Chaudhuri SR et al. In vitro and in vivo reduction of sodium arsenite induced toxicity by aqueous garlic extract. *Food Chem Toxicol* 2008; 46:740-751.

73. Nasim SA, Dhir B, Samar F et al. Sulphur treatment alters the therapeutic potency of alliin obtained from garlic leaf extract. *Food Chem Toxicol* 2009; 47:888-892.
74. Salman H, Bergman M, Bessler H et al. Effect of a garlic derivative (alliin) on peripheral blood cell immune responses. *Int J Immunopharmacol* 1999; 21(9):589-597.
75. Kim JW, Kyung KH. Antiyeast activity of heated garlic in the absence of alliinase enzyme action. *J Food Sci* 2006; 68(5):1766-1770.
76. Kuettner EB, Hilgenfeld R, Weiss MS. The active principle of garlic at atomic resolution. *J Biol Chem* 2002; 277(48):46402-46407.
77. Oommen S, Anto RJ, Srinivas G et al. Allicin (from garlic) induces caspase-mediated apoptosis in cancer cells. *European Pharmacol* 2004; 485:97-103.
78. Miron T, Wilchek M, Sharp A et al. Allicin inhibits cell growth and induces apoptosis through the mitochondrial pathway in HL60 and U937 cells. *J Nutr Biochem* 2008; 19:524-535.
79. Okada Y, Tanaka K, Sato E et al. Kinetic and mechanistic studies of allicin as an antioxidant. *Org Biomol Chem* 2006; 4:4113-4117.
80. Yamada Y, Azuma K. Evaluation of the in vitro antifungal activity of allicin. *Antimicrob. Agents Chemother* 1997; 11:743-749.
81. Jiang XW, Hu J, Mian FI. A new therapeutic candidate for oral aphthous ulcer: Allicin. *Med Hypoth* 2008; 71:897-899.
82. Gonen A, Harats D, Rabinkov A et al. The antiatherogenic effect of allicin: possible mode of action. *Pathobiology* 2005; 72:325-334.
83. Li M, Ciu JR, Ye Y et al. Antitumor activity of Z-ajoene, a natural compound purified from garlic: anti-mitotic and microtubule-interaction properties. *Carcinogenesis* 2002; 23(4):573-579.
84. Shimon LJV, Rabinkov A, Miron T et al. Alliin lyase (alliinase) from garlic (*Allium sativum*): crystallization and preliminary X-ray characterization. *Acta Cryst* 2002; 58:1335-1337.
85. Kuettner EB, Hilgenfeld R, Weiss MS. Purification, characterization and crystallization of alliinase from garlic. *Arch Biochem Biophys* 2002; 402(2):192-200.
86. Smeets K, Van Damme M, Van Leuven F et al. Isolation and characterization of lectins and lectin-alliinase complexes from bulbs of garlic (*Allium sativum*) and ramsons (*Allium ursinum*). *Glycoconjugate J* 1997; 14:331-343.
87. Lawson LD, Wang JZ. Low allicin release from garlic supplements: a major problem due to the sensitivities of alliinase activity. *J Agric Food Chem* 2001; 49:2592-2599.
88. Li Y, Xu SY, Sun DW. Preparation of garlic powder with high allicin content by using combined microwave-vacuum and vacuum drying as well as microencapsulation. *J Food Engin* 2007; 83(1):76-83.
89. Ayala-Zavala JF, Soto-Valdez H, González-Leon A et al. Microencapsulation of cinnamon leaf (*Cinnamomum zeylanicum*) and garlic (*Allium sativum*) oils in β -cyclodextrin. *J Incl Phenom Macrocycl Chem* 2008; 60(3-4):359-368.
90. Gibbs BF, Kermasha S, Alli I et al. Encapsulation in the food industry: a review. *Int J Food Sci Nutr* 1999; 50:213-224.
91. Becker TA, Kipke DR, Brandon T. Calcium alginate gel: a biocompatible and mechanically stable polymer for endovascular embolization. *J Biomed Mater Res* 2001; 54(1):76-86.
92. Orive G, Ponce S, Hernandez RM et al. Biocompatibility of microcapsules for cell immobilization elaborated with different type of alginates. *Biomaterials* 2002; 23(18):3825-3831.
93. Li B, He J, Evans DG et al. Enteric-coated layered double hydroxides as a controlled release drug delivery system. *Int J Pharm* 2004; 287(1-2):89-95.
94. Choy JH, Park M, Oh JM. Bio-nanohybrids based on layered double hydroxide. *Curr Nanosci* 2006; 2(3):275-281.
95. Crepaldi EL, Valim JB. Layered double hydroxides: Structure, synthesis, properties and applications. *Quim Nova* 1998; 21:300-311.
96. Trikeriotis M, Ghanotakis DF. Intercalation of hydrophilic and hydrophobic antibiotics in layered double hydroxides. *Int J Pharm* 2007; 332(1-2):176-184.
97. Kantonis G, Trikeriotis M, Ghanotakis DF. Biocompatible protoporphyrin IX-containing nanohybrids with potential applications in photodynamic therapy. *J Photochem Photobiol A Chem* 2007; 185(1):62-66.
98. Choy JH, Kwak SY, Jeong YJ et al. Inorganic layered double hydroxides as nonviral vectors. *Angew Chem Int Ed* 2000; 39:4041-4045.
99. Sankalia MG, Mashru RC, Sankalia JM et al. Papain entrapment in alginate beads for stability improvement and site-specific delivery: Physicochemical characterization and factorial optimization using neural network modeling. *AAPS Pharm Sci Tech* 2005; 6(2):E209-E222.
100. Orive G, Hernandez RM, Gascon AR et al. Encapsulation of cells in alginate gels. In Guisan JM, ed. *Methods in biotechnology. Immobilization of enzymes and cells*, 2nd ed. Totowa: Humana Press, 2006; 22:345-355.