

# GARLIC SULFUR COMPOUNDS SUPPRESS CANCEROGENESIS AND OXIDATIVE STRESS: A REVIEW\*

M. Dvořáková, I. Weingartová, J. Nevoral, D. Němeček, T. Krejčová

*University of Life Sciences Prague, Faculty of Agrobiolgy, Food and Natural Resources, Prague, Czech Republic*

Garlic has long been considered a food with many health benefits. Several studies have confirmed that sulfur compounds are responsible for the positive effects of garlic on organisms. Garlic acts as an antioxidant by increasing antioxidant enzyme activity, reducing reactive oxygen species generation, and protecting proteins and lipids from oxidation. Garlic suppresses carcinogenesis through several mechanisms: (1) it reduces oxidative stress, and therefore, prevents damage to DNA; (2) it induces apoptosis or cell cycle arrest in cancer cells; and (3) it modifies gene expression through histone acetylation. The positive effects of garlic could be mediated by several mechanisms. It influences signalling pathways of gasotransmitters such as hydrogen sulfide. Garlic enhances hydrogen sulfide production both through its direct release and through an increase in activity of enzymes which produce hydrogen sulfide. Hydrogen sulfide acts as a signalling molecule in various tissues and participates in the regulation of many physiological processes. We can presume that garlic, which is able to release hydrogen sulfide, exhibits effects similar to those of this gasotransmitter.

cancer; diallyl disulfide; hydrogen sulfide; reactive oxygen species S-allyl cysteine



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## INTRODUCTION

The first statements about the beneficial health effects of garlic originate from 2600–2100 BC. Ancient medical books from Greece, Rome, Egypt, India, and China recommended garlic consumption as a way to improve health (G o r i n s t e i n et al., 2007).

Garlic stimulates the immune system; it has antibacterial, antiviral, antiparasitic (I c i e k et al., 2009), hepatoprotective (B a n e r j e e et al., 2003), and neuroprotective properties (B o r r e l l i et al., 2007), and decreases oxidative stress in cells. Garlic reduces the proliferation of some kinds of cancer cells (B a n e r j e e et al., 2003). It lowers blood cholesterol and triacylglycerol levels, reduces blood pressure, and prevents the development of atherosclerosis. It impairs thromboxane synthesis and therefore acts as an anticoagulant (I c i e k et al., 2009), and also decreases plasma glucose levels, which in turn prevents the development of diabetes mellitus (B a n e r j e e et al., 2003).

## Biosynthesis and metabolism of biologically active compounds from garlic

Garlic contains several sulfur compounds, which are known for their positive physiological effects. Interestingly, the effects of garlic differ depending on whether crushed fresh garlic or aged garlic extract are applied.

Whole garlic bulbs contain gamma-glutamyl cysteine, which undergoes two important reactions: (1) through hydrolysis and oxidation it is converted into S-alkenyl sulfoxides, which include alliin (S-allyl cysteine sulfoxide); (2) through gamma-glutamyl transpeptidase activity it is converted into S-allyl cysteine (SAC). This second reaction occurs during the long-term extraction of the garlic bulb (C o r z o - M a r t i n e z et al., 2007).

Fresh garlic extract is formed when garlic bulbs are crushed. Alliin is converted into sulfonic acid, pyruvate, and ammonia during the process (A m a g a s e ,

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2006). The reaction is conditioned by the presence of vacuolar enzyme alliinase. Whole alliin has been detected in the stomach, intestine, and liver of mice after its consumption without any further conversion (E g e n s c h w i n d et al., 1992; L a c h m a n n et al., 1994). The optimal pH level for alliinase activity is 6.5; levels under 3.6 are typical for the stomach environment, in which alliinase is ineffective. Alliinase activity is completely and irreversibly inhibited in stomach acid (L a w s o n , H u g h e s , 1992). Heat-processed garlic contains mainly alliin because alliinase is destroyed at temperatures of 60°C and higher.

Sulfonic acid, created from alliin through alliinase activity, is highly reactive and therefore it undergoes condensation, creating allicin (diallyl thiosulfinate) molecules (L a n z o t t i , 2006). Allicin is a volatile and highly unstable compound. It is instantly decomposed into oil-soluble diallyl monosulfide (DAS) and polysulfides, mainly diallyl disulfide (DADS) and diallyl trisulfide (DATS). Polysulfides are compounds likely to be responsible for the positive biological effects of garlic (F r e e m a n , K o d e r a , 1995).

Allicin is also converted into ajoene, a compound considered responsible for the anticoagulant effects of garlic (A p i t z c a s t r o et al., 1983; B l o c k et al., 1984).

Besides fresh garlic extract produced through the crushing of garlic bulbs, garlic could be processed through long-term extraction in 15–20% ethanol, which produces aged garlic extract (AGE). AGE contains less allicin in comparison with fresh garlic. Other compounds are generated during long-term extraction; gamma-glutamyl cysteine from intact garlic bulbs is converted into S-allyl cysteine (SAC) (A m a g a s e , 2006; C o l i n - G o n z a l e z et al., 2012). SAC has been detected in blood after AGE consumption in a dose-dependent manner (R o s e n et al., 2001; S t e i n e r , L i , 2001).

Sulfur compounds from AGE are stable and odorless; they have a milder, less specific flavour. They exhibit greater and more stable positive effects within the organism. They are safer in comparison with raw garlic (C o r z o - M a r t i n e z et al., 2007). Daily consumption of AGE at a dose of 1.8–10 g increases immune response in humans without any toxic side-effects (A m a g a s e , 2006)

The metabolism of garlic compounds has yet to be fully understood. Garlic sulfur compound levels in blood after garlic consumption are good markers of garlic metabolism.

#### **Garlic protects cells from oxidative stress**

Oxidative stress is caused by an imbalance between free radical generation and endogenous antioxidative activity (S t e a r e , Y e l l o n , 1995). DNA, protein and lipid oxidation through reactive oxygen species (ROS)

plays an important role in the ageing and development of many diseases. Endogenous antioxidants prevent cell damage through ROS. Reduced glutathione, superoxide dismutase, catalase, and glutathione peroxidase are among the most important endogenous antioxidants (B a n e r j e e et al., 2003).

Regular consumption of garlic significantly increases cell antioxidative activity (B a n e r j e e et al., 2002). Garlic prevents ROS generation and protects the mitochondrial membrane from oxidative stress, which alters membrane potential (C e r v a n t e s et al., 2013).

Alliin scavenges free radicals, protects lipids from oxidation, and increases antioxidative enzyme activity (B a n e r j e e et al., 2003).

Fresh garlic extract scavenges free radicals in a dose-dependent manner (P r a s a d et al., 1996), and therefore it protects brain and heart cells from oxidative stress-induced ischemia (B a t i r e l et al., 1996; B a n e r j e e et al., 2003). Fresh garlic reduces lipid peroxidation in the heart, liver, and kidneys (B a n e r j e e et al., 2001, 2002) and low-density lipoprotein (LDL) oxidation (L a u , 2001).

Allicin is the major compound of fresh garlic extract, however it is highly unstable. It reacts with cysteine and glutathione (R a b i n k o v et al., 2000), and creates substances which have antioxidative effects. Allicin and its derivatives can influence the activity of proteins through S-thiolation, which is one of many cell antioxidative mechanisms (P i n t o et al., 2006). Allicin binds LDL, and therefore prevents LDL oxidation in blood-vessel walls (G o n e n et al., 2005).

DAS and polysulfides increase the activity of antioxidative enzymes, and therefore protect cells from oxidative stress (F u k a o et al., 2004).

AGE exhibits the highest antioxidative activity. It prevents damage to DNA by free radicals and therefore protects cells from cancer development (B o r e k , 2001). AGE reduces the risk of cardiovascular and cerebrovascular diseases (L a u et al., 1987). It prevents damage to cell membranes and lipid peroxidation in pulmonary endothelial cells subjected to oxidized LDL. AGE reduces hydrogen peroxide and superoxide production. It increases the activity of superoxide dismutase and glutathione peroxidase in pulmonary endothelial cells (I d e et al., 1997).

The antioxidative effects of garlic can be mediated by nitric oxide (NO) production. SAC, the main compound of AGE, regulates NO production by two different pathways:

Oxidative stress triggers nuclear factor kappa (NFκB) activity, which is involved in the expression of proinflammatory enzymes such as inducible nitric oxide synthase, producing NO in cells. SAC inhibits NFκB activity, and therefore it exhibits an antiinflammatory effect (I d e , L a u , 2001).

However, SAC can enhance NO production in cell through the activation of calcium-dependent nitric

oxide synthase. NO inhibits hydrogen peroxide and superoxide production. SAC increases NO production in endothelial cells, and therefore enhances blood-vessel elasticity and protects them from inflammation and development of atherosclerosis (Das et al., 1995; Sooranna et al., 1995).

#### Garlic inhibits carcinogenesis

Garlic inhibits the growth of some tumors and cell proliferation. DAS and DATS suppress benzo(a)pyren-induced development of stomach cancer in mice (Sparnins et al., 1988). DADS prevents intestine and kidney cancer in carcinogen-treated mice (Takahashi et al., 1992). Other studies confirm that garlic monosulfides and polysulfides are effective against *in vitro*-induced carcinogenesis (Wargovich et al., 1988; Schaffer et al., 1996; Suzui et al., 1997). Consumption of AGE at a dose of 2.4 ml daily for 12 months reduces the size and number of colorectal adenomas in humans (Tanaka et al., 2006). Garlic has an antiproliferative effect on human cancer cells in transgenic animal models (Singh et al., 1996; Sundaram, Milner, 1996; Xiao et al., 2006a).

Considering the toxic effects of garlic sulfur compounds on cancer cells, it is necessary to investigate their potential toxic effects on normal cells. Certain studies demonstrate a higher tolerance of normal cells against the cytotoxic effects of garlic (Karmakar et al., 2007; Kim et al., 2007). However, other studies show toxic effects of garlic compounds on normal cells, especially those of the gastrointestinal tract (Joseph et al., 1989; Banerjee et al., 2003).

The anticarcinogenic effects of garlic compounds can be mediated by several mechanisms. Garlic induces an immune response in the organism (Lamm, Riggs, 2001). Garlic compounds prevent oxidative cell damage, and inhibit cell proliferation by induction of apoptosis or cell cycle arrest (Percelle et al., 1990). They enhance the activity of detoxification enzymes, which improve excretion of carcinogens (Guyonnet et al., 1999), and suppress the activity of P450 enzymes, which mediate the activation of procarcinogens (Dion, Milner, 1997). Garlic has anticlastogenic effects and it contributes to repairing damaged DNA (Khanum et al., 2004). It influences gene expression through post-translational modifications, and therefore alters the activity of cell cycle regulating proteins (Druesne-Pecollo et al., 2007). It also suppresses blood supply to tumors. AGE prevents proliferation of endothelial cells and enhances their adhesion to collagen and fibronectin, which in turn reduces their mobility. AGE inhibits blood supply to human colorectal carcinoma through this mechanism (Matsura et al., 2006). Alliin and DATS suppress angiogenesis by reducing vascular endothelial growth factor (VEGF) secretion (Mousa, Mousa, 2005).

#### Garlic induces apoptosis in cancer cells

Garlic polysulfides induce oxidative stress in cancer cells through increased production of ROS. Enhanced oxidative stress triggers apoptotic signalling pathway. Cancer cells are especially sensitive to garlic compounds, since they possess a small amount of molecules with antioxidative properties (Filomeni et al., 2003; Xiao et al., 2004).

DADS enhances ROS generation and therefore activates the c-Jun N-terminal kinase (JNK) pathway, which triggers cell death in neuroblastoma cells (Filomeni et al., 2003). DADS increases the expression of proapoptotic factors and reduces the expression of antiapoptotic factors in breast cancer cells (Nakagawa et al., 2001). It enhances intracellular levels of calcium ions, which in turn increases hydrogen peroxide production and activates caspases in human leukemic cells (Park et al., 2002).

DATS is more effective in the induction of apoptosis in human prostate cancer cells in comparison with DAS and DADS (Xiao et al., 2004). It degrades ferritin and therefore enhances cellular labile iron, which is in turn followed by ROS generation (Antosiewicz et al., 2006). It hyperphosphorylates and inactivates antiapoptotic factors through JNK and extracellular signal-regulated kinase 1/2 (ERK 1/2) signalling pathways (Xiao et al., 2004). It causes conformational changes of proapoptotic factors, which lead to their transfer into mitochondria (Kim et al., 2007).

Ajoene activates NFκB, stimulates ROS generation, induces apoptosis and cell cycle arrest in the G2 phase (Xu et al., 2004), and decreases intracellular levels of antiapoptotic factors (Li et al., 2002).

#### Garlic induces cell cycle arrest in cancer cells

A large amount of studies have confirmed the ability of garlic to induce cell cycle arrest, which could be a mechanism of cancerogenesis inhibition.

Garlic polysulfides inhibit cyclin-dependent kinase 1 (Cdk1) and enhance cyclin B1 expression in colon cancer cells (Knowles, Milner, 2000). They reduce Cdk-activating kinase activity in liver cancer cells (Wu et al., 2004) and Cdc25 activity in prostate cancer cells (Arunkumar et al., 2006). They activate mitogen-activated protein kinase (MAPK) p38, which reduces Cdc25 phosphatase activity in stomach cancer cells (Yuan et al., 2004). DATS is more effective in the induction of cell cycle arrest in the liver (Wu et al., 2004) and prostate cancer cells in comparison with DAS and DADS. Normal epithelial prostate cells are more resistant to the toxic effect of DADS than cancer cells (Xiao et al., 2005).

Garlic polysulfides can induce cell cycle arrest in the S phase, G2 phase or prometaphase. Cell cycle arrest in G2 is caused by a reduction in the cdc25 activity (Xiao et al., 2005). Cell cycle arrest in the

prometaphase is connected with hypermethylation of key subunits of the anaphase-promoting complex/cyclosome (APC/C) (Herman-Antosiewicz et al., 2007).

Garlic induces microtubule depolymerization and changes in cytoskeleton in interphase cancer cells and disrupts mitotic spindle formation in mitosis (Hosono et al., 2005; Zhang et al., 2006). Garlic polysulfides initiate changes in the microtubule network, chromatin condensation, and phosphorylation of histone H3 in the M phase (Herman-Antosiewicz, Singh, 2005). DATS induces oxidative changes in beta-tubulin cysteine residues *in vitro* (Hosono et al., 2005).

#### **Garlic regulates gene expression through histone acetylation**

Garlic compounds mediate histone acetylation through an increase of histone acetylase activity and a reduction of histone deacetylase activity, and can therefore alter gene expression. Histone modification influences the expression of proteins in cancer cells and expression of proteins involved in cell cycle regulation.

DADS induces histone acetylation in human breast cancer cells and rat liver cancer cells through a reduction of histone deacetylase activity (Lea et al., 1999), and affects the activity of cell cycle regulating proteins, such as p21 kinase, MAPK3, inhibitors of DNA-binding proteins, and proteins involved in DNA repair through histone modification (Druesne-Pecollo et al., 2007). DADS enhances histone H3 and H4 acetylation in human leukemic cells and inhibits histone deacetylases in liver and breast cancer cells (Lea et al., 1999). Allicin and SAC enhance histone acetylation in breast and intestine cancer cells (Lea et al., 2002).

#### **Garlic influences cell signalization through hydrogen sulfide production**

Hydrogen sulfide is well known as a toxic gas. In recent times, its physiological function as a gaseous signalling molecule, a gasotransmitter within the organism, has been discovered (Wang, 2002). Hydrogen sulfide is a signalling molecule in the nervous (Abe, Kimura, 1996), cardiovascular (Zhao et al., 2001) and reproductive systems (Srilatha et al., 2007). Cystathionine-gamma-lyase (CSE) and cystathionine-beta-synthase (CBS) are enzymes synthesizing hydrogen sulfide within the organism (Wang, 2002).

Positive garlic effects can be mediated through hydrogen sulfide production. Human red blood cells produce hydrogen sulfide in anoxic conditions from garlic extract, allicin, DADS, and DATS in the presence of glutathione. The highest amount of hydrogen sulfide is generated from DATS. Addition of DADS into blood enhances amounts of exhaled hydrogen sulfide in rats (Insko et al., 2009). DADS and DATS

vasodilate blood-vessels through hydrogen sulfide production (Benavides et al., 2007).

Hydrogen sulfide can be generated from cysteine derivatives through the activity of cystathionine gamma-lyase (CSE) (Wang et al., 2010). One of these cysteine derivatives, SAC, can act as a direct substrate for hydrogen sulfide production, as well as a compound regulating CSE activity. SAC enhances CSE activity, and therefore protects heart cells against infarction (Chuah et al., 2007). S-propargyl cysteine (SPRC), a structural analog of SAC, enhances CSE expression. Created hydrogen sulfide enhances p53 and Bax expression. SPRC reduces the viability and division of cancer cells through this mechanism (Ma et al., 2011).

#### **CONCLUSION**

Sulfur compounds from garlic have a significant positive effect on organisms. The method of processing is a key factor determining the biological activity of garlic. Garlic could be administered as a fresh or aged garlic extract (AGE). Fresh garlic extract possesses both prooxidative and antioxidative properties, which depends on a type of treated tissue. Cancer represents one of the most investigated diseases in the developed world. Cancer cells exhibit high sensitivity to oxidative stress-induced apoptosis mediated by garlic compounds when compared to healthy cells. Many currently widespread diseases, such as cardiovascular diseases, are caused by high content of reactive oxygen species in the environment which could be balanced by garlic compounds, especially those contained in aged garlic extract. Hydrogen sulfide is a gaseous molecule exhibiting antioxidative activity. It stabilizes mitochondrial membranes, and therefore protects cells against apoptosis. Garlic sulfur compounds are able to release hydrogen sulfide, which could represent their mechanism of action. This presumption should be approved more thoroughly using different types of tissues for wider implication of garlic health benefits.

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#### Abbreviations:

AGE = aged garlic extract, CSE = cystathionine-gamma-lyase, DADS = diallyl disulfide, DAS = diallyl monosulfide, DATS = diallyl trisulfide, ERK = extracellular signal-regulated kinase, JNK = c-Jun N-terminal kinase, LDL = low-density lipoprotein, MAPK = mitogen-activated protein kinase, NFκB = nuclear factor kappa, ROS = reactive oxygen species, SAC = S-allyl cysteine, SPRC = S-propargyl cysteine, VEGF = vascular endothelial growth factor

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#### Corresponding Author:

Ing. Markéta Dvořáková, Czech University of Life Sciences Prague, Faculty of Agrobiolgy, Food and Natural Resources, Department of Veterinary Sciences, Kamýcká 129, 165 21 Prague 6-Suchbát, Czech Republic, phone: +420 224 282 932, e-mail: dvorakova2@af.czu.cz

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