A COMPREHENSIVE SURVEY OF GARLIC FUNCTIONALITY

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ABSTRACT

Garlic (*Allium sativum* L.) is the edible bulb from a plant of the *Allium* genus, commonly used for flavouring in cooking and for its beneficial effects for human health. Although garlic cloves are usually eaten raw or cooked, different garlic dietary supplements including dried or powdered formulations, oils and liquid extracts have being recently incorporated into the market to satisfy the demand of consumer for garlic bio-active compounds.

Despite the numerous therapeutic effects attributed to garlic, the chemistry behind its health-promoting effects is still poorly understood. Garlic is a major source of sulfur-containing compounds, particularly S-alk-(en)yl-L-cysteine sulphoxides (ACSOs), being alliin the major one. Volatiles such as allicin, and lipid-soluble sulphur compounds such as diallyl sulphide, diallyl disulphide, diallyl trisulphide, dithiins, ajoene and others, are originated from ACSOs by different metabolic pathways after tissue damage of garlic by cutting, crushing or bitting. These compounds provide to garlic its characteristic odour and flavour, as well as most of its biological properties. The effect of garlic on cardiovascular diseases, including hypocholesterolemic, anti-hypertensive, antithrombotic, and anti-hyperglycaemic activities, is one of its most extensively studied benefits. Garlic intake has also been described to reduce the risk for developing several types of cancer, especially those of the gastrointestinal tract (colon and stomach). Other bioactivities previously described in garlic include antimicrobial, antioxidant, antiasthmatic, immunomodulatory and prebiotic effects.

Recently, it has been demonstrated that additional garlic constituents such as organo-selenium compounds, steroid saponins and sapogenins (e.g. β-chlorogenin), vitamins B_6 and B_{12}, flavonoids (e.g. allixin), lectins and N-fructosyl-aminoacids, may contribute, along with organo-sulphur compounds, to the above mentioned biological effects of this vegetable.

Despite garlic can cause side effects, including gastrointestinal distress, allergic and asthmatic reactions, and interfere with a few medications, its use as therapeutic

agent seems to be safe, since these adverse effects appear with an excessive and prolonged consumption. Thus, the efforts of research should be directed to determine the effective intake to note the beneficial properties as well as the most suitable preparation to avoid undesirable effects.

INTRODUCTION

Genus *Allium* is formally classified in the family Liliaceae, represented by 280 separate genera and 4000 species. However, recent taxonomic revisions have seen members of this genus placed in the family Alliaceae. Of the approximately 700 species *Allium*, the edible members, including onion (*A. cepa* L.), garlic (*A. sativum* L.), chives (*A. schoenoprasum* L.), leek (*A. porrum* L.) and Welsh onion (*A. fistulosum* L.) are highly prized (Fenwick & Hanley, 1985). Among them, garlic is one of the oldest cultivate plants. Its possible ancestor appears to be *A. longicuspis*, a native in the mountainous regions of central Asia, which later spread to China, the Near East, and the Mediterranean regions before moving west to Central and Southern Europe, Northern Africa (Egypt) and Mexico (Lutomski, 1987). Today, garlic cultivation is distributed throughout most regions of the temperate world.

Garlic has been used as spice and food ingredient in cooking all over the world because of it combines well with an enormous range of foods, adding its own aroma and flavour as well as enhancing the flavours of the foods with which it is mixed (Woodward, 1996). Besides to be used like food, garlic has long been used in folk medicine with protective and curative purposes.

The earliest indication of the use of garlic is in clay models in Egyptian cemeteries, dated to as early as 3,750 BC (Woodward, 1996). It was part of the staple diet of the Egyptian pyramid builders and several cloves of garlic were also found in the tomb of Tutankamen. The pharaohs believed that by taking garlic to the afterlife, the food there would always be well seasoned. The *Codex Ebers*, an Egyptian medical papyrus dated to about 1550 B.C. and translated in 1937, contains over 800 therapeutic formulas of which 22 mention garlic as an effective remedy for a variety of ailments including heart problems, headache, bites, worms and tumors (Block, 1985). Garlic is also mentioned in the literature of Ancient Israel (The Talmud) and in the Bible during the time of the exodus. 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, tumors and constipation. Dioscorides, a chief physician to the Roman army in the first century A.D., prescribed garlic as a vermifuge or expeller of intestinal worms. Likewise, in Babylonian and Greek civilizations, use of garlic has been recorded by Hippocrates, “the Father of Medicine”, as an effective laxative and diuretic, by Aristophanes and Galen as excellent for the treatment of uterine tumors, and by Aristotle as a cure for rabies. During the first Olympic Games in Greece in 776 B.C., athletes ingested garlic as stimulant (Fenwick & Hanley, 1985; Block, 1985). In China, garlic tea has long been recommended for fever, headache, cholera, dysentery and prolonging longevity (Srivastava *et al*., 1995) and in India, garlic has been used for centuries for the treatment of hemorrhoids, rheumatism, dermatitis, abdominal pain, cough and as an antiseptic lotion for washing wounds and ulcers, due to its antibacterial properties. Indee, the realisation in 1858 by the French Louis Pasteur that garlic had potent antibacterial properties later led to its use in the First and Second World Wars, when penicillin and sulfa drugs were scarce, as an antiseptic to disinfect open wounds and prevent gangrene.

Nowadays, garlic is being still employed in folk medicine for over the world for the treatment of various ailments such cardiovascular diseases, cancer and microbial infections (Ali *et al*., 2000).

THE CHEMISTRY OF GARLIC

Some of the nutritional and chemical properties of garlic bulbs are given in **Table 1**. Garlic has been analysed for moisture, carbohydrates, protein, fat, minerals, vitamins, energy, ash, pH, acidity and essential oil contents (Haciseferogullari *et al.*, 2005). Protein content was found to be considerably higher than that in other vegetables such as bean and pea (Cemeroglu & Acar, 1986), but crude oil content was considerable lower. Garlic moisture was also low as compared to other vegetables (Cemeroglu & Acar, 1986) and caper bud and caperberries fruits (Ozcan & Akgül, 1998; Ozcan, 1999). Among minerals, garlic is known to contain high levels of potassium (21,378.84 mg/kg), phosphorous (6009.37 mg/kg) followed by magnesium (1056.15 mg/kg), sodium (532.78 ppm), calcium (363.61 ppm) and iron (52.91 ppm). In addition, garlic also contains the minerals selenium and germanium. The amount of these minerals in the bulb depends on the content of the respective minerals in the soil where the bulb is grown. Vitamins like riboflavin, thiamine, nicotinic acid, vitamin C and vitamin E are other important chemical constituents.

The biological effects of some of these constituents in intact garlic, such as lectins (the most abundant proteins in garlic), prostaglandins, fructan, pectin, adenosine, vitamins B_1, B_2, B_6, C and E, biotin, nicotinic acid, fatty acids, glycolipids, phospholipids and essential amino acids, have been studied for over several decades (Fenwick & Hanley, 1985). Recently, special attention has been given to certain steroid saponins and sapogenins such as β-chlorogenin. Several studies have demonstrated the importance of their biological and pharmacological activities such as antifungal, antibacterial, antitumor, anti-inflammatory, antithrombotic and hypocholesterolemic properties (Matsuura, 2001; Lanzotti, 2006). Since β-chlorogenin is bioavailable *in vivo* and detected in blood, this indicates that β-chlorogenin may be a bioactive compound in garlic. Other characteristic chemical constituents of garlic include allixin and organo-selenium compounds. These chemical compounds are reported to exhibit several biological effects, including cholesterol reduction, cancer prevention and others (Amagase, 2006).

However, despite the fact that the above mentioned compounds contribute in part to garlic bioactivity, evidence from several investigations suggests that the biological and medical functions of garlic are mainly due to their high content in organo-sulphur compounds (Augusti & Mathew, 1974; Wargovich *et al.,* 1988), which likely work synergistically with other compounds such as organo-selenium compounds.

Intact garlic cloves contain only a few medicinally active compounds (Block, 1992; Lawson, 1993). The primary sulphur-containing constituents in whole garlic are the S-alk(en)yl-L-cysteine sulfoxides (CSs, 1.8%) and γ-glutamyl-S-alk(en)yl-L-cysteine peptides (0.9%), both non-volatile and, therefore, odour-free sulphur compounds (**Figure 1**). It has been stimated that S-allyl-L-cysteine sulphoxide (alliin [1]) and S-methyl-L-cysteine sulphoxide (methiin), the major CSs in garlic, together with S-(2-carboxypropyl)glutathione, γ-glutamyl-S-allyl-L-cysteine, γ-glutamyl-S-(trans-1-propenyl)-L-cysteine and γ-glutamyl-S-allyl-mercapto-L-cysteine, make up more than 82% of the total sulphur content of whole garlic (Sugii *et al*., 1964; Fenwick & Hanley, 1985; Sendl, 1995). The γ-glutamylcysteine peptides are biosynthetic intermediates for corresponding CSs (Lancaster & Shaw, 1989). On prolonged storage or during germination, the enzyme γ-glutamyl transpeptidase acts on γ-glutamylcysteine peptides to form thiosulfinates (Sendl, 1995) such as S-allyl-cysteine (SAC [2]), which is also present in intact garlic and contributes heavily to the health benefits of some garlic preparations (Amagase *et al.,* 2001). The thiosulfinates other than SAC (e.g. allicin [3]) as well as other oil-soluble components such as ajoenes [4] (e.g. *E*-ajoene and *Z*-ajoene), vinyldithiins [5] (e.g. 2-vinyl-(4*H*)-1,3-dithiin and 3-vinyl-(4*H*)-1,2-dithiin), and sulfides (e.g. diallyl sulphide, DAS [6], diallyl disulphide, DADS [7], and diallyl trisulphide, DATS [8]), provide to garlic its characteristic odour and flavour as well as most of their biological properties (Lanzotti, 2006), but they are not naturally occurring compounds in intact garlic. When garlic is cut, crushed, chewed, dehydrated or otherwise processed, the vacuolar enzyme, alliinase, is released and rapidly lyses the cytosolic CSs (mainly alliin), which are converted into hundreds of organo-sulphur compounds in a short period of time. First, it is formed the reactive intermediate allylsulfenic acid (R-SOH), which immediately condenses to form the odoriferous alkyl alkane- thiosulfinates, among which, allicin represents 70-80% of total. Then, allicin (allyl 2-propene thiosulfinate) and other thiosulfinates such as allyl methane thiosulfinate, which are very unstable products, instantly undergo a number of transformations, giving rise to other sulphur-compounds derivatives (e.g. products [4-10]), depending on environmental and processing conditions (as temperature, pH and solvent polarity) (Block, 1985; Reuter & Sendl, 1995; Amagase, 2001) (**Figure 1**). Sulphur-containig compounds in commercial garlic preparations vary, depending on their manufacturing processes. Likewise, the variety of garlic determines the composition and quantity of each CS identified in garlic, which, in turn, determine the odour, flavour variation and biological activities observed for garlic.

In addition to odoriferous oil-soluble compounds, less odorous water-soluble organosulphur compounds such as SAC and S-allylmercaptocysteine (SAMC) have shown to be biologically active in several areas. The non-volatile sulphur-containing compounds SAC and SAMC are present in several garlic preparations, although the content varies considerably (Lawson, 1993; Imai *et al*., 1994).

Given such chemical diversity, garlic has received considerable attention from both chemist and biologist alike as new source of bioactive compounds.

GARLIC CONSUMPTION AND GARLIC SUPPLEMENTS

The worldwide trade of garlic has increased in the last years due to changes in consumer habits. The global production displayed an increase of 35% over the period 1998-2003 (from 9.1 to 12.1 million tons), which resulted in an increase of 13% in the yield and of 18% in the cropped area (from 0.95 to 1.125 million hectares). According to FAO 2005, global production of garlic is close to 15 million tons and it is estimated that the cropped area has not undergone great changes in recent years.

Several products of garlic are available in the international market and their popularity has increased in the last decade. The strong odour of fresh garlic has influenced to the consumers towards these commercial products as an optimal choice for increasing daily garlic intake.

The variety and manufacturing process of garlic are important considerations when choosing a garlic supplement, since, as indicated previously, they can markedly influence the composition of the garlic product and, therefore, its biological effects and toxicity (Fenwick & Hanley, 1985; Kritchevsky, 1991; Banerjee *et al*., 2003). Garlic products that contain the most safe, effective, stable, and odourless components are the most valuable as dietary supplements. Documentation of the safety and effectiveness is crucial in the evaluation of all garlic products that are proposed for use health promotion (Amagase, 2001).

Garlic supplements can be classified into four groups: garlic essential oil, garlic powder, garlic oil macerate and garlic extract (**Table 2**). **Garlic essential oil** is obtained by steam distillation of garlic and consists of a variety of sulfides such as DAS, DADS and DATS (Block, 1985; Yan *et al.*, 1992). Commercially available garlic oil capsules generally contain vegetable oil and a small amount of garlic essential oil because of pungent odors. **Garlic powder** is mass-produced as a flavouring agent for condiments and processed foods. Garlic cloves are sliced or crushed, dried and pulverized into powder. Garlic powder is thought to retain the same ingredients as (crushed) raw garlic, mainly alliin; however, amounts may vary significantly (Amagase, 2001). **Oil macerates** were originally developed for use as condiments. There are two types of oil macerate products on the market and both are packaged in soft gel capsules. One is made by simply mixing a garlic flavoring powder with vegetable oil. Its constituents are almost the same as the capsule and tablet forms of garlic powder. Another one is made by grounding raw garlic into vegetable oil. This type of product contains leftover alliin and allicin-decomposed compounds such as dithiins, ajoene and sulfides and, therefore, it has a strong garlic odor. For garlic extract, whole or sliced garlic cloves are soaked in an extracting solution (e.g. purified water and diluted alcohol) for varying amounts of time. After separation of the solution, the extract is generally concentrated and used. Powdered forms of the extract are also available. These aqueous or alcoholic extracts contain primarily water-soluble sulphur-compounds. In particular, KYOLIC **aged garlic extract** (AGE) is one of the most popular brands on the market. AGE is obtained by storage at room temperature of sliced and soaked in a water/ethanol mixture raw garlic for longer than 20 months (Amagase, 2006). It contains mainly the water-soluble sulphur-compounds SAC and SAMC, as well as small amounts of oil-soluble sulphur compounds.

One of the most important considerations in the above mentioned products is their standardization, which is the key to delivering consistent quality and efficacy of garlic products to consumers. It was initially thought that allicin was the main active substance *in vitro* of garlic; however, its effects *in vivo* are questionable. Several studies have revealed that the bioavailability of allicin is poor due to its great instability, not being detected in the blood or urine after the oral ingestion of raw garlic or pure (Lawson *et al*., 1992). Currently, it is well known that allicin is simply a transiet compound that is rapidly descomposed to other compounds. These findings clearly indicate that allicin does not contribute to the *in vivo* effects of garlic. Though no garlic supplement on the market can contain allicin due to its instability and high reactivity, some garlic powder products contain alliin and the enzyme, alliinase, and, therefore, could generate a certain amount of allicin (the so-called "allicin potential"). However, only a very small amount of allicin (< 5%) has been produced in simulated gastric fluid compared with water (Freeman & Kodera, 1995), demonstrating that is not generated in appreciable amounts. Therefore, allicin cannot be an appropriate marker compound to the standardization of garlic supplements. SAC is a stable water-soluble organosulphur compound and, unlike allicin, can be detected in the plasma, liver and kidney after oral intake (Nagae *et al*., 1994). SAC is the only reliable human compliance marker used for studies involving garlic consumption because it is detectable and increases quantitatively in the blood after oral intake of garlic products (Steiner & Li, 2001). Because it is found in many preparations, it might be used for standardization of garlic preparations and/or to compare various sources. AGE is the only product standardized for SAC.

EFFECTS RELATED TO CARDIOVASCULAR DISEASE

Cardiovascular disease is a complex and multifactorial disfunction characterized by multiple factors. Nowadays it is the most important cause of death in the developed countries and consequently, most research efforts were conducted to prevent it, thus, most breakthrough discoveries from natural products have been in the cardiovascular area (Gilani *et al*., 1997). There are many factors associated with cardiovascular diseases, among which can be included: elevated blood cholesterol and triglycerides levels; increased platelet activity, which can give rise to arteriosclerotic plaques formation; elevated blood homocysteine; alteration on glucose metabolism; hypertension; and obesity. These cardiovascular disease risk factors are mainly determined by uncontrollable causes (heredity, gender and age) and lifestyle-related causes (smoking, inactivity, stress and diet), which are possible to be modified. For this reason, a potential approach to the prevention and treatment of cardiovascular disease could be based on the diet. Epidemiologic studies indicate that diets rich in fruits, vegetables, and spices are associated with lower risk of all-cause cancer and cardiovascular-disease death. It has also been suggested that the benefits of fruit and vegetable consumption appears to be primarily related to cardiovascular disease and not to cancer. These foods contain phytochemicals that have anticancer and antiinflamatory properties, which confer them many heart benefits. One source of such phytochemicals is garlic, which in the prevention and treatment of cardiovascular diseases (and cancer) is well-known through the world. Preparations of garlic and its chemical constituents have been investigated for possible effects on the cardiovascular diseases mentioned above. In 2000, in the third National Health and Nutrition Examination Survey garlic was listed more frequently than other dietary supplements (Radimer *et al*., 2000). These supplements include garlic powder tablets, oil of steam-distilled garlic, oil of macerated garlic, ether-extracted oil of garlic and aged garlic extract (AGE). Some studies suggest that even the uncontrollable factors which cause the cardiovascular disease can actually be controlled or modified (Gómez del Arco *et* *al*., 1997; Waleh *et al*., 1998). For instance, S-allylcysteine (SAC) (one of the garlic active compounds, the major sulphur compound in AGE), for example, has been shown to regulate transcriptional factors that are required for gene expression (Geng *et al*., 1997). Thus, Chuah *et al*. (2007) found that SAC is protective in myocardial infarction because it regulates the expression of a protein which is responsable for the H(2)S production in the heart. Hence, dietary modification may help keep undesirable genes suppressed and desirable genes activated.

The role of garlic and its chemical constituents in preventing cardiovascular disease has been extensively acclaimed by several authors.

Effects on levels of serum lipids (cholesterol and triglycerides)

Cholesterol is an extremely important biological molecule that has roles in membrane structure as well as being a precursor for the synthesis of the steroid hormones and bile acids. Both, dietary cholesterol and that synthesized *de novo* are transported through the circulation in lipoprotein particles, being stored as cholesteryl esters in cells.

The synthesis and utilization of cholesterol must be tightly regulated in order to prevent over-accumulation and abnormal deposition within the body. Slightly less than half of the cholesterol in the body derives from biosynthesis *de novo*. Biosynthesis in the liver accounts for approximately 10%, and in the intestines approximately 15%, of the amount produced each day. Cholesterol synthesis occurs in the cytoplasm and microsomes from the two-carbon acetate group of acetyl-CoA (King & Marchesini, 2007) as shown in **Figure 2**.

Of particular clinical importance is the abnormal deposition of cholesterol and cholesterol-rich lipoproteins in the coronary arteries. Such deposition, eventually leading to atherosclerosis, is the complex interaction of serum cholesterol with the cellular components of the arterial wall. Cholesterol is the pathogenic substratum of many cardiovascular diseases and it continues to be the leader cause of death in development countries (Fabris *et al.*, 1994). Diseases related to atherosclerosis, such as ischemic heart disease (IHD) and stroke, are mainly, associated with elevated serum lipids (Medical Research Council Working Party, 1988) but also with male gender, age, hypertension, cigarette smoking, diabetes, etc.

Thus, total serum cholesterol is an important factor in the development of these diseases. Cholesterol present in the β-lipoprotein (LDL, Low Density Lipoprotein) and pre-β-lipoprotein (VLDL, Very Low Density Lipoprotein) fractions finds its way into the arterial wall, whereas α-lipoprotein (HDL, High Density Lipoprotein or commonly known as "good cholesterol") cholesterol helps to reduce the serum cholesterol (Vinay *et al*., 2008).

Several *in vitro* studies have indicated that garlic and its constituents inhibit certain enzymes involved in the cholesterol and fatty acids biosynthesis in cultured rat hepatocytes and human hepatyc cells (Gebhardt, 1993; Liu & Yeh, 2001; Yeh & Liu, 2001). It has also been shown that more water soluble compounds like S-allylcysteine (SAC) present in AGE are less cytotoxic and more efficient in inhibiting cholesterol biosynthesis than the lipid-soluble sulphur compounds such as diallylsulfide (DAS) (Yeh & Liu, 2001).

The antihyperlipidemic effect of garlic has been extensively studied and different trials carried out in animals, mainly rats and rabbits, and different commercially available garlic preparations, such as garlic essential oil and raw garlic, have reported that garlic consumption decreases significantly the content of total serum cholesterol (Chang & Johnson, 1980), LDL and VLDL and also significantly increases the level of HDL. In a study with cholesterol-fed rabbits, it was shown that AGE reduces vessel wall cholesterol accumulation and arteriosclerotic plaques development in arterial wall (Effendy *et al*., 1997; Campbell *et al*., 2001). Also, in a more recent study, Ashraf *et al*. (2005) demonstrated that a dietary supplementation of garlic and turmeric reduced the atherogenic properties of cholesterol and maintained the NO-mediated endothelial function in rats.

An increase in HDL/LDL ratio is a preventive effect of the development of IHD. However, garlic's antiatherosclerotic activity is probably due to its direct effect on the processes occurring in the vascular wall as it does not depend on blood cholesterol lowering. Some studies as those carried out by Lau *et al*. (1987) and Campbell *et al*. (2001) verified this theory. Cholesterol reduction (as well as the other risk factors) can be considered as an indirect approach to the treatment of atherosclerosis, but the effects observed at the arterial wall level provide a promising basis for the development of direct antiatherosclerotic therapy (Alexander *et al*., 1997)

In studies carried out in humans, garlic, its powder extracts or its oil extracts have shown their capacity to reduce the cholesterol and triglycerides blood levels due to the intake of high fat meals (Bordia *et al*., 1974; Basksh *et al.*, 1984).

Thus, in volunteers with normal blood levels of lipids, Bhushan *et al*. (1979) reported that eating 10 g of fresh garlic per day for two months significantly decreases (15%) serum cholesterol levels. Augusti (1977) found a diminution of 29% cholesterol levels among hypercholesterolemic patients. In another studies carried out with patients with coronary artery disease, medication with garlic essential oil during five months produced a 10% of diminution on serum cholesterol and a 21% on triglicerydes (Damnau, 1941).

In a broad metha-analysis, Silagy and Neil (1994) concluded that garlic decrease cholesterol levels about 12% (triglycerides too) after 4 weeks of treatment, remained then unchanged for the rest of the experiment. Moreover, these authors found a maximal reduction of cholesterol with raw garlic (3 garlic cloves daily) or with garlic oil (8 mg daily).

Although the most of the studies carried out in this area have revealed the cholesterol-lowering effects of raw garlic and garlic supplements, such as garlic essential oil and AGE (Lau *et al*., 1987; Warshafsky *et al*., 1993; Neil *et al*., 1996), more recent publications have showed different results. Thus, Mulrow *et al.* (2000) reported that garlic powder is ineffective in lowering blood-cholesterol levels probably due to varied levels of allicin potential in the garlic-powder supplements used in the clinical studies (Lawson & Wang, 2001). As above indicated, the amount of allicin is not a constant during the elaboration of the different garlic supplements (Amagase *et al*., 2001)

Active compounds and anti-cholesterolemic pathway by garlic derivatives

Organo-sulphur compounds are the main active substances responsible for the hypolipidemic and hypocholesterolemic effects of garlic, as much in humans as in experimentation animals (Yeh *et al*., 1997; Liu & Yeh, 2002). Several decades ago, Gebhardt (1993) reported the multiple inhibitory effects of garlic extracts in several different steps in cholesterol biosynthesis pathway in human hepatic cells. According to him, defined compounds (allicin) present in water soluble extracts of garlic inhibit the biosynthesis of cholesterol in hepatocytes, thus contributing to the reduction of serum cholesterol. Thus, it was demonstrated that allicin extracted from garlic decreases total serum lipids, cholesterol and phospholipids contents in rats fed allicin as compared to control animals (Augusti & Mathew, 1974). Some allicin-derived compounds in garlic that have demonstrated to possess a beneficial effect on cardiovascular variables are ajoene, methyl ajoene, DAS, DATS, 2-vinyl-4H-1,3-dithiin and SAC. Methiin and flavonoid quercetin (Glasser *et al*., 2002) have also shown to have the ability to reduce serum cholesterol levels and arteriosclerosis severity. Moreover, other no sulphur components of garlic, such as steroid saponins, have also demonstrated to be able to reduce serum cholesterol concentrations (Koch, 1993).

All these compounds may exert their hypocholesterolemic effect by three different mechanisms; by inhibiting hepatic cholesterol biosynthesis (Gebhardt *et al*., 1994; Gupta & Porter, 2001; Singh & Porter, 2006), by enhancing cholesterol turnover to bile acids and its excretion through gastrointestinal tract (Srinivasan & Sambaiah, 1991), or, in the case of plant saponins, by inhibiting cholesterol absorption from intestinal lumen without changing HDL cholesterol levels in hypercholesterolemic animal models (Matsuura, 2001; Slowing *et al*., 2001).

Conversely to the above mentioned studies, Lawson *et al*. (1998) found negative results possibly due to the preparations with reduced bioavailability of allicin. Recently, Gardner *et al.* (2007) have reported that neither raw garlic nor powdered garlic and AGE supplements, in reasonable doses, have statistically significant effects on LDL cholesterol or other plasma lipid concentrations in adults with moderate hypercholesterolemia. Therefore, although garlic appears to hold promise in reducing parameters associated with cardiovascular disease, more in-depth investigations are required (Rahman & Gordon, 2006).

Anti-hypertensive effect

Hypertension (systolic blood pressure (SBP) ≥ 140 mm Hg; diastolic blood pressure (DBP) ≥ 90 mm Hg), a typical lifestyle-related disease, has been considered the most important risk factor for chronic circulatory disease (Japanese Ministry of Health and Welfare, 2005) and is one of the major risk factors of atherosclerosis (Srivastava *et al*., 1995), affecting an estimated 1 billion individuals worldwide (Chobanian *et al*., 2003). Primary management should include relevant lifestyle modifications such as increased exercise, weight loss and dietary changes which could incorporate dietary supplementation. Garlic (*Allium sativum*) has played an important dietary as well as medicinal role in human history (Lawson, 1998). Blood pressure reducing properties of garlic have been linked to its hydrogen sulphide production (Benavides *et al*., 2007) and allicin content (Banerjee *et al*., 2003; Higdon & Lawson, 2005) which has angiotensin II inhibiting and vasodilating effects, as shown in animal and human cell studies (Kaye *et al.*, 2000; Al-Qattan *et al*., 2003; Mohamadi *et al.,* 2000; Sharifi *et al*., 2003; Al-Qattan *et al*., 2006; Benavides *et al*., 2007). Preliminary studies in humans and reviews on garlic preparations and blood pressure have been inconclusive. Das *et al*. (1995) founded some evidences that suggested garlic reduces blood pressure by inhibiting platelet nitric oxide synthase. Nitric oxide (NO) is an important local vasodilatador which controls several physiological functions of the cardiovascular system. Three kinds of NO synthases (NOSs): neuronal constitutive NOS (ncNOS), inducible NOS (iNOS) and endothelial constitutive NOS (ecNOS), are responsible for NO biosynthesis. A meta-analysis published in 1994 reported promising results in subjects with mild hypertension but found insufficient evidence to recommend garlic for clinical therapy (Silagy & Neil, 1994). Later, anti-hypertensive effect of garlic was determined in multiple studies with hypertensive rats using AGE, aqueous garlic extracts and garlic powder (Fallon *et al*., 1998; Al-Qattan *et al*., 1999; Harauma & Moriguchi, 2006). In contrast, other investigations carried out with ethanolic extracts of garlic in hypertensive rats reported that oral administration of extracts during a normal salt diet or during a high salt diet do not influence blood pressure (Kivirantava *et al*., 1989).

Currently, many medical supplies and health foods have been researched and developed to prevent or improve hypertension (Harauma & Moriguchi, 2006). The increasing use of these alternative and complementary therapies for hypertension (Ernst, 2005; Yeh *et al*., 2006) make it timely to provide an updated systematic review and meta-analysis of trials investigating the effect of garlic preparations on blood pressure (Ried *et al*., 2008). Inclusion of additional data from studies published since 1994 has enabled subgroup meta-analyses of hypertensive and normotensive subjects. This systematic review and meta-analysis suggests that garlic preparations are superior to placebo in reducing blood pressure in individuals with hypertension. Many clinical trials find no significant antihypertensive effect despite form, dose or duration of treatment (Valli & Giardina, 2002). Future large scale long-term trials are needed to investigate whether standardised garlic preparations could provide a safe alternative or complementary treatment option for hypertension in clinical practice.

Active compounds and anti-hypertensive pathway by garlic derivatives

Several investigations have allowed the determination of the mechanism by which garlic exerts its anti-hypertensive action. Some studies of garlic effect on muscular contraction *in vitro* have concluded that its hypotensive action may be, at least partly, due to a direct relaxant effect on smooth muscles (Aqel *et al*., 1991). On the other hand, other studies have suggested that garlic may also exert an indirect vasodilator effect, inducing the NO and hydrogen sulphide synthesis, both potent vasodilators. The latter is synthesized from sulfhydryl-containing amino acids, presents in large amounts in garlic extracts, such as cysteine (that it is the most abundant) and the S-alk(en)yl derivatives as SAC, SEC (S-ethylcysteine) and SPC (S-propylcysteine) (Liu & Yeh, 2002). Likewise, a recent study with several rat models of hypertension has indicated that quercetin and its methylated metabolite isorhamnetin can reduce blood pressure and prevent angiotensin II-induced endothelial dysfunction by inhibiting the overexpression of p47 (phox), a regulatory subunit of the membrane NADPH oxidase, and the subsequent increased superoxide production, resulting in a highest NO bioavailability (Sanchez *et al*., 2007).

A novel drug assayed in hypertensive rats has been recently synthesised through the reaction of the pharmaceutical drug Captopril with allicin (**Figure 3**). The reaction product, called allylmercaptocaptopril (CPSSA), provides better protection against hypertension, since it has the Captopril ability to inhibit the angiotensin-converting enzyme (ACE) and the allicin ability to reduce serum cholesterol and triglycerides levels (Miron *et al*., 2004).

<u>Anti-hyperglycaemic or anti-diabetic potential</u>

Diabetes Mellitus, often referred to simplify, as diabetes, is a disease in which the body does not produce or properly use insulin. Insulin is a hormone that is needed to convert sugar, starches and other food into energy needed for daily life. Thus, diabetes resulting in abnormally high blood sugar levels (hyperglycemia). Its cause continues to be a mystery, although both genetics and environmental factors such as obesity and lack of exercise appear to play roles.

The relationship between *diabetes Mellitus* and atherosclerosis is likely based on the interactions between arterial cells and atherogenic glycosylated LDL lipoproteins originated during diabetes development, that play a key role in the initiation of an atherosclerotic lesion, inducing cholesterol accumulation in arterial cells (Ide & Benjamin, 2001) and other more severe atherosclerotic manifestations at cellular level that lipoproteins from no diabetic subjects (Winocour, 1994; Sobenin *et al*.,1994).

The garlic effectiveness as hypoglycaemic agents has been scarcely investigated and the existing data are controversial, having not found evidence of its effectiveness in all cases (Sheela & Augusti, 1992; Mansell *et al*., 1995).

The hypoglicemic effects of garlic and its individual components have been demonstrated in animal models (Jain *et al*., 1973; Zacharias *et al*., 1980; Sheela & Augusti, 1992) whereas other researchers found no significant alteration of hyperglycaemia in animals (Swanston *et al*., 1990). Recently, it has been reported that long-term absorption of natural flavonoids as quercetin could be useful to prevent advanced glycation of collagens, which contributes to development of cardiovascular complications in diabetic patients (Urios *et al*., 2007). Type II *diabetes Mellitus* is characterized by premature accelerated atherosclerosis development leading to early invalidization and high mortality in this category of patients (Krolewski *et al*., 1991; Burchfiel *et al*., 1993). In a study on the use of natural remedies for type II *diabetes Mellitus* treatment in a diabetic women group from United States, garlic appeared among the most used vegetables (Johnson *et al.*, 2006) and in a recent double-blinded placebo controlled study with a new garlic-based formulation (namely, time-released garlic powder tablets Allicor), Sobenin *et al*. (2008), established that this product is recommended for the treatment of type II *diabetes Mellitus* along with dietary treatment and/or sulfonylurea derivatives to achieve better metabolic control. In addition garlic supplement may improve the other risk factors (reduction of serum triglycerides, inhibition of cholesterol synthesis, etc). Thus, the use of this vegetable is suggested in conjunction with anti-diabetic drugs to increase their therapeutic potential and to minimize their oral dosage.

Active compounds and anti- hyperglycaemic pathway by garlic derivatives

The bioactive constituents from garlic, such as methiin and S-allyl cysteine sulphoxide (SACS) (Sheela & Augusti, 1992), exert their anti-diabetic action by 3 different ways: (i) stimulating the insulin production and secretion by pancreas, (ii) interfering with dietary glucose absorption, and (iii) favouring the insulin saving (Srinivasan, 2004a, 2004b).

Anti-platelet or anti-thrombotic effect

As it is known, platelets (or thrombocytes), are the cells circulating in the blood that are responsible for mantain the haemostatic integrity of blood vessels and the stop of bleeding after injury (Ali *et al*., 2000) through vasoconstriction, clot formation and blood coagulation. High levels of platelets may increase the risk of thrombosis: the formation of a clot or thrombus into a blood vessel obstructing the flow of blood through the circulatory system (see **Figure 4**). Therefore, it is evident that platelet circulation is much related to certain cardiovascular diseases (Becker, 1999).

Garlic and its components are known to possess antiplatelet activity which has been demonstrated mostly *in vitro* (Lawson *et al.*, 1998) and several platelet inhibitors have been isolated and characterized from this vegetable. The inhibitory effects of garlic extracts as well as allicin, ajoene and other individual garlic compounds on thrombus formation and platelet aggregation has been also investigated (Srivastava, 1986; Mayeux *et al*., 1988; Apiz-Castro *et al*., 1992). Cavagnavaro *et al*. (2007) studied the effect of cooking on garlic antiplatelet activity and its content in thiosulfinates. Their results suggested that allicin and thiosulphinates are responsible for the *in vitro* antiaggregatory activity and that crushing garlic before moderate cooking can reduce the loss of activity. This partial loss of antithrombotic effect in crushed-cooked garlic may be compensated by increasing the amount consumed.

The study carried out by Chang *et al.* (2004) showed that the alkenyl thiosulfate sodium 2-propenyl thiosulfate (2PTS) obtained from boiled garlic has the potential to prevent cardiovascular disease by inhibiting platelet aggregation in dogs and humans *in vitro*. As these compounds are not volatile, these compounds are considered heat-stable platelet-inhibitory factors.

Aqueous and organic garlic extracts are also able to inhibit platelet aggregation induced by a number of physiologically important aggregating agents, as collagen and adrenaline, and the thromboxanes synthesis *in vivo* (Mohammad & Woodward, 1986) by several mechanisms, such as inhibition of several steps of the arachidonic acid pathway in platelets (Ali *et al*., 2000), which is the thromboxanes precursor. Due to the variations in methods of preparation, the different garlic products commercially available may show different inhibitory effect on platelet aggregation (Lawson *et al*., 1992).

It was found that garlic oil administration to healthy subjects and patients with coronary artery disease (CAD) inhibited platelet aggregation *ex vivo*. Though garlic components leave the body quickly, a slow building up of the active ingredients may take place. This was evident from the observation that though a 2-3 fold higher dose was not effective in inhibiting platelet aggregation when administred once, whereas lower dose became effective in long-term administration (Bordia *et al.*, 1996).

Two clinical studies reported reductions in platelet aggregation of 16.4% and 58% respectively with garlic oil obtained from 9-10 g fresh garlic cloves (Boullin, 1981; Barrie *et al*., 1987). In a randomized double-bind study of normal healthy subjets, the effect of three different doses of AGE compared with placebo on platelet aggregation and adhesion were measured after 6 weeks of supplementation. AGE supplementation reduced platelet function, and this inhibitory effect was selective, affecting collagen and epinephrine but not ADP-induced aggregation. Not all studies show a favourable effect of garlic on platelet function. A placebo-controlled, double-bind, randomized study on healthy men showed no effect of garlic extract on platelet aggregation, serum tromboxane and platelet activating factor (Morris *et al.*, 1998).

Active compounds and anti- platelet pathway by garlic derivatives

Antiplatelet activity is substantially affected by genotype, environment and storage duration of vegetable. It has been reported by several epidemiologic studies that, in garlic, the antiplatelet activity is determined, in part, by the native concentration of organo-sulphur compounds and genotypically determined sulphur content of the bulb (Goldman *et al*., 1996).

These compounds have structural similarity to ajoene, considered the major antiplatelet compound in garlic extracts. In addition, other no sulphur compounds, such as β-chlorogenin and quercetin, have been also shown to inhibit platelet aggregation (Rahman *et al.*, 2006).

The mechanism of platelet aggregation inhibition is associated at least with reduction of tromboxane formation from exogenous arachidonate (Srivastava, 1986) and perturbation of the physicochemical properties of platelet plasma membrane (Apiz-Castro *et al.*, 1983). Gillian *et al*. (2006), in a preliminary study, reported the mechanisms that may be involved in the inhibition of platelet aggregation by AGE when platelets are stimulated with adenosine diphosphate (ADP). These authors founded that the mechanism involved appear to be multiple in nature, involving membrane fluidity changes, inhibition of phospholipase C, inhibition of calcium mobilization, increase in NO and cAMP (cyclic adenosine monophosphate) production, and inhibition of TXA2 (tromboxane A2), all of which can lead to an inhibition of platelet aggregation.
The different results obtained are probably due to the use of different garlic preparations and variable amounts of the active constituents in garlic in these studies (Rajaram, 2003).

<u>Effect on hyperhomocysteinemia</u>

Homocysteine (Hcy) is a sulphur-containing amino acid formed during metabolism of methionine, an essential amino acid derived from the diet. The determination of total plasma homocysteine (Hyc) has become a very useful tool because moderately elevated values of circulating homocysteine constitute an important risk factor for the development and progress of occlusive vascular affections as it is shown in **Figure 5** (Fischer *et al*., 2000). In addition, hyperhomocysteine is a risk factor for ischaemic heart disease in diabetic patients (Okada *et al*., 1997).

Homocysteine exists in normal human plasma in several different forms. Approximately 70% is bound to plasma proteins, mainly albumin, through disulphide bounds. The remaining homocysteine circulates as a free thiol compound, reduced or combined by oxidation with other thiols, as cysteine, resulting in mixed disulphide, or another molecule of homocysteine, to form the dimmer homocystine (Mansoor *et al*., 1992b). Hence measurement of total plasma homocysteine as a cardiovascular risk factor involves assay of bound, free, reduced and oxidized forms. The concentration of total homocysteine is regulated by disulphide-disulphide exchange and thiol-disulphide exchange reactions. Cysteine plays an essential role in modulating thiol-disulphide exchange (Ozkan *et al*., 2002), whereas protein-bound cysteine and cysteinylglycine participate in disulphide-disulphide exchanges (Mansoor *et al*., 1992a).

There are several factors that cause increase of Hyc. Hyperhomocysteinemia can be congenital, due to hereditary metabolic affections (Mudd & Levy, 1983), or acquired and to have a multifactor origin. The commonest cause of acquired hyperhomocysteinemia is the folate, vitamin B_6 and/or B_{12} deficiency (Durand *et al*., 1996; Jacobsen, 1996; Ubbink *et al*., 1996; Sumner *et al*., 1996) and the drugs consumption that interfere with these vitamins metabolism.

Because garlic contains vitamins B_6 and B_{12} and a large amount of aminothiol compounds, such as SAMC, DAS, diethyl disulphide (DEDS) and dipropyl disulphide

(DPDS) (Liu & Yeh, 2000), it was thought that garlic intake may be an effective way to reduce plasma homocysteine levels.

Several hyperhomocysteinemia has been reported in individuals with genetic defects in enzymes such as cysthatione β-synthase (Clarke *et al*., 1991; Aguilar *et al*., 2004) and N5, N10-methylenetetrahydrofolate reductase (Aguilar *et al*., 2004; Takenata, 1993). Conversely, folic acid supplementation is effective in reversing elevated homocysteine level (Doshi *et al*., 2002; Boers, 2000; Moat *et al*., 2004).

Garlic contains a variety of aminothiol compounds that may interact with free and protein-bound homocysteine. Yeh *et al.* (2005) indicated that a reduction in plasma level of homocysteine could not be attributed to disulfide-disulfide exchange and thiol-disulphide exchange among aminothiol compounds and homocysteine. Several recent studies (Yeh *et al*., 2005; Yeh & Yeh, 2006; Weiss *et al*., 2006; Ide *et al.*, 2006) have demonstrated the effectiveness of AGE to reduce the plasma concentration of homocysteine in rats with hyperhomocysteinemia induced by severe folic acid deficiency, but the action mechanism is not yet known with absolute certainty. Yeh and Yeh (2006) established the reduction in total homocysteine of the everaly folate-deficient rats was accompanied by a proportional decrease in protein-bound and free homocysteine, resulting in an unchanged protein-bound: free homocysteine ratio. AGE added to the diet not alter plasma concentrations of other aminotiol compounds: cysteine gluthatione and cysteinylglycine. These data, together with the increase of S-adenosylmethionine and the decrease of S-adenosylhomocysteine concentrations in the liver, suggest that the hypohomocysteinemic effect of AGE most likely steams from impaired remethylation of homocysteine to methionine and enhanced transsulfuration of homocysteine to cystathione.

Smoking, alterations in serum lipid profiles, hypertension and diabetes are the risk factors that are conventionally associated to the early appearance of cardiovascular disease. However, many patients with clinical manifestations of premature arteriosclerosis do not show any of these risk factors. In the last ten years, new risk factors for arteriosclerotic vascular disease such as hyperhomocysteinemia have been described, which have allowed to develop new measures of prevention. Cardiovascular risk is further increase by a combination of hyperhomocysteinemia, hypertension and smoking (Boers, 2000). It has been documented that plasma total-homocysteine levels in patients with cardiovascular disease are significantly higher than those of normal subjects (Ueland *et al*., 1992). Similarly, patients with myocardial infarction had increased levels of homocysteine as compared to other free of infarction (Stampfer *et al*., 1992) The risk for cardiovascular diseases caused by hypercholesterolemia is associated with atherosclerosis. However, the mechanism underlying homocysteine-induced cardiovascullar diseases is still controversial (Yeh & Yeh, 2006). It has been suggested that homocysteine may impair production of endothelium-derived relaxing factor, stimulate proliferation of smooth cells, retard endothelial NO activity, and induce cardiovascular fibrosis (Massy *et al.*, 1994; Tsai *et al*., 1994; Das, 2003; Tyagi, 1999).

Endothelial dysfunction (ED) due to decreased bioavailable NO by increased vascular oxidant stress plays a critical role in the vascular pathobiology of hyperhomocysteinemia (hhcy). Aged Garlic Extract (AGE) can minimize intracellular oxidant stress and stimulates NO generation in endothelial cells. Weiss *et al*. (2006) carried out a placebo-controlled, blinded, cross over study to examine whether AGE prevents macro- and micro ED during acute hhcy induced by an oral methionine challenge in healthy subjets and the results allowed concluded that AGE may at least partly prevent a decrease in bioavailable NO during acute hhcy.

In addition Nagatoshi *et al.* (2006) demonstrated the effectiveness of AGE in the homocysteine inhibition and, hence, in modulation of formation of early atherosclerotic lesions in a study carried out with human cells.

Evidences, here showed, from different clinical trials point toward garlic having, mostly, a role to play in either preventing or delaying cardiovascular disease. However, more research is still required to convince health works, consumers, and regulatory bodies.

EFFECTS ON CANCER AND MUTAGENESIS

Numerous scientific reports imply that vegetable intake may affect cancer incidence. In reviews of epidemiologic studies there is convincing evidence that high consumption of certain vegetables reduces the risk of colorectal, stomach, lung and esophageal cancers; in addition, there is probable evidence for cancers of the breast and bladder (World Cancer Research Fund, American Institute for Cancer Research, 1997). Garlic is one of the most ancient spice plants reputed to have an effect on cancer. As recorded around 1550 B.C. in the Ebers Papyrus, garlic was applied externally for the treatment of tumours by ancient Egyptians and internally by Hippocrates and Indian physicians (Hartwell, 1967, 1968; Block, 1985). However, the modern era of the use of garlic as anticancer agent begins in the 1950s when Weisberger and Pensky (1958) demonstrated *in vitro* and *in vivo* that thiosulfinate extracts from garlic inhibited the tumour cells growth. Since these investigations, many epidemiological and laboratory studies have been developed to evidence the chemopreventive or anticarcinogen effects of garlic and related *Allium* species. Interestingly, China provides an ideal “Field Laboratory” for epidemiological studies of cancer incidence. Stomach cancer was found to rank higher for males and females in cancer mortality (Wang *et al.*, 1985; Lau *et al*., 1990) than other cancer incidence in China (Mei *et al*., 1982). They suggested that garlic consumption may inhibit nitrate reduction by bacteria. Subsequently, the lower gastric nitrite (a nitrosamine precursor) concentration may reduce the risk of developing stomach cancer. Likewise, You *et al*. (1989) identified that smoking, salty foods and moldy grains are associated with increased risk of stomach cancer (You *et al.,* 1989). A significant reduction of stomach cancer risk was found to be associated with increasing consumption of garlic, scallicens and Chinese chives (You *et al*., 1988). In addition, it has been also shown an inverse relationship between garlic consumption and the incidence of sarcoma (Lau *et al*., 1990) and carcinoma in colon (Lau *et al*., 1990; Steinmetz *et al*., 1994), oesophagus (Lau *et al*., 1990; You *et al*., 1998), prostate (Hsing *et al*., 2002), bladder, liver (Lau *et al*., 1990; Lamm & Rings, 2001), lungs (Le Marchand *et al*., 2000), mammas (Lau *et al*., 1990; Challier *et al*., 1988), and skin (Lau *et al*., 1990).

Several investigations have shown that both water- and lipid-soluble sulphur compounds from garlic provide anticarcinogen benefits, however, generally, the lipid-soluble sulphur compounds such as DAS and its metabolites, diallyl sulphoxide (DASO), diallyl sulfone ($DASO_2$), DADS and DATS are the most effective antitumorogenic agents. Although the question of how these compounds result in chemoprevention has not yet been fully answered, several mechanisms of action have been proposed (Knowles & Milner, 2001; Griffiths *et al.,* 2002; Thomson & Ali, 2003) (**Figure 6**).

Garlic compounds can **alter the carcinogen metabolism** either increasing the detoxifying enzymatic systems activity that increase the carcinogen polarity, facilitating its excretion from the body (Guyonnet *et al*., 1999), or inhibiting the procarcinogens activation by cytochrome P_{450} (Dion & Milner, 1997; Khanum *et al*., 2004). Glutathione-S-transferase (GST) is a well-known detoxifying enzyme in Phase II metabolism of drugs that removes harmful electrophiles by conjugating them with glutathione. Therefore, GST can play a detoxifying role in metabolism of carcinogens that may be electrophilic in nature. Sparnins and coworkers (1986, 1988) studied the effect of oral administration of allyl methyl trisulfide (AMTS) on glutathione-S-transferase (GST), a detoxifying enzyme, in the liver, forestomach, small intestine and lung of mice. They observed that 96 h after oral administration of AMTS, GST activity was increased in all tissues and, in addition, benzo[a]pyrene induction of forestomach tumors was suppressed. Similarly, three other garlic-derived compounds (allyl methyl disulfide, DATS and DADS) stimulated GST activity in these organs. In contrast, saturated (propyl) derivatives did not affect GST activity in these organs of mice. These results suggest that allyl groups are important for the stimulation of GST. Such anticarcinogenic activity of DADS against benzo(a)pyrene in mice has been also reported by Srivastava *et al*. (1997). Similarly, Sumiyoshi and Wargovich (1989) reported that the oral administration of DAS (400 mg/Kg) stimulated mouse hepatic GST activity. They also reported elevated colonic GST activity. In both the liver and colon, the increased GST activity was DAS dose-dependent. In an earlier study, Wargovich and Goldberg (1985) also found that DAS affects aflatoxin B_1 metabolism and DNA binding and prevents nuclear damage to colon epithelial cells *in vivo* induced by chemical carcinogens such as DMH (1,2-dimethylhydrazine) and NMBA (N-nitroso methylbenzylamine), by inhibiting the conversion of procarcinogens to ultimate carcinogens in the liver.

Manson *et al.* (1997) also studied the effect of oral administration of garlic oil to rats on a number of drug metabolizing enzymes in liver tissues. They reported that garlic oil induced phase II enzymes such as GST and the conjugating enzyme, gamma-glutamultranspeptidase.

In other study, Singh *et al.* (1998) observed that treatment of mice with DADS and DATS, which are potent inhibitors of benzo(a)pyrene-induced forestomach and pulmonary tumorogenesis, resulted in a statistically significant increase in forestomach and lung NAD(P)H: quinone oxidoreductase (NQO) activity, an enzyme implicated in the detoxification of actived quinone metabolites of benzo(a)pyrene. In addition, DADS and DATS were much more potent inducers of forestomach NQO activity than DAS, which is a weaker inhibitor of benzo(a)pyrene-induced tumorogenesis than the former compounds. Ajoene has been also shown to be able to inhibit aflatoxin B_1-, benzo(a)pyrene- and 4-nitro-1,2-phenylenediamine-induced mutagenesis *in vitro* models as well as prevent *in vivo* skin tumor of mouse by 12-O-tetradecanoylphorbol-13-acetate (Tadi *et al.*, 1991; Ishikawa *et al.*, 1996; Nishikawa *et al*., 2002).

Anticarcinogen compounds from garlic have also an **anticlastogenic effect**, preventing the chromosomal damage (Lau *et al.*, 1990; Khanum *et al*., 2004). Several authors have studied the anticlastogenic effects of garlic. In several studies with mice, Choudhary *et al.* (1997) have observed that aqueous garlic extract administered orally either alone or in combination with mustard oil significantly reduced the frequency of chromosomal aberrations resulting from intravenous injection of sodium arsenate, a strong clastogen. It has been suggested that trivalent arsenate induces toxicity by binding to thiol ions which ultimately leads to inhibition of certain enzymatic reactions. Therefore, the sulphur-containing compounds in crushed garlic may be the principal factors responsible for the significant reduction of the clastogenic effects of sodium arsenate by crude garlic extract (Sharma & Talukder, 1987; Choudhary *et al*., 1997a, 1997b). Chowdhury *et al*. (2008) found several evidences, including reduction of intracellular ROS level in human tumor cells, inhibition of tissue lipid peroxide generation, and increase of total tissue sulfhydryl groups, glutathione and antioxidant enzymes level, which indicated that AGE can be a potential protective regimen for arsenic mediated toxicity.

Garlic compounds can also **inhibit the tumor growth**, by inhibition of cell division and induction of apoptosis (Perchellet *et al.*, 1990; Izzo *et al.*, 2004). Apoptosis, also known as programmed cell death, is a means by which living organisms control abnormalities in cells. It is of interest that in numerous human pathological conditions including cancers, that apoptotic signalling cascades are often impaired (Rose *et al*., 2005). Both garlic extracts and their phytochemical constituents can induce apoptosis in several in vitro cell culture models. From the available data, activation of the proteolytic enzymes, changes in intracellular redox homeostasis, generation of reactive oxygen species (ROS) and the activation of stress signaling cascades are all implicated in the apoptotic response of cancer cells to garlic sulphur compounds. Li *et al.* (1995) investigated the effect of AGE and two of its components, SAC and SAMC, on human breast cancer cells. They observed an anti-proliferative response of these compounds and an alteration in glutathione level without significant concurrent changes in the glutathione metabolizing enzymes (Li *et al.*, 1995). In a more recent study, Katsuki *et al*. (2006) reported that AGE has chemopreventive effects on DMH-induced colon carcinogenesis through modulation of cell proliferation. Likewise, studies have shown that SAMC can inhibit cell proliferation in human erythroleukaemia cell lines as well as in human colon cancer cells (Sigounas *et al.*, 1997; Shirin *et al.*, 2001). Xiao *et al*. (2003) later found that SAMC exerts anti-proliferative effects by arresting cells in mitosis and triggering apoptosis. Similarly, garlic-derived sulfides (DAS, DADS and DATS) have also been shown to be potent inducers of apoptosis in cancer cells. Many reports have shown that DAS has antitumor efficacy in cultured carcinoma cell lines, such as lung cancer cells and mouse skin tumors (Wargovich *et al*., 1992; Hong *et al*., 2000; Arora & Shukla, 2003). Likewise, Xiao *et al.* (2006) have observed that DATS induces apoptosis in human prostate cancer by activation of pro-apoptotic proteins. Both, DADS and DATS, induce apoptosis in cultured human neoplastic and non-neoplastic lung cancer cells (Sakamoto *et al*., 1997; Hong *et al*., 2000) and human leukaemia HL-60 cells exposed to DADS undergo apoptotic cell death (Kwon *et al.*, 2002). At micromolar concentrations, DADS also inhibits cell proliferation and induces apoptosis *in vitro* in estrogen receptor positive and negative breast cell lines, as well as in in human gastric cell lines (Li *et al.*, 1998; Nakagawa *et al*., 2001). Moreover, DADS has been shown to inhibit cell proliferation in human colorectal cells by inducing the pro-apoptotic gene NAG-1 (Bottone *et al.*, 2002) and it has been reported to be as effective as the colon anticancer compound 5-fluorouracil in nude mice at equivalent doses (Sundaram & Milner, 1996; Singh *et al*., 1996). Ajoene has been also shown to exhibit antitumor activities either *in vitro* on breast cancer, hepatocellular, gastric and colon carcinoma, or *in vivo* on hepatocarcinoma and sarcoma, through both cell cycle blockage and apoptosis of tumor cells (Li *et al*., 2002). Another interesting property of ajoene is its selective cytotoxic action on neoplastic (vs. normal) cells (Li *et al*., 2002; Dirsch *et al*., 1998). Indeed, ajoene induces apoptosis in human leukemic HL60 cells but not in peripheral mononuclear cells of healthy donors (Dirsch *et al*., 1998). Recently, Terrasson *et al*. (2006) have demonstrated a cytotoxic effect of Z-ajoene against a large spectrum of cell lines (astrocytoma, lymphoma, neuroblastoma, etc.) by inducing apoptosis. This effect was mediated by accumulation of pro-apoptotic proteins in Z-ajoene-treated cells which was likely due to both increase in gene transcription and in inhibition of their proteolysis by proteasome enzymes. These authors also investigated a new activity of *Z*-ajoene against human cytomegalovirus (HCMV), a DNA virus of the herpesvirus family that has been associated with several tumor cells including those from glioblastoma and colorectal cancers. Data demonstrated a potent anti-HCMV activity of *Z*-ajoene *in vitro* that was mediated by an increase of apoptotic cells after infection. Regarding to allicin, it has been determined that this lipid-soluble volatile organo-sulphur compound, but not its precursor alliin, inhibits proliferation of human mammary, endometrial, and colon cancer cells through induction of apoptosis, cell cycle blockage and transient drop in the intracellular glutathione level (Hirsch *et al*., 2000; Oommen *et al.*, 2004).

Recently, a number of researchers have focussed on garlic **antimutagenic activity**, observing that certain sulphur compounds such as DAS have an effect on DNA repair mechanisms, protecting the DNA from activated mutagens and preventing, thus, the initiation of carcinogenesis (Wargovich *et al*., 1988; Hong *et al*., 1991; Khanum *et al.*, 2004).

Another mechanism of action is the **effective stimulation of the immune response**. To date, this latter action mechanism is thought to be the most important direct anticarcinogen action of garlic (Lamm & Riggs, 2001), which has been documented in cultures of different cancerous tissues, including colon, prostate, bladder and stomach (Pan *et al*., 1985; Knowles & Milner, 1997). Given the importance of this mode of action, it will be treated more in depth later.

Moreover, it is accepted that phytochemicals of garlic (and other foods) with antioxidant properties minimize DNA damage by reacting with free radicals and in this way they could prevent cancer (Perchellet *et al.*, 1990). However, in some studies antioxidants increase incident of cancers instead of lowering it. It is therefore likely that antioxidants are acting in different way than expected. One of the possibilities is that they are disrupting specific pathways or inhibit enzymes that are important in carcinogenesis (Jankun *et al.*, 2003). In particular, the pro-inflammatory enzyme lipoxygenase, is a regulator of human cancer development and it is overexpressed in a variety of tumors including breast, colorectal and prostate cancer, and cancer cell lines (Pidgeon *et al*., 2002) and that its inhibition trigger tumor cell apoptosis, reduce tumor cell motility and invasiveness, or decrease tumor angiogenesis and growth (Nie *et al.*, 2001). Belman *et al*. (1989) investigated the inhibition of soybean lipoxygenase (LOX) by onion and garlic components. They found that the di- (1-propenyl) sulphide was the only irreversible inhibitor. DATS, allyl methyl trisulfide and DADS were competitive inhibitors, while 1-propenylpropyl sulphide and ajoene were mixed inhibitors. Sendl *et al.* (1992) also studied LOX inhibitory activity of garlic. They used extracts of wild garlic (*Allium ursinum*) and garlic (*Allium sativum*) with defined chemical compositions to assess their inhibitory potential on LOX. The inhibition rates as IC_{50} values of these extracts showed a good correlation with the %-content of the major sulphur-containing compounds (thiosulfinates and ajoene).

In addition to organo-sulphur compounds, eruboside-B, a steroid saponin isolated from garlic bulb, and allixin (phytoalexin), are largely responsible for the anticarcinogenic activity of garlic (Yamasaki *et al*., 1991; Matsuura, 1997). Allixin, being a phenolic compound, is an effective inhibitor of phospholipid metabolism stimulated *in vitro* by the tumor promoter (Kodera *et al*., 1989). Garlic is also risch in flavonols, particularly kaempferol, which have antineoplastic effects by helping in the detoxification of carcinogenic compounds, by inducing apoptosis (Brisdelli *et al*., 2007), by inhibiting bioactivating enzymes (Lautraite *et al.*, 2002; Muto *et al*., 2001) and due to its antioxidant and anti-inflammatory activities (Mutoh *et al*., 2000; Raso *et al*., 2001). Moreover, garlic is one of the best natural sources of germanium. It is of interest to note that this trace metal has also been reported to prevent and cure cancer. Garlic is also an excellent source of selenium (Se), which has potential therapeutic value in cancer treatment (Bolton *et al.*, 1982; Lawson, 1993; El-Bayoumy *et al.*, 2006). Epidemiological studies have indicated a relationship between Se intake and the incidence of certain cancers. Se-enriched garlic has higher anticarcinogenic activity than the common plant (Ip *et al.,* 1992). This increased effect of cancer prevention is achieved at least partly by S substitution with Se. The pure Se-compounds have proved to be superior anticancer agent than their corresponding S-analogues. For example, diallyl selenide is at least 300 times more active than DAS in the reduction of tumours of mammal cancer (El-Bayoumi *et al*., 1996). Se-methyl selenocysteine is the major organo-Se-compound in garlic bulb and, along with γ-glutamyl-Se-methyl selenocysteine, the major Se-compound possessing anti-cancer activity (Block *et al*., 2001). In mammary tumor model, Se-methyl selenocysteine was shown to be the most effective Se-compound so far in reduction of tumors (Whanger, 2004). Identification and quantification of Se-compounds in Se-enriched *Allium* are particularly important in order to study the anti-cancer mechanisms in detail. For this reason, new analytical techniques are necessary to gain more insight in the identification of Se-compounds (Arnault & Auger, 2006).

Large-scale gene expression analysis in combination with functional assays yields a considerable amount of information on anticarcinogenic and antimutagenic potential of garlic active components. Thus, for example, data from cDNA array studies reveal that the antiproliferative effects of DADS may be related to changes in gene expression of aggrecan 1, tenascin R, vitronectin and cadherin 5 (Knowles & Milner, 2003). Likewise, it has been recently reported that the response to garlic and its components depends on the consumer's genetic backgrounds (nutrigenetic effects), DNA methylation and histone regulation (nutritional epigenomic effects), ability to induce or repress gene expression patterns (nutritional transcriptomics effects), occurrence and activity of specific proteins (nutriproteomic effects), and/or dose and temporal changes in cellular small-molecular-weight compounds (metabolomics effects). Knowledge about each of these variables and the identification of biomarkers that can be used to predict who will and will not respond to garlic or other *allium* foods will be essential for the development of tailored strategies for reducing cancer burden and for effective intervention to occur (Milner, 2006).

ANTIOXIDANT PROPERTIES

Research studies evidence that plant-based diets, in particular those rich in vegetable and fruits, provide a great amount of antioxidant phytochemicals, such as vitamins C and E, phenolic compounds (flavonoids), vegetable pigments (antocianins and carotenoids), as well as thiols (as sulphur compounds) (Yang *et al.*, 2004; Sharma *et al.*, 2005; Dimitrios, 2006). As antioxidants, all of these are compounds able to slow down, stop or reverse oxidation of nucleic acids (DNA), proteins and lipids by scavenging oxidizing agents such as reactive oxygen species (ROS) (Wilson & Demming-Adams, 2007). These oxidation processes play an important role in aging and in a wide range of common diseases, including cancer and cardiovascular, inflammatory and neurodegenerative diseases, such as Alzheimer's disease and other age-related degenerative conditions (Borek, 1997; Gutteridge, 1993; Richardson, 1993). It has been demonstrated that endogenous levels of ROS increase during chronic infection and inflammation, strenous physical exercise, hypermetabolic states seen in stress, trauma and sepsis, and during exposure to exogenous sources of ROS such as tobacco smoke, UV light or polluted air (Borek, 2001).

Among garlic-derived products, AGE is the preparation with the highest antioxidant activity, even more than fresh garlic and other commercial garlic supplements (Imai *et al.*, 1994). This is due to its own extraction procedure, since the long-term extraction of garlic ages the extract, modifying unstable molecules with oxidant activity such as allicin (Freeman & Kodera, 1995) and increasing stable and highly bioavailable water-soluble organo-sulphur compounds content such as SAC and S-allylmercaptocysteine (SAMC), which have potent antioxidant activity (Imai *et al.*, 1994). SAC and SAMC are the major organo-sulphur compounds found in AGE, nevertheless, this garlic preparation has other compounds with antioxidant effect, such as stable lipid-soluble allyl sulphides derived from allicin (e.g. DAS, DATS, DADS and diallyl polisulphides) (Awazu & Horie, 1997; Amagase *et al.*, 2001); tetrahydro-β-carboline derivatives, which are formed during the natural aging process (Ichikawa *et al.*, 2006); flavonoids (as allixin); saponins; and essential micronutrients (selenium, Se) and macronutrients, as lectins, whose antiperoxide effect of lectins has been demonstrated in the liver, kidney and heart of rats (Rajasree *et al.*, 1999; Amagase *et al.*, 2001; Borek, 2001). Another recently identified antioxidant compounds of AGE are N-fructosyl glutamate, N-fructosyl arginine (Ryu *et al.*, 2001) (whose antioxidant activity is comparable to that of ascorbic acid) and N-fructosyl lysine (Moreno *et al.*, 2006), Amadori rearrangement products, originated during the first steps of the Maillard reaction as a result of processing and storage, mainly to high temperatures.

Phytochemicals in AGE may act in synergistic or additive way. In addition to scavenging ROS (Awazu & Horie, 1997; Borek, 2001), they exert their antioxidant action by enhancing the activities of the cellular antioxidant enzymes superoxide dismutase (SOD), catalase and glutathione peroxidase (Awazu & Horie, 1997), and increasing glutathione in the cells (Liu *et al.*, 1992), important defence mechanism in living cells, since, in addition to protecting against oxidative stress and being a cofactor for the antioxidant enzyme glutathione peroxidase, is one of the detoxification systems of the body and induces the detoxifying enzyme glutathione-S-transferase (GST). Thus, they provide additional protection to own antioxidant defences of organism against oxidant damage, decreasing the risk of injury to vital molecules and helping to prevent, thus, the onset and progression of diseases (Gutteridge, 1993; Borek, 1997). According to this, a study carried out by Kempaiah & Srinivasan (2004) showed that the sulphur compounds in garlic are effectively able to protect the endogenous thiol pool. In this study, rats were given a high-fat diet with or without garlic, and blood levels of triglycerides and thiols such as glutathione were assessed. Food intake *per se* was not affected by garlic. The high-fat diet increased the levels of blood triglycerides, decreased the levels of thiols such as glutathione and increased lipid oxidation. Authors found that all of these adverse effects of the high-fat diet were effectively reduced by regular addition of garlic to the diet. When garlic was added to the high-fat diet, total endogenous thiols increased by 16 per cent, glutathione increased by 28 per cent, and the level of catalase, which is depleted under oxidative stress, also increased.

Particularly, due to its antioxidant action, AGE decreases the risk of cardiovascular and cerebrovascular disease inhibiting the lipid peroxidation and oxidation of LDL, which play an important role in the initiation and progression of arteriosclerosis (Steinberg, 1997; Amagase *et al*., 2001; Lau, 2006). Oxidation of lipids can also cause direct effects such as destabilization of lipid membranes, e.g. of red blood cells (Yang *et al*., 2004; Kempaiah & Srinivasan, 2004). Thus, AGE protects the erythrocytes membrane against oxidative stress inhibiting the formation of abnormally dense erythrocytes, which are believed to play an important role in the clinical manifestations (painful crisis and anaemia) of sickle cell anaemia patients (Ballas & Smith, 1992). It also inhibits free radical and mutations-mediated DNA damage, decreasing, therefore, the onset and development of tumors (Borek, 1997). Moreover, AGE has radioprotective effects (Lau, 1989), protecting against ionising radiation and UV light-induced damage. Likewise, AGE limits the biosynthesis of pro-inflammatory enzymes such as inducible nitric oxide synthetase (NOS), cyclooxygenase (COX) and lipoxygenase (LOX) (Janssen-Heininger *et al*., 2000). Chronic over-production of either COX or LOX causes excess inflammation and increased endogenous levels of ROS, contributing to chronic pro-inflammatory diseases such as cardiovascular disease, diabetes, arthritis rheumatoid and others (Goodsell, 2005). COX and LOX also play physiological roles in processes such as growth, development, wound healing and senescence. The messengers produced by LOX can either stimulate or prevent the programmed cell death or apoptosis. For this, an over- production of this enzyme could give rise to an insufficient cell death, which could lead to development of cancer

(Hannun, 1997), or to an excessive cell death, which is involved in neurodegenerative diseases, such as Alzheimer's disease or dementia. The synthesis of these pro-inflammatory enzymes (COX and LOX) is regulated by gene regulatory factors (transcription factors), whose expression is, in turn, controlled by reduction (via antioxidants) and oxidation (via ROS). One of these transcription factors is nuclear factor kappa B (NF-κB), a master control gene of the immune/inflammatory response (Janssen-Heininger *et al*., 2000). Under normal conditions, NF-κB remains inactivated by another factor, its I-κB inhibitor. However, when NF-κB is stimulated, this is under insufficient levels of antioxidants, particularly sulphur-containing ones (such as those present in garlic) (Janssen-Heininger *et al*., 2000), or an excess of ROS, more COX/LOX is synthesized and inflammation is triggered. Lang *et al*. (2004) found that allicin can inhibit the production of pro-inflammatory cytokine messengers in a study of inflammatory bowel disease, apparently by inactivating the pro-inflammatory factor NF-kB via its I-κB inhibitor. By virtue of sulphur-based antioxidants found in garlic, NF-kB was maintained in its inactive state, thus preventing the synthesis of excess COX/LOX. The role of garlic in preventing age-related diseases has been also investigated extensively over the last 10-15 years. It is now accepted that aging and age-related diseases are, at least in part, caused by free radical reactions. Thus, because of its strong antioxidant properties, AGE has been suggested that it can prevent age-related chronic diseases of the cardiovascular, immune and brain systems, which can cause loss of autonomy, dependence and high social costs for individuals and society. In fact, it can inhibit the thrombus and cataract formation, improve blood circulation and energy levels, rejuvenate skin, and prevent arthritis and cancer. Moreover, other studies have demonstrated that it promotes neuronal cells survival by inhibition of the pro-inflammatory enzyme LOX and protection against oxidative damage, increasing cognitive functions, memory and longevity and slowing down age-related impairment of learning behaviour and memory (Moriguchi *et al*., 1997). However, more experimental evidence is required to confirm this last hypothesis (Sumi *et al.*, 2001). Due to this neurotrophic activity attributed to AGE, the garlic potential as natural alternative for the treatment of neurodegenerative diseases, such as Alzheimer's disease or dementia, has been recently studied (Chauhan, 2005, 2006).

IMMUNOMODULATORY ACTIVITY

Garlic has been suggested as a promising candidate for maintaining the homeostasis of immunomodulatory activity (Burger *et al*., 1993; Kyo *et al*., 2001; Lamm & Riggs, 2001). Since the immune dysfunction plays an important role in the development and progress of several diseases, modification of immune functions by garlic can contribute to their treatment and prevention.

Several studies have been carried out on animal models to examine the effect of different garlic components and formulations on immunomodulatory activity. AGE has shown to exert an anti-allergic effect (Kyo *et al*., 1997, 2001), as it may directly and/or indirectly modify the functions of mast cells, basophiles and lymphocytes, which play a leading role in the allergic cascade reactions, including inflammation.

Patya *et al.* (2004) found that multiple intraperitoneal administration of synthetic allicin elicited a marked antitumor effect in mice inoculated with B-16 melanoma and MCA-105 fibrosarcoma. They postulated that such immune-stimulatory effect of allicin was mediated by its activation of the proto-oncogene $p21^{ras}$, which has been identified as a key molecular switch involved in regulating lymphocyte activation.

The pharmacologic effect of AGE to inhibit the tumor cell growth through immune stimulation has also been described (Lamm & Riggs, 2001; Kyo *et al*., 2001). The recognized toxicity of effective therapies against cancer and the absence of toxicological effects observed for garlic treatment have made garlic a valuable alternative therapy for cancer (Lamm & Riggs, 2001).

Garlic appears to be effective for restoration of the immune suppression by different agents such as chemotherapy, UV irradiation and physical and psychological stress (Reeve *et al*., 1997; Ushijima *et al*., 1997; Kyo *et al*., 1999; Kyo *et al*., 2001;; Dwight *et al*., 2006). Age-related deterioration of learning behaviour (Zhang *et al*., 1997), and abnormal impairment of immune response, as occurs with acquired immunodeficiency syndrome (AIDS) (Lamm & Riggs, 2001), have been reported to be improved by the immunomodulatory effect of this vegetable.

The component in garlic that is responsible for the effective immune stimulation is not known conclusively, and it is likely that multiple ingredients are immunologically active. Nakata & Fujiwara (1975) identified a carbohydrate in the garlic extract that appeared to be responsible for the antitumor immunity. In a later study, Hirao *et al*. (1987) isolated a protein fraction from garlic with a clear immune-stimulating effect *in vitro*. However, these compounds are not the only active ingredients, since results of other studies suggest that several low-molecular-weight sulfur compounds from garlic such as DAS, SAC, etc have also immune-stimulating properties (Sundaram & Milner, 1996; Geng *et al*., 1997).

EFFECTS ON MICROORGANISMS

Effects of garlic on different categories of microbes are discussed in the following. In folk medicine, garlic has long been associated with the treatment of viral, bacterial, fungal, and parasitic infections. Nowadays, the antimicrobial properties of garlic have been the focus of several recent studies. It is apparent from recent chemical characterisation of their sulphur compounds that the therapeutic effects, particularly with regards to the antimicrobial properties, are due to the allicin-derived compounds (Rose *et al.*, 2005). However, some proteins, saponins and phenolic compounds can also contribute to this activity (Griffiths *et al*., 2002). Due to the great antimicrobial activity that garlic possesses, this vegetable could be used like natural preservatives, to control the microbial growth (Pszczola, 2002).

Antiviral activity

Garlic has long been stated to possess antiviral properties; however, hardly any work has been done to investigate these properties. Nagai (1973) reported *in vivo* antiviral effect of garlic in mice against intranasally-inoculated influenza virus. Garlic extract also enhaced the production of neutralizing antibody when it was inoculated with the influenza vaccine. Weber *et al*. (1992) reported the effectiveness *in vitro* of allicin and its various transformation products against Herpes Simplex Virus 1 and 2, Vesicular Stomatitis Virus, Vaccinia Virus and Parainfluenza Virus type 3. Garlic extract was effective against each virus tested, and, at the highest concentration tested (1000 mg/mL), the infectivity of each virus was substantially reduced (Weber *et al*., 1992). Moreover, garlic extract also shows *in vitro* activity against human cytomegalovirus, human rhinovirus type 2, Human Immunoeficiency Virus (HIV), viral pneumonia and rotavirus (Tsai *et al*., 1985; Meng *et al*., 1993). Allicin, ajoene, DATS, allyl methyl thiosulfinate and methyl allyl thiosulfinate have been reported to possess antiviral activity, being ajoene the most effective of them all (Hughes *et al*., 1989; Weber *et al*., 1992). In the case of HIV, it is thought that ajoene acts by inhibiting the integrin-dependent processes (Tatarintsev *et al.,* 1992) and DADS has also proven effective against HIV-infected cells (Shoji *et al*., 1993). The antiviral activities of various commercial garlic products against herpes simplex virus type 1 and parainfluenza virus type 3, including garlic powder tablets and capsules, oil-macerated garlic, steam-distilled garlic oils, garlic aged in aqueous alcohol and fermented garlic oil, have been also studied. Antiviral activities of these commercial products seem to be dependent upon their preparation process and those products with the highest levels of allicin and other thiosulfinates have the best antiviral activities (Weber *et al*., 1992).

Antibacterial activity

Garlic has been used for centuries in various societies to combat infectious diseases. Louis Pasteur (1858) and Lehmann (1930) provided the first modern scientific evidences on medicinal an antibacterial use of garlic extract. More recently, a number of studies have proven the garlic effectiveness to inhibit the growth of gram-positive, gram-negative and acid-fast bacteria, as well as toxin production.

The antibacterial activity of garlic is widely attributed to allicin. This is supported by the observation that if garlic extract is stored at room temperature its antibacterial effectiveness is greatly reduced. This reduction occurs to a much lesser extent if the extract is stored at 0-4ºC, suggesting thermal instability of the active components (Harris *et al*., 2001). Because of its relative instability and high reactivity, allicin may not have antibacterial activity *in vivo*. The allicin-derived organo-sulphur compounds such as DAS, DADS and ajoene (Naganawa *et al*., 1996), as well as other thiosulfinates isolated from oil-macerated garlic, as 2-propene-1-sulfinothioic acid *S-(Z,E)*-1-propenyl ester [AllS(O)SPn-(*Z,E*)], 2-propenesulfinothioic acid S-methyl ester [AllS(O)SMe] and metanesulfinothioic acid *S-(Z,E)*-1-propenyl ester [MeS(O)SPn-(*Z,E*)] (Yoshida *et al*., 1999), are also largely responsible for the antibacterial activity of garlic.

The antibacterial effect of garlic apparently results from thiol-disulphide exchange reactions between these sulphur compounds and free thiol groups of bacterial enzymes such as alcohol deshydrogenase, thioredoxin reductase, trypsin, other proteases and RNA and DNA polymerases (needed for the replication of the bacterial chromosomes). This disruption can affect to cell essential metabolism and, therefore, to bacterial virulence and growth (Jonkers *et al*., 1999; Bakri & Douglas, 2005).

The bacterial strain *Staphylococcus aureus* causes pus-producing infections, such as boils, as well as pneumonia and urinary tract infections (Todar, 2005). Cultures of this strain, as well as *Streptococcus* (including *S. viridans* and *S. haematyticus*), *Vibrio cholerae, Pseudomonas, Proteus vulgaris, Klebsiella pneumoniae, Salmonella enteriditis* (the bacterium responsible for salmonella food poisoning), *Mycobacterium*, *Clostridium* and *Micrococcus*, are effectively inhibited by fresh garlic, vacuum dried powdered garlic preparations and garlic oil. Garlic has been also shown to inhibit the bacterial growth of *Bacillus* (including *B. typhosus, B. dysenteriae, B. enteriditis, B. subtilis, B. megaterium, B. pumitus, B. mycoides, and B. thurigiensis*), *Sarcina lutea, Serratia marcescens amd Escherichia coli* (a common toxin-producing) (Cavallito & Bailey, 1944; Johnson & Vaughn, 1969; Delaha & Garagusi, 1985; Tsao *et al*., 2003; Zhou, 2003; Benkeblia, 2004). Chowdhury *et al.* (1991) also investigated tha ability of garlic to inhibit antibiotic-resistant strains of bacteria. They showed that garlic extract was effective *in vitro* against *Shigella dysenteriae, S. flexneri, S. sonnei* and *E. coli*, being the minimum inhibitory concentration of extract 5 μL/mL. Promising *in vivo* activity was also shown against drug-resistant *S. flexneri*. Moreover, several authors have used multiple resistant strains of bacteria to investigate antibiotic potential of garlic. They found that garlic was more effective than any of the test antibiotics (penicillin, ampicillin, doxycycline, streptomycin and cephalexin) against clinical strains of *Staphylococcus, Escherichia, Proteus*, *Pseudomonas and Klebsiella* bacteria (Bakri & Douglas, 2005; Lai & Roy, 2004). Moreover, DAS and DADS have been shown to be potent therapeutic agents for the treatment of infections originated by *S. aureus* resistant to methicilin (Tsao & Yin, 2001; Tsao *et al*., 2003) and allicin has demonstrated to exert bacteriostatic effects on some vancomycin-resistant enterococci. An inhibitory synergism was observed when used in combination with vancomycin (Jonkers *et al*., 1999). It is thought that allicin modifies the sulfydryl groups on the enzymes of the TN*1546* transposon, which encodes vancomycin resistance, enhancing susceptibility to vancomycin.

Recently, it has been reported that garlic extracts inhibits the growth of oral pathogens, concretely *Streptococcus mutans* and *S. sobrinus* and *Porphyromonas gingivalis* and *Prevotella intermedia* (gram-positive bacteria), considered as the main bacteria responsible for dental caries and adult periodontitis, respectively (Kim, 1997; Bakri & Douglas, 2005; Groppo *et al*., 2007).

The use of garlic extracts as effective agents for inhibition of the growth of *Helicobacter pylori*, which is responsible for serious gastric diseases as ulcers and even stomach cancer development, has been also proposed. Cellini *et al.* (1996) demonstrated that aqueous garlic extract effectively inhibited sixteen clinical isolates and three reference strains of *Helicobacter pylori.* The concentration of garlic extract required for 90% inhibition of the microbes was 5 mg/mL. More recently, several studies have shown that *H. pylori* could be efficiently controlled, even better than the commercial antibiotics for *H. pylori*, when ethanol and acetone were used for extraction instead of water (O'Gara *et al.*, 2000; Sivam, 2001; Canizares *et al.,* 2002, 2004). Epidemiological studies have demonstrated that allicin, allyl-methyl and methyl-allyl thiosulfinate, isolated from acetonic garlic extracts, as well as DAS and DADS can reduce the risk of gastric neoplasia induced by *H. pylori*, and inhibit the gastritis due to this bacterium (You *et al.*, 1998). Likewise, a number of studies have reported that garlic exerts a differential inhibition between beneficial intestinal microflora and potentially harmful enterobacteria (Rees *et al*., 1993). Inhibition observed in *E. coli* was more than 10 times greater than that seen in *Lactobacillus casei* for the same garlic extract dose (Skyrme, 1997). This behaviour is not clear, but may be due to a greater sensitivity of enterobacteria to allicin possibly because of the different composition and the increased permeability to allicin of their cell membrane (Miron *et al.*, 2000).

Antifungal activity

Several *in vitro* and *in vivo* studies have shown the great effectiveness of garlic against a broad spectrum of yeasts (Davis & Perrie, 2003) and fungi, including *Epidermophyton* and *Trichophyton,* two of the three filamentous fungal genera classified as dermatophytes (Schmidt & Marquardt, 1936), *Candida, Torulopsis, Cryptococcus, Rhodotorula* and *Trichosporon* (Tansey & Appleton, 1975). Likewise, Adetumbi and Lau (1986) reported that aqueous extract of dehydrated garlic preparation inhibits the growth of the dimorphic fungus *Coccidioides immitis* and *in vitro* fungal spore germination.

Aqueous extract of garlic has been successfully used in treating cryptococcal meningitis, which is caused by the fungus *Cryptococcus neoformans* (Singh & Singh, 1997). Davis *et al.* (1990) reported a significant *in vivo* response to intravenous injection of garlic extract in two patients with *C. neoformans* and three patients with other types of meningitis. In these cases, plasma titres of anti-*C. neoformans* activity rose two-fold over pre-administration titres. In a later report, Davis *et al.* (1994) investigated the use of a concentrated garlic extract that contained 34% allicin, 44% total thiosulfinates and 20% vinyldithiins. This extract displayed significant *in vitro* fungicidal and fungistatic activity against 3 different isolates of *C. neoformans*, as well as an *in vitro* synergism with amphotericin B. This *in vitro* synergistic activity of garlic with amphotericin B, one of the main antifungal drugs, was also reported by Shen *et al.* (1996) in a later study. Likewise, garlic has proven to be more effective than nystatin in retarding growth of the fungi, including *Aspergillus* and *Penicillium* (Srivastava, 1984). Moreover, aqueous extract of garlic has been also demonstrated to inhibit the growth of other zoopathogenic fungi such as *Histoplasma capsulatum*, a fungus that produces a disease similar to tuberculosis, dermatophytes that cause athletics' foot and ringworm and *Candida albicans*, commonly involved in vaginitis (Srivastava *et al.*, 1995). Venugopal and Venugopal (1995) also studied the ability of garlic to treat ringworm. They concluded that garlic could be used as an effective antidermatophytic agent, and suggested that advance extraction and purification steps could prove garlic to be as effective as standard antifungal drugs.

Such antifungal activity of garlic extracts depends on their concentration in allicin and its breakdown sulphur products such as DAS, DADS, DATS, and ajoene. Tansey and Appleton (1975) determined the activities of DATS, DATeS, DADS and DAS against three species of *Candida* and three of *Aspergillus*, which were ordered as follows: DATeS> DATS> DADS> DAS. Ajoene also possesses antifungal activity against *Aspergillus*. Reimers *et al*. (1993), studying the antifungal activity of ajoene, observed that the addition of ajoene to some fungal growth mixtures, including

Aspergillus niger, *Candida albicans* and Paracoccidiodes, resulted in inhibition at concentrations lower than that experienced with allicin, suggesting that ajoene has stronger activity than allicin. Such findings are in agreement with those obtained in an earlier study by Yoshida *et al.* (1987). Likewise, in a recent study, allicin has demonstrated to synergize the fungicidal activity of Cu^{2+} ions against various strains of fungus, by inducing Cu^{2+} complexation with a plasma membrane protein (Ogita *et al.*, 2006). Tadi *et al.* (1990), studying the antifungal activity of AGE and its major constituents, SAC and SAMC, found no *in vitro* activity. However, when AGE was administrated to infected mice, the number of organisms was reduced up to 80%.

Adetumbi *et al.* (1986) and Lemar *et al*. (2002) reported that reduction of *Candida albicans* growth by garlic extracts is due to the inhibition of lipids, proteins and nucleic acid synthesis. Active compounds of garlic have also shown to destroy fungal cells by inhibiting of succinate dehydrogenase and decreasing, thus, the oxygen uptake (Szymona, 1952), reducing the organism growth, changing the lipid profile of the cell membrane (Ghannoum, 1988) and inhibiting the synthesis of the fungal cell wall by the alilamines. These compounds inhibit the squalene monoxygenase, an enzyme involved in the formation of fungal cell wall, besides being essential for the cholesterol synthesis (Gupta & Porter, 2001).

In addition to sulphur compounds, a great variety of antifungal proteins and peptides have been isolated from several *Allium* species, such as the peptide Ace-AMP1 from onion seeds (Phillippe *et al.*, 1995), the protein allivin from bulbs of the round-cloved garlic (Wang & Ng, 2001), and chitinases from garlic, leek (*Allium porrum*) and chive (*Allium tuberosum*) (Van Damme *et al.*, 1993; Lam *et al*., 2000). Likewise, it is necessary to consider certain steroid saponins, such as eruboside-B, isolated from the garlic bulb that also exhibit antifungal activity for *Candida albicans* (Matsuura *et al*., 1988).

Therefore, garlic and its derivatives appear to meet all criteria for being considered antifungal agents, since, in addition to their effectiveness against a broad spectrum of fungi and yeast, they are cheaps and safe.

Antiparasitic activity

Literature on the antiparasitic capacity of garlic focuses mainly on protozoan parasites. African Tripanosomiasis, Amoebiasis and Giardiasis are all serius threats to humans and livestock in vast regions of Africa, South America and Asia. Due to the occurrence of unpleasant side effects and increasing resistance to the synthetic pharmaceuticals recommended for the treatment of these diseases, garlic has been investigated as a potential alternative. Results of a clinical study (Lun *et al.*, 1994) carried out on patients with tripanosomiasis, amoebiasis and giardiasis demonstrated that DATS, an allicin breakdown product, is effective against *Tripanosoma brucei* (*ssp. brucei, ssp. rhodesiense, ssp. gambiense, ssp. evansi, ssp. congolense* and *ssp. equiperdum*), *Entamoeba histolytica, Giardia lamblia* and *Giardia intestinalis*.

Moreover, several studies have demonstrated that garlic extracts are also effective against *Opalina ranarum, O. dimidicita, Balantidium entozoon, Leishmania, Leptomonas* and *Crithidia* (Reuter *et al*., 1996).

In China, DATS, easily synthesised and more stable than the extremely volatile allicin, is commercially available as a preparation, called Dasuansu, prescribed for the treatment of giardiasis (Lun *et al*., 1994) and infections by *Entamoeba histolytica* and *Trichomonas vaginalis* (Lang & Zhang, 1981). In addition, ajoene and other organo-sulphur compounds from garlic are also effective antiprotozoals.

OTHER BENEFITS

The prebiotic effect of garlic and other plant sources has recently received considerable attention (Sharma *et al.*, 2006; Mussatto & Mancilha, 2007). Fructans are non-reducing water-soluble fructooligo/polysaccharides which are naturally present in garlic and are used by garlic as a carbohydrate reserve for osmoregulation, adaptation to low temperature photosynthesis, and protection from freezing stress (Darbyshire & Henry, 1981; Chow, 2002; Fujishima *et al.*, 2005). Concentrations ranging from 125 to 235 mg/g on a wet weight basis have been reported for garlic fructans, which make up 96% of total non-structural garlic carbohydrates (Losso & Nakai, 1997).

Fructo-oligosaccharides (FOS) are fructans consisting of β (2→1) linked fructosyl units with a terminal sucrosyl moiety, which are obtained either by hydrolysis from inulin or from sucrose by transfructosylation. FOS have been described to selectively stimulate the growth and/or activity of beneficial bacteria (bifidobacteria and lactobacilli) in the colon, and thus improve host health (Ernst & Feldheim, 2000). In a study by Cardelle-Cobas *et al.* (2008), FOS with degree of polymerisation (DP) from 3 to 7 including 1-kestose, neokestose, nystose, etc were determined in commercial dehydrated garlic. The presence of FOS in garlic with a DP higher than 7 is well known (Darbyshire & Henry, 1981). Although no identification has been done, polyfructosaccharides with a DP as high as 38 or even 50 have also been described (Darbyshire & Henry, 1981; Losso & Nakai, 1997). However, highly polymerized fructans are not efficiently utilized by bifidobacteria (Losso & Nakai, 1997).

In addition to their prebiotic character, garlic FOS present other important beneficial properties to the health of consumers. They have been associated with a lower risk of infections and diarrhoea, with an improvement of the immune system response (Mussatto & Mancilha, 2007) and with a non-cariogenic effect (Yun, 1996). FOS have also been described to increase ferrum, calcium and magnesium absorption (Hidaka *et al.*, 1991) and to decrease the levels of cholesterol, phospholipids and tryglicerides in serum (Yun, 1996). As many oligosaccharides are not digested by humans because the human body lacks the enzymes required to hydrolyze the β-links formed among the units of some monosaccharides, these garlic components are suitable for use in sweet, low caloric diet foods, and for consumption by individuals with diabetes (Mussatto & Mancilha, 2007).

SAFETY

Adverse effects

Despite the extensive research supporting the numerous beneficial biological properties of garlic and garlic supplements, several papers dealing with their adverse effects and toxicity and interactions with different drugs and chemicals have also been published (Tattelman, 2005).

Garlic pungent smell, reflected in both breath and body odors, is the most common adverse effect associated with the intake of small amounts of garlic. Long-term supplementation of garlic and/or consumption of excessive amounts of this vegetable may cause other less frequent undesirable effects such as gastrointestinal upsets (indigestion, diarrhea, etc), flatulence and changes in the intestinal flora (Ackermann *et al.*, 2001). The use of certain garlic preparations such as enteric-coated garlic supplements, designed to deliver allicin (1-5 mg depending on the product label claim) directly into the intestinal tract, has also been reported to be hazardous for stomach mucosa (Hoshino *et al.*, 2001; Amagase *et al.*, 2001). The effect of several of these garlic preparations (raw garlic powder, boiled garlic powder and pulverized enteric-coated garlic product) directly delivered into the estomach, as described by Hoshino *et al*. (2001), is shown in **Figure 7.**

Allergic reactions to garlic are rare but might cause contact dermatitis, rhinoconjunctivitis, asthma, urticaria, etc in susceptible individuals (Lybarger *et al.*, 1982; Añibarro *et al.*, 1997; Asero *et al.*, 1998; Kao *et al.*, 2004). Burns and contact dermatitis are the most noted adverse effects after topical application of raw or crushed garlic (Parish *et al.*, 1987; Canduela *et al.*, 1995; Davis, 2005; Friedman *et al.*, 2006). Most of allergic symptoms are hypothesized to occur due to garlic's primary allergens: allicin, diallyl disulfide, and allylpropyl disulfide (Farrell & Staughton, 1996), being diallyl sulphide the most allergenic compound when it is topically applied.

A study on a group of workers exposed to garlic and clinically diagnosed with asthma and rhinitis, revealed IgE-mediated allergy as the cause of their occupational allergy (Añibarro *et al.*, 1997). Although very few papers try to identify allergenic proteins in garlic, a combination of proteomics and immunologic methods has been used to identify alliin lyase (a glycoprotein) as a major allergen of garlic (Kao *et al.*, 2004).

Drug and chemical interactions

Several studies have shown contradictory results related to garlic's interaction with drugs (Piscitelli *et al.*, 2002; Gallicano *et al.*, 2003). Due to its antithrombotic properties, it has been suggested that patients taking anti-clotting drugs such as Warfarin use caution when taking raw garlic or certain garlic supplements, since their anticoagulant activity may be enhanced and originate prolonged bleeding (Ackermann *et al.*, 2001). High doses of garlic should therefore be avoided prior to surgery (Burnham, 1995). However, recent clinical trials have reported the safety of aged garlic extract as a complementary therapy for several drugs, including Warfarin, Aspirin, statins (cholesterol-lowering drugs), etc (Macan *et al.*, 2006; Budoff *et al.*, 2004).

It has been reported that the intake of a garlic powder supplement reduced the blood concentrations of Saquinavir and Ritonavir, protease inhibitors used as antiviral HIV drugs, due to the stimulation of P450 isozymes (Piscitelli *et al.*, 2002; Gallicano *et al.*, 2003). Unlike garlic-powder products that contain oil-soluble sulfur compounds derived from allicin (DAS, DADS, etc), the water-soluble AGE active components neither cause P450-induced contraindications nor produce severe gastrointestinal toxicity (Amagase, 2006).

Horie *et al*. (2001) reported that AGE may protect the small intestine against the side effects (nauseas, vomits, diarrhoea, stomatitis and gastrointestinal ulceration) induced by antitumor drugs. AGE and diallyl disulfide in steam-distilled garlic oil have been shown to protect against the cardiotoxic effects and oxidative injuries caused by doxorubicin, an antineoplastic agent widely used in cancer therapy (Kojima *et al*., 1994; Awazu & Horie, 1997; Dwivedi *et al*., 1998). The utility of AGE against liver damage caused by different environmental chemicals and medicinal substances, all of them producing free radicals, has also been proved (Nakagawa *et al*., 1988; Wang *et al*., 1998).

It has been recently reported that cooking garlic with meat seems to reduce the production of carcinogenic chemicals that may occur in meat as a result of cooking methods, such as grilling, that expose meat to high temperatures (Wilson *et al*., 2005). Diallyl sulfide, the garlic phytonutrient responsible for garlic's pungency, may help prevent cancer by inhibiting the effects of one such carcinogen: 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). The production of the liver enzymes that transform PhIP into activated DNA-damaging compounds is decreased by DAS. In addition, DAS signals the genes responsible for producing two protective antioxidant enzymes (glutathione-S-transferase and superoxide dismutase), which help to protect the body against harmful compounds such as those produced from PhIP.

Dosage, administration route and formulation type

Conditions of extraction have shown to greatly affect the chemical composition of garlic preparations (Khanum *et al*., 2004) (**Figure 8**). A desirable extraction process should eliminate the toxic compounds while retaining the most active components. However, to further establish a garlic formulation as a safe and effective treatment, dosage and administration route should be taken into account.

It has been taken for granted that garlic is safe in a wide range of doses. However, several studies have reported that the use of high concentrations and/or prolonged administration of garlic may cause undesirable effects. In a study by Agusti (1996), prolonged feeding of high levels of raw garlic to rats resulted in anaemia, weight loss and failure to grow due to lysis of red blood cells. A significant loss of the normal cellular architecture of the heart, liver and kidneys after 30 days feeding of garlic homogenate at a dose of 1 g/kg/day was also reported by Banerjee *et al*. (2001, 2002). Chronic administration of garlic powder (50 mg/day) also resulted in inhibition of spermatogenesis in rats, reflecting the antiandrogenic nature of garlic (Dixit & Joshi, 1982).

However, the toxic effects of garlic may be appreciably reduced at lower concentrations. Oral dosages recommended in the literature to promote health in adults are 4 g (1-2 cloves) of raw garlic per day, one 300-mg dried garlic tablet (standardized to 1.3% alliin or 0.6% allicin) 2-3 times per day, or 7.2 g of aged garlic extract per day (Tattelman, 2005).

Although a number of researchers have shown the inhibitory effect of AGE on tumour growth in a dose related manner (Belman, 1983; Lamm & Riggs, 2001), repeated injections have been described to become toxic (Lamm & Riggs, 2001). Different outcomes depending on the administration route have also been reported by Lau *et al*. (1986), with intratumoral injections of garlic being more effective than intraperitoneal admissions for the treatment of mouse bladder tumours. Recently, a reversal of antioxidant effect has also been described with an increase in the dose of raw garlic homogenate (Banerjee *et al*., 2002).

The above mentioned dosage-dependent toxicity can not be explained fully, but it could be related with the ability of some allicin-derived sulfur compounds present in garlic to cross the cell membranes and spontaneously combine with the SH-groups of amino acids and proteins, thus interfering with the cell metabolism. In moderate amounts, human cells are not poisoned by these garlic compounds as they contain glutathione, a sulphur-containing amino-acid that combines with the allicin derivatives, preventing cell damage. However, at higher doses, interaction between garlic compounds and enzymes in the body could inhibit their activity, explaining garlic toxicity (Banerjee *et al*., 2003; Stephen, 2005).

The study of the bioavailability and metabolic fate of the active ingredients (or their metabolites) in garlic preparations is essential, since their concentration and their effects *in vitro* may not determine their effectiveness *in vivo*. Dried garlic preparations are required to be enteric coated to be effective because allicin formation is inhibited by a low gastrointestinal pH (Tattelman, 2005; Li *et al*., 2007). However, microencapsulation can give rise to a significant loss in bioactivity and, as previously mentioned, can cause gastrointestinal upsets (Hoshino *et al*., 2001).

Similarly, oil-based preparations are presumably less efficacious because of the instability of sulfur compounds in this media (Freeman & Kodera, 1995). Compounds such as allicin, sulfides, ajoene, vinyldithiins, etc have not been found in blood or urine, even after consumption of a large amount of garlic and, therefore, are likely not to be the active compounds *per se*. The instability and/or metabolism of these compounds could contribute to the inconsistent results found in several clinical studies on hypocholesterolemic effect of garlic oil and garlic powder products (Breithaupt-Grögler *et al*., 1997; Berthold *et al*., 1998).

SAC, the water-soluble organosulfur compound used to standardize AGE can be detected in plasma, liver and kidney after oral intake; its bioavailability being higher than 87% for the different animals tested (Nagae *et al*., 1994). N-acetyl-SAC, a metabolite of this compound due to the action of N-acetyltransferase, was also identified in urine. The usefulness of these compounds as adequate markers for clinical studies involving garlic is therefore proved (Steiner & Li, 2001).

With regards to processing conditions, the deactivation by heat of alliinase has questioned the therapeutic efficacy of cooked garlic. In a study with rats, Prasad *et al*. (1996) demonstrated that garlic subjected to a cooking temperature of 100ºC for 20 min preserves its bioactive compounds (sulfur compounds, dietary fibre and essential trace elements such as selenium and copper), antioxidant potential and protein profile. The decrease in the total content of antioxidants is, however, significant after heating at 100 ºC for more than 40 min.

Several studies on the effect of controlled storage of commercial dehydrated garlic samples on Maillard reaction evolution have been carried out (Cardelle-Cobas *et al*., 2005; Moreno *et al*. 2006). In general, dehydrated garlic exhibited a very slow progress of the reaction which did not lead to any noticeable change in its antioxidant activity upon storage. Therefore, processing and storage conditions should be taken into account to determine the quality and effectiveness of the different garlic products marketed.

CONCLUSION

Although used primarily today as a food flavouring agent in cooking, there is good evidence that garlic may be beneficial for a wide variety of conditions and diseases. Nowadays, the trend towards the use of natural remedies with fewer side effects has also promoted garlic consumption as an alternative therapy for certain diseases. However, before garlic can be considered as a safe and effective therapy, further research into several questions is required.

Despite garlic cloves are usually eaten raw or cooked, different garlic dietary supplements including dried or powdered formulations, oils and liquid extracts have been recently incorporated into the market to satisfy the demand of consumer for garlic bioactive compounds. However, it is worth noting that these components are highly dependent on the garlic preparation and, therefore, no single garlic dietary supplement may cover the wide range of biological activities here reported (**Figure 9**). Furthermore, several aspects such as garlic variety, growing location, manufacturing processing and storage conditions, etc may also affect the content of garlic active components, their stability and health benefits.

Future research should also be done to standardize the content of active compounds in garlic supplements. This would help to establish the effective dosage and type of garlic (dehydrated, aged, etc) most appropriate for the health-promoting effect wanted.

The search for active preparations with undesirable pungent odour and taste kept at a minimum would allow the use of this vegetable and its derivatives as functional ingredients with therapeutic function in many processed foods. For instance, they could be employed in the manufacturing of highly consumed products (e.g. fast foods or ready-to-eat foods) with the aim of providing them with antioxidants, prebiotics, mineral nutrients, etc of usefulness in the prevention of nutritional deficiencies.

Garlic has been extensively studied *in vitro* and *in vivo* using animal models. However, human clinical trials are scarce and they are often of short duration and including a small number of patients (Fleischauer & Arab, 2001). Therefore, there is a need to gain reliable scientific credibility based on well designed trials of the actual and potential health benefits ascribed to standardized preparations of garlic with known active components (Tattelman, 2005).

Finally, garlic products are marketed both as foodstuffs and as herbal medicinal products. Whereas garlic consumption is generally accepted as safe, the lack of toxicity of garlic supplements is now to be guaranteed prior to their use as bioactive products. At the current time, a standardized regulation of nutrition and health claims on foods is being introduced with the purpose of defining a set of generally applicable criteria for the scientific substantiation of these claims (Asp & Bryngelsson, 2008). This would assure that the consumer benefits without risk from all the nutritional and health-promoting effects of an old natural remedy: garlic.

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