

Aged Garlic Extract™

*Research Excerpts from Peer Reviewed
Scientific Journals & Scientific Meetings*

**Wakunaga of America Co., Ltd.
23501 Madero
Mission Viejo, CA 92691 USA**

**Phone: (949) 855-2776
Fax: (949) 458-2764
1-800-421-2998 (USA)**

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AGED GARLIC EXTRACT

Kyolic® Aged Garlic Extract™ is truly the only odorless garlic supplement in the market. The production of Kyolic Aged Garlic Extract begins with garlic grown on Wakunaga farms under strictly controlled organic conditions without the use of chemical fertilizers, herbicides or pesticides. Wakunaga's garlic crop is cultivated with time-tested procedures to enhance its beneficial constituents. Quality control begins when the harvested raw garlic is tested for conformity to specified quality standards. Only the finest garlic is used for the production of Kyolic. The unique aging process applied in manufacturing Aged Garlic Extract distinguishes Kyolic from other garlic products available on the market. First, the garlic cloves are cleaned and sliced. Then, under carefully controlled conditions, the sliced garlic is stored in an aqueous ethanol solution in stainless steel tank and naturally aged, without heating, for up to 20 months. Through this unique process, the harsh and unstable organosulfur compounds are converted into mild and effective compounds including the sulfur-containing amino acids (e.g. S-allylcysteine, S-allylmercaptocysteine) and Maillard reaction products that are responsible for Kyolic's health benefits, and the extract is reported to increase the antioxidant effects during the natural aging process. This conversion eliminates odor-causing components, resulting in the truly odorless Kyolic Aged Garlic Extract that contains safe, stable, bioavailable and beneficial compounds. Kyolic Aged Garlic Extract is manufactured and quality controlled under International Standard Organization (ISO) 9001 and Good Manufacturing Practices (GMP) as detailed in Part 211 of Title 21 of The Code of Federal Regulations. Aged Garlic Extract also complies with the specifications established in the US Pharmacopeia/National Formulary (USP/NF) monograph. Aged Garlic Extract, and its various constituents, has been the subject of almost 600 scientific studies around the world including Japan, the United States and Europe since its development in 1955. The following are short summaries of published data, which confirm the safety and effectiveness of this unique garlic preparation.

PHARMACOLOGICAL PROPERTIES OF AGED GARLIC EXTRACT

Cardioprotective Effects Seen in Clinical Studies

The following clinical studies have shown the reduction of cardiovascular disease risk factors including reduction of blood lipids or fats, such as serum cholesterol, and triglycerides, inhibition of platelet aggregation or blood-thinning effects, enhancement of circulation and reduction in blood pressure following the intake of Aged Garlic Extract:

Inhibits Plaque Formation in the Coronary Artery

A randomized double-blind placebo controlled pilot clinical study has shown significant inhibition of plaque formation in the coronary artery of heart surgery patients by consuming 4ml of Kyolic® Aged Garlic Extract™ a day, for about a year. Approximately 65% of significant reduction in plaque formation was observed in the Kyolic group compared to placebo. Blood homocysteine and LDL have a tendency to reduce via Kyolic supplementation as well. In addition, HDL has a tendency to increase. Thus, Kyolic AGE may be very beneficial and useful for prevention of stopping blood flow to the heart, especially in high-risk people including heart surgery patients.

*Budoff, M., Takasu, J. et al.
FASEB J. 2003, April 15, 2003,
San Diego.*

Budoff et al. found in their one year, placebo-controlled, double-blind, randomized pilot study that patients with known coronary artery disease who were given 4 ml of Kyolic® Aged Garlic Extract™ for one year have a significantly lower calcium score (mean change: $7.5 \pm 9.4\%$) than the placebo group ($22.2 \pm 18.5\%$). This pilot study indicates the potential ability of AGE to inhibit the rate of progression of coronary calcification.

*Budoff, M., Takasu, J. et al. 2004.
Prev. Med. 39(5): 985-91.*

*Budoff, M. et al. FASEB J. April
2003, San Diego.*

Using electron beam computed tomography (EBCT) in a placebo-controlled, double-blind, randomized pilot study, aged garlic extract (AGE) has been shown to inhibit coronary plaque formation by 7.5% in patients on statin therapy.

*Budoff M. Aged Garlic Extract
Retards Progression of Coronary
Artery Calcification. J. Nutr.
2006;136 Suppl 3:S741-4.*

A randomized, double-blind, placebo-controlled clinical study has shown more than 65% significant inhibition of plaque formation of cardiac patients consuming 4ml of Kyolic® Aged Garlic Extract™ a day for over a year compared to the placebo. Significant reductions of blood pressure, platelet aggregation, LDL oxidation and homocysteine level were also observed in the Kyolic AGE group in many other clinical studies, which was done in a same -blind, placebo-controlled manner. This suggests that Kyolic AGE may be useful and beneficial in the treatment and prevention of cardiac atherosclerosis through multiple risk reduction mechanisms.

*Amagase, H., Budoff, M. et al.
FASEB J. April 2004, 18(6):
600: 6.*

*Amagase, H., Budoff M. and
Rosen, R.T. ICNPR. July 31-
August 4, 2004.*

Lipid Lowering Effects

- Lau, B., Lam, F. et al. 1987. *Nutr. Res.* 7:139-149.
- Lau et al. (1987) administered four capsules of Kyolic® Aged Garlic Extract liquid daily to subjects with elevated levels of cholesterol (220-440 mg/dl) while another group received a placebo indistinguishable from the Kyolic capsules. Serum levels of cholesterol and triglycerides in the Kyolic-taking group significantly dropped, while the placebo group showed no change. After six months, 11 of 15 subjects or 73% experienced a significant reduction in serum cholesterol compared to baseline (\downarrow 12-31%), while 10 of 15 subjects experienced drops of greater than 10% in triglycerides. Moreover, subjects' serum levels of LDL cholesterol, a major risk factor for heart disease, were decreased by Kyolic supplementation. Concurrently, an increase in HDL cholesterol, which is known to protect against heart disease, was observed in the Kyolic-taking group.
- Silagy, C. and Neil, A. 1994. *J. Royal Coll. Physic. Lond.* 28(1): 39-45.
- Lau's paper (1987) met the stringent criteria established for the meta-analysis of various clinical studies conducted by Silagy et al. (1994) to determine the effects of garlic on cholesterol. Pooling Lau's data in with a total of 16 trials including more than 900 subjects confirmed that garlic-treated subjects experienced a 12% reduction in cholesterol beyond the effect of placebos alone.
- Warshafsky, S., Kamer, R. et al. 1993. *Ann. Intern. Med.* 119: 599-605.
- Another meta-analysis conducted by Warshafsky et al. (1993) included Lau's paper (1987) in the pooled data and found a 9% reduction in cholesterol from garlic intake.
- Kawashima, Y., Ochiai, Y. et al. 1989. *Shinryou To Shinyaku (Treat. New Med.)* 26: 377-388.
- After taking four capsules of a Kyolic® Liquid Aged Garlic Extract preparation containing vitamin B₁, vitamin B₁₂ and liver extract, each day for 16 weeks, a 9% reduction in serum cholesterol (234±10 mg/dl → 213±10 mg/dl), a 17% reduction in triglycerides (229±20 mg/dl → 190±16 mg/dl) and a 9% reduction in LDL cholesterol (152±10 mg/dl → 138±10 mg/dl) were seen in 19 patients studied by Kawashima et al. (1989).
- Yeh, Y., Lin, R. et al. 1995. *J. Am. Coll. Nutr.* 13:545
- In a five-month, double-blind, randomized, placebo-controlled intervention study of men with high cholesterol (241±21 mg/dl) by Yeh et al. (1995, 1997), Aged Garlic Extract was found to reduce total plasma and LDL-cholesterol by 7% and 10%, respectively. During the study, the men maintained their normal diets.
- Yeh, Y., Lin, Ret al. 1997. In *Food Factors for Cancer Prevention*. Ohigashi, et al. eds., p 226-230. Springer-Verlag, Tokyo.
- Steiner, M., Lin, R. et al. 1996a. *Shinyaku To Rinsho (New Drug Clin.)* 45(3): 456-466.
- Steiner et al. (1994, 1996a) in a six-month, double-blind, crossover study found that aged garlic extract powder supplementation led to a modest reduction in LDL cholesterol (8%) in free-living men with high cholesterol levels (230-290 mg/dl) on a lowfat/low cholesterol diet. No change in HDL was noted. In participants, who had good compliance in their regimes, triglycerides were significantly reduced 18.9% compared to baseline and 6.7% compared to placebo.
- Steiner, M., and Lin, R. 1994. *J. Amer. Coll. Nutr* 13(5): 524.
- In this double-blind, crossover study of hyperlipidemic patients (cholesterol 220-290 mg/dl) on the NCEP¹ Step I Diet, Steiner et al. (1996b) found a 6-7% reduction in total serum cholesterol and a 4-5% reduction in LDL cholesterol following six month oral intake of Aged Garlic Extract powder.
- Lee, YY, YY Yeh. 2003. *FASEB J* 17(4): A752, # 455.1
- The lipid-lowering effect of garlic in humans is explored via the effects of aged garlic extract and water-soluble sulfur containing compounds of garlic in HepG-2 cells, a human hepatoma cell line. Kyolic contains S-allyl cysteine (SAC), S-ethyl cysteine (SEC), and S-propyl cysteine (SPC) and gamma glutamyl S-alk(en)yl cysteines. Kyolic inhibits cholesterol synthesis due to these water-soluble organosulfur compounds. The results of this study show that the maximal inhibition on cholesterol synthesis by garlic requires a concerted action of these various compounds in HepG-2 cells.
- Allison GL, Lowe GM, Rahman K. *Aged Garlic Extract and Its Constituents Inhibit Platelet Aggregation through Multiple Mechanisms. J. Nutr.* 2006;136 Suppl 3:S782-8.
- Aged Garlic Extract (AGE) and its constituents inhibits platelet aggregation in a concentration-dependent manner by working in a synergistically and exerting multiple effects on biochemical pathways. Calcium movement from/into the cells will be the key mechanism.
- Thomson M. et al. 2006. *J Nutr.* 136(3S): 800S-802S
- In a study by Thomson et al. (2006), raw and boiled aqueous extracts of garlic were administered to subjects orally and intraperitoneally for 4 weeks. A significant reduction in the cholesterol level (11-14%) of subjects was observed in the group that received a low dose of raw aqueous extract of garlic. A significant reduction in triglyceride levels (38%) was also noted in subjects who received garlic orally and intraperitoneally. Glucose, cholesterol, and triglyceride levels were also significantly reduced in subjects treated with a high dose of raw garlic.

Circulation-Enhancing/Blood-Thinning Clinical Effects

Overview of Cardioprotective Effects of Aged Garlic Extract Found in Various Clinical Studies

Anemia (RBC, Hemoglobin) ¹	↓ in 61% of patients
Blood Pressure ^{2,3}	↓ 6-9%
Chest Pain ⁴	↓ in 90% of patients
Chill (limbs) ⁴⁻⁸	↓ in 40-100% of patients
Cholesterol Oxidation ^{9,17, 19, 25}	↓ 38%
Circulation (Hands/Feet) ^{8,10}	↑ in 67% of patients
Dizziness ^{4,5,7,8}	↓ in 50-85% of patients
Endothelial Function ^{22,23}	↑ in 44-66% of patients
Headaches ^{4,5,7,8}	↓ in 50-80% of patients
Homocysteine ²²	↓ in 27% of patients
Heart Palpitations ^{1,4,5,7,8}	↓ in 45-75% of patients
LDL Cholesterol ^{2,11-13}	↓ 5-12%
Numbness in Limbs ^{5,7}	↓ in 40-80% of patients
Oxidative Stress by Smoking ²⁴	↓ in 290-37% of patients
Platelet Aggregation ^{9,12, 20}	↓ 10-25%
Platelet Adhesion ^{9,12}	↓ 30-58%
Plaque formation in artery ²¹	↓ 67%
Safety with Warfarin ^{14, 26}	Confirmed
Shortness of Breath ^{5,7}	↓ in 45-80% of patients
Sickle Cell Anemia ^{15, 18,20}	↓ dense rbc by 30% ↓ Heinz bodies by 30%
Total Serum Cholesterol ^{2,3,7,11-13,16,17}	↓ 6-31% ⁺
Triglycerides ^{3,4,11}	↓ 10-19%

- Hasegawa Y, et al. 1983. Japanese J. New Remedies Clinic 32:365-76.
- Steiner M, et al. 1996. Am J Clin Nutr. 64:866-70.
- Steiner M, et al. 1996. Shinyaku to Rinsho. 45(3):456-66.
- Kawashima H, et al. 1985. Shinryou to Shinyaku. (Treatment and New Medicine). 22:3012-24.
- Miyoshi A, et al. 1984. Shinryou to Shinyaku. 21:1806-20.
- Kohno M, et al. 1976. Yakuri to Chiryō 4:700-8.
- Hasegawa Y, et al. 1984. Shinryou to Shinyaku. (New Drug Clin.). 21:2021-35.
- Kikuchi T, et al. 1994. Shinyaku to Rinsho. (Jpn J New Remedies Clin). 43(1):146-58.
- Steiner M, et al. 1998. J Cardiovascular Pharmacol. 31:904-8.
- Okuhara T. 1994. Jpn Pharmacol Therapeut. 22(8):3695-3701.
- Lau B, et al. 1987. Nutr Res. 7:139-49.
- Steiner M, et al. 1994. J Am Coll Nutr. 13(5):524.
- Yeh Y, et al. 1995. J Am Coll Nutr. 14(5):545.
- Rozenfeld V, et al. 1999. 18th Annual Eastern States Conference for Pharmacy Residents and Preceptors. Baltimore, MD. April 21-24.
- Ohnishi ST, Ohnishi T, et al. 2000. Nutrition. 16:330-8.
- Yeh Y, et al. 1997. In Food Factors for Cancer Prevention.
- Kawashima Y, et al. 1989. Shinryou to Shinyaku. 26:377-88.
- Munday J, et al. 1999. Atherosclerosis. 143:399-04.
- Takasu J, et al. 2002. BMC Blood Disorders. 2:3.
- Lau BHS, 2001. J. Nutr. 131:985S-8S
- Rahman K, Billington D. 2000. J. Nutr. 130:2662-5.
- Budoff M, Takasu J, et al. 2004. Prev Med. 39(5):985-91.
- Weiss N, et al. 2006. J Nutr. 136(3S):750S-4S.
- Williams, et al. 2005. Phytother. Res. 19(4):314-9.
- Dillon SA, et al. 2002. J. Nutr. 132:168-71.
- Lau B, et al. 2001. J Nutr. 131:168-71.
- Macan H, et al. 2006. J. Nutr. 136(3S):793S-5S.

Cardioprotective Effects Seen in Preclinical Studies

The following preclinical studies have shown the reduction of cardiovascular disease risk factors including lowering of serum cholesterol and triglycerides, inhibition of platelet aggregation, lowering of serum homocysteine levels and inhibition of fatty streak development and smooth muscle cell proliferation² following intake of Aged Garlic Extract.

Lipid Lowering Effects

The following studies showed that Aged Garlic Extract and *S*-allyl cysteine,³ a key compound in Aged Garlic Extract, demonstrated lipid lowering effects in models and cell cultures:

The avian model is an ideal model for cholesterol because its lipoprotein profile is more closely related to the human than that of others. Moreover, according to Khalid et al. (1994), atherosclerosis of a type closely resembling that found in humans can be produced easily and consistently in models fed a high cholesterol diet.

Research by Qureshi et al. (1990a) Abuirmeileh et al. (1991) and Yu et al. (1991) found that Aged Garlic Extract and, a key constituent in Aged Garlic Extract, lowered total serum cholesterol and LDL cholesterol in hypercholesterolemic models. Cholesterol reduction was achieved by inhibition of the activity of key enzymes involved in cholesterol synthesis (β -hydroxy- β -methylglutaryl CoA synthetase and reductase) in the liver. According to Qureshi and Yu et al. (1991), among garlic preparations tested, maximum inhibition of cholesterol-producing enzyme activities was observed in this order:

Kyolic[®] > SAC > commercial garlic oil > garlic powder

Khalid, Q., Sultana, L., et al. 1994. Pak. J. Sci. Ind. Res. 37(12): 524-527.

Qureshi, A., Lin, R. et al. 1990a. First World Congress on the Health Significance of Garlic and Garlic Constituent., Washington, D.C. August 28-30, p. 16.

Abuirmeileh, N., Lin, R. et al. 1991. FASEB J. 5: A1756.

Yu, S., Qureshi, N., et al. 1991. National Conference on Cholesterol and high blood pressure. Sponsored by Cholesterol Education Program of the National Institutes of Health. April 8-10, 1991, Washington, D.C.

Immune Enhancement and Anti-Infection Effects

Aged Garlic Extract has been shown to mitigate infectious diseases through enhancement of the immune system. As seen in the following studies, it has been found to enhance various immune factors such as the phagocytic (cell-killing) activity of macrophages, T-lymphocyte activity, natural killer cell activity and antibody generation. It has also demonstrated antiviral and anti-fungal activities and has been shown to modify, both directly and indirectly, the function of immune cells, which play a leading role in allergic cascade reactions including inflammation. In addition, Aged Garlic Extract has been shown to improve age-related deterioration of the immune response.

Immune Enhancement Seen in Clinical Studies

Abdullah, T., Kirkpatrick, D. et al. 1989. *Onkologie* 21:52-53.

Abdullah et al. (1989) found that Aged Garlic Extract enhanced natural killer cell activity and improved helper/suppressor T cell ratios in AIDS patients. After only six weeks intake, natural killer cell activity was within the normal range for all subjects. Further, patients in this study noted improvements in diarrhea, candidiasis, pansinusitis with recurrent fever and interruption of recurrent cycles of genital herpes.

Kandil, O., Abdullah, T. et al. 1988. *Potential role of Allium sativum in natural cytotoxicity. Arch. AIDS Res* 1:230-231.

After a three-week period of garlic intake, Kandil et al. (1987, 1988) and Abdullah et al. (1988) found that Aged Garlic Extract (1800 mg of Kyolic® capsules) was effective at enhancing Natural Killer cell activity. Further, Aged Garlic Extract was more effective than a high dosage of raw garlic (0.5 mg/kg body weight -for a 70 kg/154 lb. man this would be equivalent to 35 g or almost 10 cloves). Natural Killer cell activity was enhanced 140% by raw garlic and 160% by garlic capsules.

Kandil, O., Abdullah, T. et al. 1987. *Fed. Proc.* 46(3): 441.

Abdullah, T., Kandil, O., et al. 1988. *J. Nat. Med. Assoc.* 80(4): 439-445.

Kohno, M., Shirakura, T. et al. 1976. *Yakuri To Chiryō* 4: 700-708.

In patients 46-50 years of age participating in the Omiya Camp of the Japanese Land Self-Defense Force, daily consumption of 4 mls of Leopin-5®, an Aged Garlic Extract preparation containing ginseng, B vitamins and other nutritional factors, significantly increased leukocyte, or white blood cell, count after 50 days intake.

Ushirotake, T. et al. 2004. *Clin. Drug and Pharmacol (Rinsho Iyaku). Suppl.* 20(7):785-793.

Following a 2 month examination period between the group taking AGE for more than one year and the control group, Ushirotake (2004) observed that the frequency of catching colds was significantly lower in the AGE group than the control group. Those in the AGE group who caught the cold recovered from the symptoms quicker as compared to the control group.

Ishikawa H. et al. 2006. *J Nutr.* 136(3S): 816S-820S.

Immune Enhancement Seen in Pre-Clinical Studies

Kyo E. 2000. 6th Annual Meeting of Jpn Mibyou System Assoc. Jan. 28, Hiroshima (Japan), pp43 (Japanese)

The various biological activities of Aged Garlic Extract (AGE) have been discussed in previous conferences. In this conference, the immune modulation effect of AGE was introduced. AGE inhibited histamine release from RBL-2H3 cells, and decreased ear swelling, indicating an IgE-mediated skin reaction. AGE also inhibited the growth of Sarcoma-180 and LL/2, and enhanced the number of natural killer (NK) cells and NK activity. Furthermore, AGE inhibited the decrease in spleen weight, cell numbers, anti-SRBC antibody production, and NK activity induced by psychological stress. All of which indicate that AGE may be useful for maintaining homeostasis of immune function as an immune modulator.

Kyo E, Uda N, Kasuga S, Itakura Y. 1999. 6th Annual Meeting on Japanese Association for Cancer Prevention. Tokyo (Japan), July 16-17, Abst#P-38 (Japanese)

Immune Enhancement Seen in Cell Culture Studies

Kyo, E., Uda, N., et al. 1998. *Phytomed.* 5(4): 259-267.

Kyo et al. (1998) found that Aged Garlic Extract stimulated the proliferation of model spleen cells and the release of cytokines. Aged Garlic Extract strongly enhanced phagocytosis of peritoneal macrophages (the ability of immune cells to engulf foreign agents) and increased natural killer cell activity both *in vitro* and *in vivo*. After 24 hours, Aged Garlic Extract doubled the ability of Natural Killer cells to destroy YAC-1 (a cancer cell line).

Kyo E, Uda N. 1999. 58th Annual Meeting of Japanese Cancer Association. Hiroshima (Japan), Sep 29-Oct. 1, Abst #2170 (Japanese)

Lau, B. 1989. *Nutr. Rev.* 9:27-31.

In Lau's study (1989) leukocytes (white blood cells) were taken from the peritoneal cavity, spleen and lymph nodes of models following intake of Kyolic Aged Garlic Extract. Using chemiluminescence, he found that the phagocytic activity (ability to engulf toxins and pathogens) of these cells was significantly enhanced.

Horie, T., Awazu, S. 1989. *Planta Med.* 55: 506-508.

When free radicals attack membranes, which are high in lipids (fat), they form lipid peroxides. Lipid peroxidation seems to be closely related to toxicity, disease and aging. TBA-RS⁵ are used as markers of lipid peroxidation and when Aged Garlic Extract was added to liver cells, Horie et al. (1989) found a reduction in TBA-RS suggesting an antioxidant effect of the Aged Garlic Extract. Specifically, at 40 mg/ml garlic extract completely inhibited the oxidant ascorbic acid/FeSO₄-induced lipid peroxidation of liver tissue.

Horie, T. 1990. *First World Congress on the Health Significance of Garlic and Garlic Constituents*. Washington, D.C. August 28-30, 1990, p. 23.

Horie (1990) found that Aged Garlic Extract added to liver tissue (microsomal membranes) exposed to peroxidation (oxidized by oxidants ascorbic acid and Iron-Fe²⁺) prevented oxidation as indicated by prevention of the formation of TBA-RS⁵ (fluorescent substances that indicate oxidation). Aged Garlic Extract also prevented the decrease in membrane fluidity (flexibility and ability to transport nutrients and wastes across the membrane) caused by peroxidation.

Horie, T., Awazu, S. et al. 1992. *Planta Medica* 58: 468-469.

Additional research by Horie et al. (1992) found that the polysulfide fraction of Aged Garlic Extract also significantly prevented lipid peroxidation of liver microsomes.

Amagase, H. et al. 2000. Ch. 6. *Phytochemicals and Phytopharmaceuticals*. AOCS Press, Champaign, Illinois, pp. 62-78.

Amagase et al. (1999) and Ide et al. (1996b) also found that Aged Garlic Extract and two of its constituents, SAC and S-allyl mercaptocysteine (SAMC), decreased emissions of low level chemiluminescence (LLC) initiated by the oxidant t-butyl hydroperoxide in liver tissue. On the other hand, water extracts of raw and heat-treated garlic enhanced such emissions. AGE reduced emissions by 30% whereas a water extract of raw garlic enhanced emissions by 110% (Amagase et al. 1999). Aged Garlic Extract, SAC and SAMC scavenged hydrogen peroxide *in vivo* and Aged Garlic Extract was more effective than raw garlic juice.

Ide, N., Itakura, Y. et al. 1996b. *2nd International Congress on Phytomedicine*. September 11-14, 1996. Munich, Germany.

Ohnishi and Kojima (1997) found that Kyolic[®] and S-allyl cysteine (SAC), one of its key ingredients, could inhibit the oxidation or rancidity of vegetable oil (a linoleic acid micelle suspension) caused by the radical generator or oxidant 2,2'-azo-bis(2-amidinopropatne HCl (AAPH). When Aged Garlic Extract or SAC were added to this oil, they prevented the oxidizing effects of AAPH. In this biologically relevant reaction, Kyolic was found to be more effective than pure SAC alone at inhibiting lipid peroxidation.

Yang, C., Yasaei, P. and Page, S. 1993. *J. Food Drug Anal.* 1(4): 357-364.

Yang et al. (1993) from the FDA found that Kyolic (50 mg) afforded protection from lipid oxidation. When Kyolic was added to a standard lipid/fat (methyl linoleate) held at 60°C (140°F), it reduced the heat induced oxidation of this fat as indicated by a decrease in methyl linoleate hydroperoxide, an intermediate in the lipid oxidation process. Aged Garlic Extract also scavenged peroxide radicals.

Dillon S, Rhaman K. 2003. *Life Sci* 72: 1583-1594.

Oxidation of LDL has been recognized as playing an important role in the development and progression of atherosclerosis. Human LDL was isolated and challenged with a range of oxidants either in the presence of AGE or its diethyl ether extract. Results of this study indicate AGE's ability to inhibit the *in vitro* oxidation of LDL by scavenging Superoxide and inhibiting the formation of lipid peroxides. AGE also reduced LDL oxidation by chelating copper ions. Thus, AGE may have a role to play in preventing the development and progression of atherosclerotic disease.

Dillion, S., Rahman, K. et al. 2003. *Life Sciences*. 72, 1583-1594.

Dillion et al. (2003) found that Aged Garlic Extract inhibits *in vitro* oxidation of isolated LDL by scavenging superoxide and inhibits the formation of lipid peroxides in cell free assays. AGE was also shown to reduce LDL oxidation by the chelation of Cu²⁺. Thus, AGE may have a significant role in preventing the development and progression of atherosclerotic disease.

Horie T. 2000. *6th Annual Meeting of Jpn Mibyou System Assoc.* Jan 28, Hiroshima (Japan), pp42. (Japanese).

Since ancient times, garlic has been used as both food and medicine. Recently, it has been determined that reactive oxygen species and lipid peroxidation cause changes in biological functions, which is associated with disease and aging. Preventing these changes is important for maintaining a healthy body. In this work, the antioxidant effects of garlic are introduced. Incubation with isolated microsome, iron and ascorbic acid caused lipid peroxidation in *in vitro* systems. Co-incubation with the aforementioned compounds and garlic compounds, such as diallyl polysulfides, has shown strong antioxidant effects, and the ability to inhibit lipid peroxidation.

Ohnishi, S. and Kojinra, R. 1997. Ch. 12. In: *Nutraceuticals: Designer Foods III Garlic, Soy and Licorice*. Paul P. Lanchance (ed.). Food & Nutrition Press, Trumbell, CT, pp. 105-115.

Mostafa, M., Mima, T., Ohnishi, S.T., Mori, K. 2000. *S-Allylcysteine Ameliorates Doxorubicin Toxicity in the Heart and Liver in Mice*. *Planta Medica*, 66: 148-151.

Awazu, S. 1997. Ch. 14. In: *Nutraceuticals: Designer Foods III Garlic, Soy and Licorice*. Paul P. Lanchance (ed.). Food & Nutrition Press, Trumbell, CT, pp. 131-138.

Ide, N. and Lau, B.H.S. . 1997a. *FASEB J*. 11(3): A122/#713.

Ide, N., Lau B.H.S. et al. 1997a. *Planta Med*. 63: 263-264.

Ide, N. and Lau, B.H.S. *Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement*. Newport Beach, CA. November 15-17, 1998.

Ide N, Lau BHS. 1998. 5th Annual Meeting of the Oxygen Society. Washington DC, November 19-23, Abst# 68, *Free Rad Biol Med*. 25: S33.

Ide, I and Lau, B.H.S. *J. Nutr.*, 131: 1020S-1026S, 2001.

Lau, B.H.S. *Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement*. Newport Beach, CA. November 15-17, 1998.

Lau, BHS. *J. Nutr.*, 131: 985S-988S, 2001.

Ohnishi et al. (1997) found that both liquid Aged Garlic Extract (0.05 ml/ 20 g body weight intraperitoneally) and Kyolic tablets (0.01-0.02 g/20 g body weight orally), given six times weekly, protected the heart against the cardiotoxicity of DOX (1.5 mg/kg body weight given three times a week for 40 days). Aged Garlic Extract assisted in maintaining a normal QRS width. Further, Aged Garlic Extract inhibited lipid peroxidation as seen by a decrease in TBA-RS.⁵ DOX alone increased TBA-RS in heart tissue by 76% whereas liquid Aged Garlic Extract reduced TBA-RS to 17%. Aged Garlic Extract also ameliorated the decrease in body weight and survival rate caused by the DOX administration.

Doxorubicin (Adriamycin), a potent anticancer drug, is effective against a wide range of human cancers. However, the clinical uses of doxorubicin have been limited due to its serious cardiotoxic adverse effects, which are likely the result of generation of free radicals and lipid peroxidation. Dr. Ohnishi's team found that S-allylcysteine (SAC), an antioxidative, organosulfur compound from Aged Garlic Extract, significantly reduced doxorubicin-induced mortality, heart and liver damage. The authors suggested that SAC research may ultimately lead to a resolution of the adverse effects of doxorubicin treatment in cancer chemotherapy.

Diallyl pentasulfide, a constituent in Aged Garlic Extract, inhibited the production of oxidants by DOX. It completely inhibited both TBA-RS5 and chemiluminescence (indicators of oxidation) in heart tissue incubated with DOX and NADH.

— Prevented Oxidation Of LDL Cholesterol in Pre-Clinical Studies

Oxidative modification of low-density lipoprotein (LDL) has been recognized as playing an important role in the initiation and progression of atherosclerosis. Ide and Lau (1997a) from Loma Linda University found that Aged Garlic Extract and its various constituents could inhibit copper-induced peroxidation of LDL cholesterol in a concentration dependent manner. Lipid oxidation was determined by measuring TBA-RS.⁵ Further, it was found that when Aged Garlic Extract and its various constituents were preincubated with pulmonary artery endothelial cells (cells which line the lungs), cell damage caused by oxidized LDL was prevented, as indicated by prevention of lactate dehydrogenase release,⁴ loss of cell viability and TBA-RS formation. Thus, these researchers found that Aged Garlic Extract and its constituents could not only prevent the oxidation of LDL, but also prevent oxidized LDL from damaging membranes, oxidizing lipids (fats) and damaging or killing cells which may contribute to the initiation/progression of cardiovascular disease.

Ide and Lau (1998, 2001) determined the effects of aged garlic extract (AGE) and its major compound, S-allylcysteine (SAC), on oxidized LDL (Ox-LDL)-induced injury in endothelial cells (EC). Lactate dehydrogenase (LDH) release⁴ as an index of membrane damage, methiazol tetrazolium (MTT) assay for cell viability, and thiobarbituric acid reactive substances (TBA-RS)⁵ indicating lipid peroxidation were measured. Ox-LDL caused an increase of LDH release, loss of cell viability and TBA-RS formation. AGE or SAC prevented all these changes. To elucidate the mechanism, effects of AGE or SAC on intracellular glutathione (GSH) level in EC, and release of peroxide from EC and macrophages (MΦ) were determined. Ox-LDL resulted in intracellular GSH depletion and in of peroxide release. AGE or SAC inhibited these changes. Effects of SAC on hydrogen peroxide (H₂O₂) or tumor necrosis factor, alpha (TNF-α)-induced nuclear factor kappa B (NF-κB) activation was determined. Pretreatment of EC with SAC inhibited NF-κB activation. AGE and SAC protected EC from Ox-LDL- induce injury preventing intracellular GSH depletion in EC and by releasing peroxides from EC and MΦ. SAC also inhibited H₂O₂ or TNF-α-induced NF-κB activation. These data suggest that AGE, and its main compound, SAC, may be useful for prevention of atherosclerosis.

Oxidized LDL, but not native LDL, promotes vascular dysfunction by exerting direct cytotoxicity toward endothelial cells, by increasing chemotactic properties in monocytes, and by transforming macrophages to foam cells via scavenger-receptors; all these events are recognized to contribute to atherogenesis. Lau (1998) presented experimental data from his own laboratory and those from other investigators showing that several garlic compounds can effectively suppress LDL oxidation. Short-term supplementation of garlic in subjects has demonstrated an increased resistance of LDL oxidation. These data suggest that suppressed LDL oxidation may be one of the mechanisms accounted for the anti-atherosclerotic properties of garlic.

Reduced Physiological Stress in Pre-Clinical Studies

Ushijima, M., Sumioka, I. et al. 1997. *Phytother. Res.* 22: 226-230.

Ushijima et al. (1997) and Amagase et al. (1999) found that in various stress tests Kyolic was more effective than raw garlic, heated garlic or powdered garlic at reducing both physiological and psychological stress. Subjects given Kyolic swam 82% (2ml) and 90% (4ml) of the time they were in water, whereas those not given garlic swam only 50% of the time. Except at a low dosage of raw garlic, only Kyolic significantly enhanced swimming time. Subjects given Kyolic were also found to run longer than those given a placebo (control), raw garlic (RGJ), heated garlic (HGJ) or garlic powder (PG) in a mechanical treadmill running test. Control ran for 929 seconds whereas those given Kyolic ran for 1611 seconds, almost twice as long.

Amagase, H. et al. 2000. *Ch. 6. Phytochemicals and Phytopharmaceuticals. AOCS Press, Champaign, Illinois, pp. 62-78.*

Ishii S, Ushijima M, Mizuno I, Nishihama T, Kashimoto N, Morihara N, Hayama M. 2006. *Oyo Yakuri (Pharmacometrics)*, 71:101-9

AGE products with Kyoleopin Neo (KLEN) attenuates the reduction of skin surface conductance (SSC), induced by immobilization stress, and increases skin pigmentation induced by UV-B in models, suggesting that it may function as a useful agent for improvement of skin condition deterioration caused by various internal or external factors.

Reduced Physiological Stress in Clinical Studies

Ishii S, Nishihama T, Sumioka I, Morihara N, Ushijima M, Mizuno I, Kashimoto N, Hayama M. 2006. *Oyo Yakuri (Pharmacometrics)*, 70: 97-105.

Ishii et al. (2006) examined a four-week human trial, where 1 ml of Kyoleopin Neo (KLEN) was administered to subjects twice a day after meals in the morning and evening. Fatigue was evaluated at 0, 2, and 4 weeks after taking KLEN and using an Advanced Trail Making Test (ATMT); a tool used to measure the performance of brain function associated with mental fatigue. KLEN decreased "brain-age" time-dependently and significantly. Demonstrating that KLEN maybe useful for ameliorating daily fatigue or for preventing the accumulation of fatigue.

Yaguchi S, Tokoro k, Tada M. 2005. *Shinyaku to Rionsho (New Drug Clin.)*. 42: 189-96 (Japanese).

Yaguchi et al. (2005) performed clinical evaluations of Aged Garlic Extract combined with ginseng, oriental bezoar, antler velet, cuscuta see, and epimedium herb (Leopin Royal; LER) on various unexplained complaints (malaise). Subjects (n=19) were given 2mL of the AGE product daily for 4 weeks. Improvement in symptoms was evaluated. Supplementation with the AGE product reduced stress indexes and improved symptoms such as chill, stiffness in the shoulders, fatigue lassitude, decline of physical strength/willpower, headache, abnormal bowel movement, and lumbago. The efficacy of the AGE product was felt by 87.5% of subjects, indicating that the AGE product is useful for various unexplained complaints and malaise.

Improved Recovery from Athletic Performance in Pre-Clinical Studies

Kasuga S, Morihara N. et al. 1998. *Hokkaido J Sports Med Sci.* 6: 29-36.

The effect of AGE for endurance performance, SDH activity in the models gastrocnemius muscle and soleus muscle and plasma NO metabolic concentrations increased significantly. The results showed that AGE would facilitate the skeletal muscle oxidative enzyme activity and contribute to enhancement of physical strength and endurance training.

Improved Recovery from Athletic Performance in Clinical Studies

Kohno, M., Shirakura, T. et al. 1976. *Yakuri To Chiryō (Jpn. J. Pharmacol. Ther.)* 4(3): 700-708.

In a double-blind study by Kohno et al. (1976), a Kyolic® Liquid Aged Garlic Extract preparation containing vitamin B₁, vitamin B₁₂ and liver extract (KLE) was given to personnel of the Omiya Camp of the Japanese Land Self-Defense Force. Subjects taking KLE recovered from exhaustion faster and made fewer complaints of exhaustion and tiredness after manual labor than those taking placebos. Results were more marked in the 45-50 year-olds than in the 25-35 year-olds.

Kawashima, H., Ochiai, Y., et al. 1986. *Kiso to Rinsho (Preclinical and Clinical Report)* 20(16): 8229-8245.

Kawashima et al. (1986) studied 20 healthy male college students and found that Aged Garlic Extract (2 ml, twice per day) improved subjective and objective symptoms of fatigue following 22 days of intense physical training. Aged Garlic Extract also improved patellar reflex and promoted serum levels of GOT, GPT, triglycerides, and lactic acid, which favor relief from fatigue. These latter effects provided further objective measurements of the anti-fatigue effects of Aged Garlic Extract. The authors concluded that Aged Garlic Extract is an effective medicine for the prevention of and recovery from fatigue.

Kimoto R, Kambayashi, Ishimura N, Nakamura T, Uchida E, Kyo E, Fuji H, Takeda H. 2005. *Hokkaido J Sports Med Sci.* 10:17-26.

Kimoto et al. (2005) investigated AGE's influence on the change of urinary 8-OHdG content, which is thought to be a marker of oxidative stress during daily regular and temporary intense exercise. Twelve healthy males were divided into two groups: AGE supplementation group and the control group. The AGE supplementation group was given AGE for 2 weeks. Urinary 8-OHdG content was found to be significantly lower with the AGE group than with the control. Also, AGE supplementation significantly increased the sum total of oxygen uptake during intense exercise.

Inhibits Stress-Induced Peptic Ulcer Formation

Nagai (1972) found that a Kyolic® Liquid Aged Garlic Extract preparation containing vitamin B₁, vitamin B₁₂ and liver extract, orally administered at dosages of 2.0, 0.2, and 0.02 ml clearly prevented stress-induced ulcer formation. The authors attributed the anti-ulcer effects to strengthening of the gastric mucosa rather than to a decrease in aggravating agents such as gastric acid and pepsin.

Nagai, K. 1972. *Kiso To Rinsho (Preclin. Clin. Reports)* 6: 1536-1538.

Anticancer and Cancer-Preventive Effects

You, W., Blot, W. et al. 1988. *Cancer Res.* 48: 3518-3523.

Buiatti, E., Palli, D. et al. 1989. *Int. J. Cancer* 44: 611-616.

Singh, S. *Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement held in Newport Beach, CA. November 15-17, 1998.*

Singh, S.V., *J. Nutr.*, 131: 1046S-1048S, 2001.

Thomson M, Muslin A. 2003. *Curr Cancer Drug Targ*, 3: 67-81.

Chu Q, Ling MT, Feng H, Cheung HW, Tsao SW, Wang X, Wong YC. 2006. *Carcinogenesis*. 27(11):2180-9.

Lau, B.H.S., Woolley, J. et al. 1986. *J. Urol.* 136: 701-705.

Riggs, D., Lamm, D. et al. 1995. *J. Urol.* 153(4): 1029.

Lau, B., Barker, G et al. 1985. *85th Annual Meeting of the American Society for Microbiology. March 3-7, 1985, Las Vegas, Nevada, E-95.*

Marsh, C., Lau, B. et al. 1987. *J. Urol.* 137: 359-362.

Case control epidemiological studies in northeast China (You et al., 1988) and Italy (Buiatti et al., 1989) showed that there are strong reverse trends in stomach cancer risk with dietary intake of garlic. Further, a number of studies have reported inhibitory effects of garlic and its constituents on the development and growth of cancer. Specifically, Aged Garlic Extract and its constituents have demonstrated anti-cancer effects in an array of cancer models including bladder tumors, melanoma cells, neuroblastoma cells, skin cancer, breast cancer, colon cancer, prostate cancer, esophageal cancer, stomach and lung cancer, erythroleukemia and aflatoxin induced mutagenesis as seen in the following studies:

Oral administration of diallyl disulfide (DADS) resulted in a dose-dependent and statistically significant inhibition of H-ras oncogene transformed tumor growth. The tumor growth inhibitory effect of DADS was apparent in terms of delay in the appearance of measurable tumors, tumor volume as well as tumor weight. DADS suppresses the growth of H-ras oncogene transformed tumors by inhibiting the membrane association of tumoral p21H-ras, and that the allyl group is an important determinant in tumor growth inhibitory effect of DADS.

Numerous studies have demonstrated the chemopreventive activity of garlic by using different garlic preparations; including fresh garlic extract, aged garlic extract, garlic oil and a number of organosulfur compounds derived from garlic. Recent research has also focused on the antimutagenic activity of garlic. It has also been observed that AGE, but not the fresh garlic extract, exhibits radical scavenging activity. The two major compounds in aged garlic, SAC & SAMC have the highest radical scavenging ability. Because of this, consumption of garlic may provide protection from cancer development.

Metastatic cancer is one of the main causes of cancer-related death since it rarely responds to available treatments. Using colony-forming, wound-closure as well as matrigel-invasion assays, Chu et al. found that two main water-soluble constituents of the garlic, S-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC), were able to suppress Pca cell proliferation and invasive abilities through restoration of E-cadherin expression in cancer cells.

Inhibited the Growth of Bladder Tumors

Lau et al. (1986) found Aged Garlic Extract to be more effective than *bacillus Calmette-Guerin (BCG)*¹¹ at inhibiting the development and growth of bladder tumors from implanted transitional cell carcinoma. Delivering the Aged Garlic Extract directly into the tumor was more effective than through the intraperitoneal route.

Aged Garlic Extract was found to be a “highly impressive nontoxic oral treatment modality” in a bladder cancer model. The effect was similar to that of BCG. At a low dosage (50 mg) Aged Garlic Extract significantly reduced tumor volume, but not mortality. At a higher dosage (500 mg) it significantly reduced both tumor volume and mortality. No adverse effects were noted in any Aged Garlic Extract groups (Riggs et al., 1995).

Lau and Marsh et al. (1985, 1987) observed that Aged Garlic Extract was equally or slightly more effective than BCG at inhibiting the development and growth of intravesically transplanted bladder tumors. Aged Garlic Extract, given one and six days after transplantation of bladder tumor cells, yielded the lowest final tumor incidence.

Reeve V., □ Bosnic, M. et al. 1993a. *Photochem. Photobiol.* 58(6):813-817.

Reeve et al. (1993a) found that Aged Garlic Extract protected bald models from photocarcinogenesis (skin carcinogenesis induced by ultraviolet radiation). Aged Garlic Extract-treated models exposed to DMBA, a cancer causing agent, then exposed to UV radiation for six weeks also developed significantly fewer tumors than untreated models exposed to the same regiment.

Inhibited the Growth of Carcinogen-Induced Tumors of the Breast

Song K.; Milner, J. 1999. *J. Nutr.* 129(3):657-661.

Song et al (1999) found that providing either 0.105 micromol diallyl disulfide or *S*-allyl cysteine, constituents in Aged Garlic Extract, by gastric gavage thrice weekly for 2 weeks was effective in retarding DMBA bioactivation. Isomolar alliin was not effective.

Pinto, J. and Rivlin, R. *Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement.* Newport Beach, CA. November 15-17, 1998.

Garlic constituents have been shown to inhibit both initiation and promotion of cancer and have attracted wide interest for their anticancer potential. Pinto and Rivlin (1998, 2001) conducted studies with the human breast cancer cell lines, MCF-7 (estrogen-sensitive) and the MCF-ras (estrogen-insensitive) and found that Aged Garlic Extract, SAC and SAMC, at increasing concentrations inhibit cell growth progressively.

Pinto, J.T and R.S. Rivlin, *J. Nutr.*, 131: 1058S-1060S, 2001.

Tiwari, R., Pinto, J. et al. 1993. *Breast Cancer Res. Treat.* 27:(1-2): 80.

Tiwari et al. (1993) found that *S*-allyl cysteine (SAC) and *S*-allyl mercaptocysteine in Aged Garlic Extract inhibited the growth and proliferation of transformed human breast cells. They also increased both glutathione-*S*-transferase¹³ and peroxidase levels in the non-transformed cells. Glutathione-*S*-transferase is critical for detoxification and gene expression.

Li, G., Tiwari, R. et al. 1995. *Oncology Rep.* 2: 787-791.

Li et al. (1995) also reported that the *S*-allyl cysteine (SAC) and *S*-allyl mercaptocysteine in Aged Garlic Extract inhibited the growth of transformed human breast cells and increased both glutathione-*S*-transferase and peroxidase levels in the non-transformed cells. Glutathione-*S*-transferase is critical for detoxification and gene expression.

Milner, J. and Liu, J. 1990. *First World Congress on the Health Significance of Garlic and Garlic Constituents p.* 25.

Milner and Liu (1990) found that DMBA treatment while receiving a diet of 4% Aged Garlic Extract powder, mammary cancer incidence was 35% compared to 90% in controls receiving no Aged Garlic Extract. The 4% diet was given two weeks prior to and two weeks following DMBA treatment. Aged Garlic Extract also significantly inhibited *in vivo* formation of adducts¹⁴ which were 96% correlated to ultimate tumor number. This research suggested that Aged Garlic Extract could alter the mammary tissue's ability to convert DMBA into an active cancer-causing agent, which can then bind to DNA.

Zoumas, C., Milner, J. et al. 1992. *FASEB J.* 6(4): 2641.

In a study by Zoumas et al. (1992), a diet supplemented with 2% Aged Garlic Extract depressed DMBA-DNA binding by 30-40%. Aged Garlic Extract given in accordance with a high fat diet (20% of total calories) depressed binding by 56%. An increase in DMBA-DNA binding is associated with an increase in fat content of the diet.

Schaffer, E. and Milner, J. 1997. *16th International Congress of Nutrition. IUNS July 27-August 1, 1997, p.* 67.

Subjects fed additional linoleic acid or oleic acid (to a standard 5% corn oil diet) had approximately 60 or 35% more (P<0.05) total mammary DNA adducts,¹⁴ respectively, than those fed the basal diet; but only 10 or 20% more adducts, respectively, when 2% Aged Garlic Extract was also supplemented in the diet. This research by Schaffer and Milner (1997) concluded that Aged Garlic Extract could block the enhancement by specific fatty acids of the initiation of DMBA-induced mammary carcinogenesis.

Amagase, H. and Milner, J. 1992. *FASEB J.* 6(4): 3229.

Amagase et al. (1992, 1993) found that the majority of the garlic preparations tested could reduce the binding of the carcinogen DMBA to mammary cell DNA. However, among the effective garlic preparations, only Aged Garlic Extract did not cause any side effects. The other garlic preparations, including commercial garlic powder, significantly decreased food intake and body weight gain. SAC, a constituent in Aged Garlic Extract, also dose-dependently reduced DMBA-DNA adduct¹⁴ formation in the mammary gland.

Amagase H., and Milner, J. 1993. *Carcinogenesis* 14: 1627-1631.

Liu J. and Milner, J. 1990. *FASEB J.* 4: A1175.

Liu and Milner (1990) found that treatment with Aged Garlic Extract (4% of diet) and selenium (1mcg/g) resulted in a synergistic 30% reduction in DNA adduct¹⁴ formation exposed to the carcinogen DMBA.

Inhibited the Growth of Carcinogen-Induced Tumors of the Colon

Shirin H, Pinto JT, Kawabata Y, Soh JW, Delohery T, Moss SF, Murty V, Rivlin RS, Holt PR and Weinstein IB. 2001. Antiproliferative effects of *S*-allylmercaptocysteine on colon cancer cells, when tested alone or in combination with sulindac sulfide. *Cancer Res.* 61(2):725-731.

Dr. Weinstein's group at Columbia and Cornell Universities compared two water-soluble derivatives of Aged Garlic Extract, *S*-allylcysteine (SAC) and *S*-allylmercaptocysteine (SAMC), to sulindac sulfide (SS), a well-established colon cancer chemopreventive agent, for their effects on two human colon cancer cell lines, SW-480 and HT-29. SAMC, but not SAC, inhibited the growth of both cell lines at doses similar to that of SS. SAMC also induced apoptosis (cell death). These effects of SAMC were accompanied a marked increase in endogenous levels of reduced glutathione (a potent antioxidant). SAMC inhibited growth progression of both cell lines. SAMC, co-administered with SS enhanced the growth inhibitory and apoptotic effects of SS. These findings suggest that SAMC may be useful in colon cancer prevention when used alone or in combination with SS or other chemopreventive agents.

Wargovich, M. 1987. *Carcinogenesis* 8: 487-489.

Wargovich (1987) found that diallyl sulfide, a constituent in Aged Garlic Extract, significantly reduced the incidence of colon cancer, by 74%, induced by 20 weekly injections of the carcinogen, 1,2-dimethylhydrazine (DMH).

Sumiyoshi, H. and Wargovich, M. 1990. *Cancer Res.* 50: 5084-5087.

Sumiyoshi and Wargovich (1990) found that two constituents in Aged Garlic Extract, diallyl sulfide and SAC, significantly reduced the incidence of colon cancer induced by a carcinogen, dimethylhydrazine (DMH). Both these compounds, as well as various other organosulfur compounds in Aged Garlic Extract, stimulated the activity of glutathione *S*-transferase, an enzyme known to assist in the detoxification of carcinogens in the liver and colon.

Sumiyoshi, H. and Wargovich, M. 1989. *Proc. Am. Assoc. Cancer Res.* 30:181.

Two constituents in Aged Garlic Extract, diallyl sulfide and SAC, significantly inhibited nuclear damage caused by the carcinogen dimethylhydrazine (DMH), thus decreasing the toxicity of this carcinogen. Further, both compounds also significantly stimulated the activity of glutathione *S*-transferase (GST) in both the liver and colon (Sumiyoshi and Wargovich, 1989).

Sundaram, S. and Milner, J. 1996. *J. Nutr* 126: 1355-1361.

Diallyl disulfide, a compound in Aged Garlic Extract, was found to be more effective than 5-fluorouracil (5-FU), a common anti-cancer drug, at inhibiting the growth of human colon tumor cells, especially when injected intraperitoneally. Further, diallyl disulfide given simultaneously with (5-FU) prevented some of the side effects induced by 5-FU such as: depression of white blood cells, spleen weight and elevated plasma urea (Sundaram and Milner, 1996).

Hatono, S., Wargovich, M. et al. 1996. *Carcinogenesis* 17(5): 1041-1044.

Aberrant crypt foci are considered to be the most likely precursors of colon cancer. SAC administration inhibited development in the colon of one third to one half of the foci induced by DMH when given prior to this carcinogen (initiation phase). Further, SAC was found to significantly enhance GST (glutathione *S*-transferase) activity not only in the liver, but also in the proximal and middle small bowel. GST is a detoxification enzyme system in the body. Thus, SAC inhibited the development of pre-cancerous lesions in the colon and enhanced the activity of enzyme systems in the liver and small intestine, which detoxify carcinogens (Hatono et al., 1996).

Hatono, S. and Wargovich, M. 1997. Ch. 15. In: *Nutraceuticals: Designer Foods III Garlic, Soy and Licorice*. Paul P. Lanchance (ed.). Food & Nutrition Press, Trumbull, CT, pp. 139-151.

The administration of 0.4 and 0.8 maximum tolerated dose of SAC incorporated into the experimental diet significantly decreased the number of aberrant crypt foci when given during initiation but not promotion induced by the carcinogens dimethylhydrazine or azoxymethane. Models given *S*-ethylmercaptocysteine, *S*-propylmercaptocysteine and *S*-propargylcysteine, exhibited increased foci, determined to be due to decreased food intake caused by these compounds.

Uda, N., Wargovich, M., et al. 1996. *Proc. Annu. Meet Am. Assoc. Cancer Res.* 37: 1871.

Additional research by Wargovich et al. team found that the Aged Garlic Extract could change the metabolism of the carcinogen azoxymethane (AOM) and thus inhibit AOM-induced aberrant crypt foci (Uda et al., 1996). Models (F344 male) were given AOM (15 mg/kg /wk, i.p.) for two weeks. In the initiation study, they were also given Aged Garlic Extract (120, 600 or 3000 mg/kg) for three consecutive days one week prior to and including the two weeks exposure to AOM. Aged Garlic Extract significantly inhibited AOM-induced aberrant crypt foci. When Aged Garlic Extract was taken 2 weeks after AOM treatment for four weeks (post-initiation) it had only a mildly preventive effect.

Knowles, L. and Milner, J. 1997. *FASEB J.* 11(3): A422/#2445

Knowles and Milner (1997) found that DADS and SAMC, constituents in Aged Garlic Extract are effective at suppressing the growth of cultured human colon tumor cells. At equimolar amounts, DADS was most effective. Twenty-five mcg of DADS and 300 mcg of SAMC caused a 23% suppression in cell growth. DADS also assisted cells in converting from a mutated to a normal state (from G1 to S phase).

Ishikawa H. 2002. *Jpn J Cancer Chemother.* 29:545-9.

Ishikawa H. 2005. *Significance of Garlic and its Constituents in Cancer and Cardiovascular Disease.* Washington DC, April 9-11, pp53.

Tanaka S, Haruma K, Kunihiro M, Nagata S, Kitadai Y, Manabe N, Sumii M, Yoshihara M, Kajiyama G, Chayama K. 2005. *Significance of Garlic and its Constituents in Cancer and Cardiovascular Disease.* Washington DC, April 9-11, pp 57.

Tanaka S, Haruma K. et al. 2006. *J Nutr.*136(3S): 821S-826S.

Ishikawa H, Saeki T, Otani T, Suzuki T, Shimozuma K, Nishino H, Fukuda S, Morimoto K. *Aged Garlic Extract Prevents a Decline of NK Cell Number and Activity in Patients with Advanced Cancer.* *J. Nutr.* 2006;136 Suppl 3:S816-20.

Uda N, Kashimoto N, Sumioka I, Kyo E, Sumi S, Fukushima S. *Aged Garlic Extract Inhibits Development of Putative Preneoplastic Lesions in Rat Hepatocarcinogenesis.* *J. Nutr.* 2006;136 Suppl 3:S855-8.

Belloir C, Singh V, Daurat C, Siess MH, Le Bon AM. 2006. *Food Chem Toxicol.* 44:827-34.

Fukushima S. 2000. 6th Annual Meeting of Jpn Mibyou System Assoc. Jan. 28, Hiroshima (Japan), pp45 (Japanese)

Hori T, Wei M, Ichihara T, Takada N, Taniyama T, Fukushima S. 1997. 56th Annual Meeting on Japanese Cancer Association. Kyoto (Japan), Sep. 26, Abst# CCO-08. (Japanese)

Ishikawa (2002) established a protocol for a randomized controlled trial for prevention of colorectal cancer. The study will include 100 patients with hereditary non-polyposis colorectal cancer that will consume either aged garlic extract or placebo, both in capsule form. The main end point of this trial is the number and size of colorectal adenomas after 2 years. Subject recruitment began in March 2002, and the trial will be completed in September 2005.

Inhibited Growth of Colorectal Carcinoma Cells and Their Angiogenesis in Clinical Studies

In a preliminary double-blind randomized clinical trial, a high dose (2.4 mL/d) and low dose (0.16 mL/d) Aged Garlic Extract (AGE) was administered to patients with colorectal adenomas for 12 months. Following the 12 months, there was a significant suppression on both the size and number of colon adenomas in patients of the high-dose treatment whereas the number of adenomas increased in the control group. Results suggest that AGE has a suppressive effect on the progression of colorectal adenomas in humans.

Increased Natural-Killer Cells and Cell Activity

In a randomized double-blind trial by Ishikawa et al. (2006), Aged Garlic Extract (AGE) was administered to patients with inoperable colorectal, liver or pancreatic cancer. It was found that both the number of natural-killer (NK) cells and the NK cell activity increased significantly in the AGE group.

Inhibited the Development of Liver Cancer

Using the medium-term bioassay system based on the 2-step model of liver cancer, a significant reduction was observed in the specific chemical-labeling indices in the livers of the Aged Garlic Extract (AGE) group than in the control group, indicating that AGE inhibited the development of early stage of liver cancer.

SAC and allyl mercaptan (AM) significantly decreased DNA breaks in HepG2 cells treated with dimethylnitrosamine. Additionally, all the garlic organosulfur compounds studied were shown to decrease the genotoxicity of the direct-acting compounds, hydrogen peroxide and methyl methanesulfonate. This study demonstrated that garlic organic sulfur compounds (OSC) displayed antigenotoxic activity in human metabolically competent cells.

The effects of Aged Garlic Extract (AGE) on the development of glutathione *S*-transferase parental form (GST-P) positive foci was determined using a diethylnitrosamine-induced hepatocarcinogenesis model system. AGE significantly inhibited GST-P positive hepatocellular foci in a dose-dependent manner. These findings indicate that AGE inhibited the development of putative preneoplastic lesions in hepatocarcinogenesis, by reducing the proliferation rate of liver cells after partial hepatectomy.

The effects of *S*-methylcysteine and cysteine on the development of glutathione *S*-transferase parental form (GST-P)-positive foci were determined using a diethylnitrosamine-induced hepatocarcinogenesis model system. Both *S*-methylcysteine and cysteine significantly inhibited GST-P positive hepatocellular foci. These findings indicate that *S*-methylcysteine and cysteine inhibited the development of putative preneoplastic lesions in hepatocarcinogenesis.

Overview of Inhibition of Cancer Growth by Aged Garlic Extract and Its Constituents		
Cancer	<i>In vivo</i> (Model)	<i>In vitro</i> (Cancer Cell Culture)
Bladder	Model ¹⁻⁵	
Breast	Model ^{9-21, 22,23}	Human ⁶⁻⁸
Colon	Model ^{24-27, 28-30}	Human ³¹
Erythroleukemia		Human ³²⁻³³
Esophagus	Model ³⁴	
Liver		Model ³⁵⁻³⁷
Lung	Model ³⁸⁻⁴⁰	Human ⁵¹
Melanoma		Human ^{41,42} Model ⁴²
Neuroblastoma		Human ⁴³
Prostate		Human ⁴⁴⁻⁴⁷
Skin	Model ⁴⁸⁻⁵⁰	
Stomach	Model ³⁸⁻⁴⁰	

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Inhibition of Vascular Smooth-Muscle Cell and Umbilical Endothelial Cell Proliferation

Yamasaki, T., Lau, B. et al., 1994. *Phytother. Res.* 8:408-412.

Yamasaki et al. (1994) found that SAC can protect cells that line the blood vessels of the lungs from oxidant injury. SAC, in this *in vitro* test, protected bovine pulmonary endothelial cells from hydrogen peroxide (H₂O₂)-induced oxidant injury. Pretreatment of cells overnight with SAC (4 mg/mL) significantly reversed the loss of cell viability, inhibited lactate dehydrogenase release (LDH)⁴ and lipid peroxidation induced by H₂O₂. The authors suggesting that these compounds may be effective in hampering the aging process and for prevention of atherosclerosis.

Lee, E., Steiner, M. et al. 1994. *Biochim. Biophys. Acta* 1221:73-77.

SAC was found to inhibit vascular smooth-muscle cell (SMC) and umbilical endothelial cell proliferation. SMC proliferation constitutes an essential aspect in the development of atherosclerosis and of restinosis (narrowing or constriction) of blood vessels subjected to angioplasty.⁶

Sickle Cell Anemia

Ohnishi, S. *Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement.* Newport Beach, CA. November 15-17, 1998.

Sickle cell anemia is a genetic disease caused by abnormal hemoglobin. In Africa, one out of 80 people suffers from this and the patients die before reaching the age of 20. In the African American population, one out of 500 suffers. Although hydroxy urea was found to have some efficacy, still there is no cure. The patients are known to have a decreased vitamin E level, suggesting oxidative stress may be involved in the disease process. By exposing sickle red blood cells to a deoxy-oxy cycling *in vitro*, Ohnishi (1998, 2001) found that dense red cells were formed. The dense cells can be found in the patients, and they may cause blood vessel occlusion. Using this method, the Ohnishi found that SAC inhibited the formation of dense cells *in vitro*. The inhibition was 30 % at the concentration of 1 mg/ml.

Ohnishi, S.T and T. Ohnishi, *J. Nutr.* 131: 1085S-1092S, 2001.

Takasu, J., et al. 2002. *BMC Blood Disorders*, 2:3.

Takasu et al. (2002) examined the potential role of Aged Garlic Extract as an antioxidant for sickle red blood cells (RBC). Unanimously, the patient's count of Heinz bodies decreased from 58.9% to 29.8% during the 4 weeks of the study. These data suggest the significant antioxidant activity of Aged Garlic Extract on sickle cell anemia, and may represent a potential therapy to combat complications of the disease.

Antioxidative Effects of SAC

Ide, N. Lau, B et al. 1997a. *Planta Med.* 63: 263-264.

Ide and Lau (1997b) from Loma Linda University found that SAC could prevent copper, a potent oxidant, from oxidizing LDL cholesterol in an *in vitro* system.

Ide, N., Itakura, Y. et al. 1996. 2nd International Congress on Phytomedicine. September 11-14, 1996a. Munich, Germany.

Ide et al. (1996a) and Amagase et al. (1999) found that SAC decreased the emission of low level chemiluminescence (LLC) initiated by t-butyl hydroperoxide. SAC inhibited LLC emissions 33% at 5 mmol/L and 45% at 10mmol/L. SAC also demonstrated radical and hydrogen peroxide scavenging activities *in vivo*.

Amagase, H. et al. 2000. Ch. 6. *Phytochemicals and Phytopharmaceuticals.* AOCS Press, Champaign, Illinois, pp. 62-78.

Ide, N., Matsuura, H. et al. 1996b. *Phytother. Res.* 10: 340-341.

SAC demonstrated a scavenging effect on hydrogen peroxide and also inhibited the chain oxidation induced by a hydrophilic radical initiator in another study by Ide et al. (1996b).

Imai, J., Itakura, Y. et al. 1994. *Planta Medica* 60:417-420.
Ide, N. Lau, B et al. 1997a. *Planta Med.* 63: 263-264.

SAC inhibited the emission of low level chemiluminescence and the early formation of TBA-RS⁵ (markers of oxidation), whereas water extracts of raw and heat-treated garlic enhanced such emissions. Imai et al. (1994) suggested that SAC has antioxidative efficacy.

Banerjee SK, et al. 2003. *Phytother Res.* 17; 97-106.

Various preparations of garlic, mainly aged garlic extract (AGE), have been shown to have promising antioxidant potential. SAC, a major compound in AGE but not in raw garlic, has been reported to have powerful antioxidant and radical scavenging effects. This review touches on several of these areas.

Hsu, C. 2004. *Five Cysteine-Containing Compounds Have Antioxidative Activity Balb/cA Mice Nutrient Interactions and Toxicity Research Communication.* 149-152.

Five cysteine-containing compounds derived from garlic, including SAC, were added to drinking water at 1 g/L for a 4 week treatment, while cysteine was used as a comparison. At the end of treatment, glutathione (GSH) levels were higher (p<0.05) in the kidney and liver than in controls. SAC and the other cysteine-containing compounds were also found to increase catalase and glutathione peroxidase (GPX) activities in the kidney and liver. When compared with the control and cysteine-treated groups, the cysteine-containing

Sumiyoshi, H. and Wargovich, M. 1989. *Proc. Am. Assoc. Cancer Res.* 30:181. (abs. #718)

Hatono, S., Wargovich, M. et al. 1996. *Carcinogenesis* 17(5): 1041-1044.

Knowles, L. and Milner, J. 1998. *Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement.* Newport Beach, CA. November 15-17, 1998b.

Knowles, L.M. and J.A. Milner. *J. Nutr.*, 131: 1061S-1066S (2001).

Balaseshthil, S and Nagini, S. 2000. *J. Biochem. Med. Biol. & Biophys.* 4: 35-39.

Sundaesan, S, and Subramanian, P. 2003. *S-allylcysteine inhibits circulatory lipid peroxidation and promotes anti-oxidants in N-nitrosodiethylamine-induced carcinogenesis.* *Pol. J. Pharmacol.* 55: 37-42.

SAC significantly inhibited nuclear damage caused by the carcinogen dimethylhydrazine (DMH), thus decreasing the toxicity of this carcinogen. Further, both compounds significantly stimulated the activity of glutathione *S*-transferase (GST) in both the liver and colon. GST is an enzyme known to assist in the detoxification of carcinogens.

Aberrant crypt foci are considered to be the most likely precursors of colon cancer. SAC administration inhibited development in the colon of one third to one half of the foci induced by DMH when given prior to this carcinogen (initiation phase). Further, SAC was found to significantly enhance GST (glutathione *S*-transferase) activity not only in the liver, but also in the proximal and middle small bowel. GST is a detoxification enzyme system in the body. Thus, SAC inhibited the development of pre-cancerous lesions in the colon and enhanced the activity of enzyme systems in the liver and small intestine, which detoxify carcinogens (Hatono et al., 1996).

Knowles and Milner (1998, 2001) found that diallyl disulfide (DADS), a constituent in Aged Garlic Extract and garlic oil, suppresses proliferation of human colon tumor (HCT-15) cells by reducing apoptosis and altering cell division through a block in the G2/M phase of the cell cycle. A marked suppression in p34^{cdc2} kinase activity and depressed protein tyrosine phosphatase (PTPase) activity accompanied the observed G2/M phase arrest. Western blot analysis revealed that 25 and 50 μ M DADS decreased cdc25C phosphatase expression, by 21 and 45%, respectively. DADS exposure caused a dose dependent decrease in p34^{cdc2} protein expression. Suppression of p34^{cdc2} and cdc25C expression likely accounts for the ability of DADS to inhibit p34^{cdc2} kinase activity. Other sulfur compounds found in processed garlic may alter tumor cell proliferation by a similar mechanism.

Sustained Circulatory Antioxidants Depleted by Cancer-Causing Agent

7,12-Dimethylbenz(a)anthracene (DMBA) enhances lipid peroxidation in the circulation. In addition, it significantly depletes circulating antioxidants such as ascorbic acid, vitamin E, reduced glutathione and glutathione peroxidase. Administration of SAC significantly decreased DMBA-induced lipid peroxidation and enhanced the levels of antioxidants.

In a study where SAC was administered to N-nitrosodiethylamine (NEDA)-induced hepatocarcinogenesis, SAC was found to decrease tumor incidence and lipid peroxidation. SAC was also found to increase antioxidant levels by decreasing the formation of free radicals.

Liver Protective Effects of SAC

Dion, M. and Milner, J. 1996. *Exp. Biol.* 96 (abs.)

Fukushima, S. *Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement held in Newport Beach, CA. November 15-17, 1998.*

Fukushima, S., et al., *J. Nutr.*, 131: 1049S-1053S, 2001.

Nakagawa, S., Kasuga, S. et al. 1988. *Phytother. Res.* 1:1-4.

Inhibited Both the Formation and Bioactivation of a Liver Carcinogen

SAC inhibited both the formation and bioactivation of the liver carcinogen nitrosomorpholine (NMOR). Adding SAC to a solution of sodium nitrite and morpholine prevented these two compounds from generating nitrosomorpholine. SAC also prevented NMOR's ability to mutate a cell model.

S-methylcysteine, a constituent in Aged Garlic Extract, suppressed chemically-induced (sodium nitrite and morpholine) liver cancer.

Protected Liver Cells from the Liver Toxins: Paracetamol (Acetaminophen), Carbon Tetrachloride and Bromobenzene

Nakagawa et al (1988) found that SAC protected the liver cells from the liver toxins, Paracetamol and carbon tetrachloride. These compounds induce acute hepatitis. SAC appeared to enhance the activity of glutathione, a detoxifying enzyme, and to act as chemical scavengers. Thus, SAC was found to be more effective than the other chemicals used.

Peng Q, et al. Neuroprotective effect of garlic compounds in amyloid- β peptide-induced apoptosis in vitro. *Med Sci Monit.* 8(8):BR328-337, 2002.

Kosuge, Y. et al. 2003, *Neuroscience.* 122(4): 885-95.

Kim JM, Chang N, Kim WK, Chun HS. 2006. *Biosci Biotechnol Biochem.* 70:1969-71.

Kosuge Y, Koen Y, Minami K, Ishige K, Edagawa Y, Saito H, Ito Y. 2001. 74th Annual Meeting of the Japanese Pharmacological Society. Yokohama (Japan), March 21-23, Abst# P-849.

Kosuge Y, Sakikubo T, Ishige K, Ito Y. 2006. *Neurochem Int.* 49: 285-93.

AGE and SAC were reported to decrease apoptosis by enhancing endogenous antioxidant defenses in a study determining their effects on A β -induced apoptosis and reactive oxygen species (ROS) generation in a pheochromocytoma (PC12) cell line.

Kosuge et al. (2003) found that S-allyl-L-cysteine (SAC) protected differentiated PC12 cells against A β - and tunicamycin-induced neuronal death and also attenuated the A β -induced increase of intracellular reactive oxygen species.

S-Allyl-L-cysteine (SAC), an active organosulfur compound derived from garlic, was found to reduce mortality with lesser incidence of stroke and also to lower the overall stroke-related behavioral score in stroke-prone spontaneously hypertensive (SHRSP) models by dietary administration.

SAC selectively protects amyloid β (A β)-induced neuronal death in hippocampal neurons due, at least in part, to suppression of A β -induced oxidative stress. Mechanistic differences exist between A β -induced cell death in hippocampal neurons and in cerebellar granule neurons.

Kosuge et al. (2006) determined that hippocampal neurons (HPN) were protected from neuronal cell death in the presence of SAC. In contrast, SAC did not exhibit any protective effects on cerebellar granule neurons (CGN). Both neurons found in the endoplasmic reticulum (ER) are vulnerable to neuronal cell death with ER stress. Research has shown ER stress to be an important factor in amyloid β peptide (A β)-induced neurotoxicity and Alzheimer's disease pathology.

Lung Protective Effects of SAC

Mizuguchi S, Takemura S, Minamiyama Y, Kodai S, Tsukioka T, Inoue K, Okada S, Suehiro S. 2006. *Biofactors.* 26:81-92.

Protected Cells from Toxic Carbon Tetrachloride in the Lungs

SAC was found to be effective in reducing CCl₄-induced lung injury through intraperitoneal injection of CCl₄ into models twice a week for 8 weeks; SAC, N-acetyl cysteine or L-cysteine was orally administered everyday for 8 weeks. SAC significantly reduced the increases of transforming growth factor beta, lipid peroxides, AST, and ALT in plasma induced by CCl₄. SAC dose-dependently and significantly attenuated CCl₄-induced systemic inflammation and fibrosis of lung. SAC also prevented the decline of thiol levels, the increase of inducible nitric oxide synthase expression, the infiltration of leukocytes, and the generation of reactive oxygen species in lungs.

SAC Has Been Confirmed to be Bioavailable and Active

Amagase, H. 2000. Inconsistent cholesterol-lowering effects of garlic powder products due to inappropriate standardization. *Phytomed.* 7(2): 51.

Nagae, S., Itakura, Y. et al. 1994. *Planta Med.* 60: 214-217.

Rosen, R. Identification of metabolites of garlic and garlic products in human body fluids, 2nd International Conference on Food Factors (2nd ICoFF), Kyoto (Japan) December 12-17, 1999.

Rosen, R. 2000a. *Phytomed.* 7(2): 51.

Rosen, R., Hiserodt, R. et al. 2000b. *Biofactors*13(1-4): 241-249.

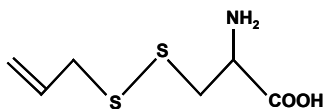
Amagase (2000) indicated that SAC is one of the water-soluble sulfur compounds in garlic derived from γ -glutamyl peptides that increases during extraction. SAC is utilized to standardize AGE. SAC is contended to be the only compliance marker compound for clinical studies involving garlic consumption. Since SAC is present in garlic preparations, has many biological effects, and is confirmed to be bioavailable, it can be considered one of the active principles in garlic preparations.

SAC is a biologically active transformation product from garlic. Nagae et al. found that SAC was rapidly and easily absorbed in the gastrointestinal tract and, then distributed mainly in the plasma, liver, and kidney after oral consumption. Its bioavailability was between 87.2% through 103% in models.

Rosen (1999, 2000a,b) established a procedure for measuring SAC in blood and urine of human subjects after consumption of garlic and garlic products. SAC was easily detected in plasma and urine by HPLC-MS using negative ion atmospheric pressure chemical ionization mode (APCI)-MS. SAC levels in the blood reach levels of 800 ppb in the blood after consumption of three Aged Garlic Extract capsules (Steiner, M., East Carolina University, personal communication, 1998).

Rosen (2000b) showed that allicin is decomposed in stomach acid into various allyl sulfides, disulfides and other volatiles. Thus allicin is not detected anywhere.

S-ALLYLMERCAPTOCYSTEINE (SAMC): A CONSTITUENT UNIQUE TO AGED GARLIC EXTRACT



Koch, H. and Lawson, L.D. 1996. *Garlic the Science and Therapeutic Application of Allium sativum L. and Related Species*. Williams & Wilkins, Baltimore, MD, pp. 104.

Heber, D. 1997. *Am. J. Clin. Nutr.* 66: 425 (editorial).

Kodera, Y. 1997. In: *Nutraceuticals: Designer Foods III Garlic, Soy and Licorice* (Lachance, P.A. ed.) Ch. 11, pp. 95-103. Food and Nutrition Press, Inc. Trumbull, CT.

Aged Garlic Extract contains a unique sulfur-containing compound, known as *S*-allylmercaptocysteine (SAMC). SAMC is produced only during aging and is not present in fresh raw garlic (Koch, 1996; Heber, 1997) or in various garlic preparations (Kodera, 1997). SAMC is a water-soluble compound that has shown an array of effects, as seen in the following studies, including anti-oxidative, liver-protective and anti-carcinogenic effects:

Antioxidative and Radioprotective Effects of SAMC

Pedraza-Chaverri, J. 2004. *S-allylmercaptocysteine scavenges hydroxyl radical and singlet oxygen in vitro and attenuates gentamicin-induced oxidative and nitrosative stress and renal damage in vivo*. *BMC Clinical Pharmacology*. 4.

In a study by Pedraza-Chaverri, SAMC treatment was found to weaken the gentamicin-induced oxidative and nitrosative stress as well as the destruction to the kidneys (nephrotoxicity) *in vivo*. SAMC was found to scavenge hydroxyl radicals and singlet oxygen *in vitro*.

Ide, N., Matsuura, H. et al. 1996a. *Phytother. Res.* 10: 340-341.

Protected Cells from Oxidant Injury

Ide et al. (1996a) found that SAMC demonstrated a scavenging effect on hydrogen peroxide. They also inhibited the chain oxidation induced by a hydrophilic radical initiator. Hydrogen peroxide yields a free radical by reacting with iron or copper (a process called the Fenton reaction). This free radical damages both membranes and DNA and/or induces lipid peroxidation.

Amagase, H. et al. 2000. Ch. 6. *Phytochemicals and Phytopharmaceuticals*. AOCS Press, Champaign, Illinois, pp. 62-78.

Amagase et al. (1999) and Ide et al. (1996b) found that SAMC, decreased emissions of low level chemiluminescence (LLC) initiated by the oxidant t-butyl hydroperoxide in liver tissue. SAMC inhibited LLC emissions 60% at 5 mmol/L and 82% at 10mmol/L, nearly the same effect as the potent antioxidant glutathione. SAMC also scavenged hydrogen peroxide *in vivo*.

Ide, N., Itakura, Y. et al. 1996b. 2nd International Congress on Phytomedicine. September 11-14, 1996. Munich, Germany.

Ide, N. and Lau, B. 1997. *FASEB J.* 11(3): A122/#713

Ide and Lau (1997) found that SAMC could inhibit copper-induced peroxidation of LDL in a concentration dependent manner. Lipid oxidation was determined by measuring TBA-RS.⁵ Further, it was found that when SAMC was preincubated with pulmonary artery endothelial cells, cell damage caused by oxidized LDL was prevented, as indicated by prevention of lactate dehydrogenase release, loss of cell viability and TBA-RS formation.

Anticancer Effects of SAMC

Thomson M, Muslim, A. 2003. *Curr Cancer Drug Targ*, 3: 67-81.

Numerous studies have demonstrated the chemopreventive activity of garlic by using different garlic preparations; including fresh garlic extract, aged garlic extract, garlic oil and a number of organosulfur compounds derived from garlic. Recent research has also focused on the antimutagenic activity of garlic. It has also been observed that AGE, but not the fresh garlic extract, exhibits radical scavenging activity. The two major compounds in aged garlic, SAC & SAMC have the highest radical scavenging ability. Because of this, consumption of garlic may provide protection from cancer development.

Borek, C. 2005. *Antioxidants and the prevention of hormonally regulated cancer. jmhg*, 2(3): 346-352.

SAC and SAMC found in aged garlic extract have been shown to destroy cancer cells by inducing apoptosis, or programmed cell death. SAC and SAMC have been shown to decrease the growth of prostate cancer cells by 80 percent while SAMC has also been shown to inhibit the growth of breast cancer cells, erythroleukemia and colon cancer cells.

Borek, C. 2004. *Int. Cancer Ther*. 3(4): 333-341.

SAMC induces apoptosis in human prostate cancer cells and in breast cancer cells, as well as colon cancer cells by activating caspase 3, inhibits antiapoptotic protein Bcl-2, and disrupts microtubules in cancer cells, preventing further growth.

Borek, C. 2004. *J. Nutr*. 134: 3207S-3209S.

Chu Q, Ling MT, Feng H, Cheung HW, Tsao SW, Wang X, Wong YC. 2006 *Carcinogenesis*. 27(11):2180-9.

Metastatic cancer is one of the main causes of cancer-related death since it rarely responds to available treatments. Using colony-forming, wound-closure as well as matrigel-invasion assays, Chu et al. found that two main water-soluble constituents of the garlic, *S*-allylcysteine (SAC) and *S*-allylmercaptocysteine (SAMC), were able to suppress Pca cell proliferation and invasive abilities through restoration of E-cadherin expression in cancer cells.

Inhibited the Growth of Carcinogen-Induced Tumors of the Breast

Tiwari, R., Pinto, J. et al. 1993. *Breast Cancer Res. Treatment* 27:(1-2): 80.

Tiwari et al. (1993) found that SAMC inhibited the growth and proliferation of transformed human breast cells. They also increased both glutathione-*S*-transferase and peroxidase levels in the non-transformed cells.

Li, G., Qiao, C., et al. 1995. *Oncol. Rep*. 2: 787-791.

Li et al. (1995) also reported that SAMC in Aged Garlic Extract inhibited the growth of transformed human breast cells and increased both glutathione-*S*-transferase and peroxidase levels in the non-transformed cells.

Inhibited the Growth of Carcinogen-Induced Tumors of the Colon

Shirin H., Pinto J. et al. 2001. *Cancer Res*. 61(2):725-731.

Pinto et al. (2001) found that SAMC inhibited the growth of two human colon cancer cell lines (SW-480 and HT-29) at doses similar to that of sulindac sulfide, a well-established colon cancer chemopreventive agent. SAMC also induced apoptosis¹⁷. In addition, SAMC cause a marked increase in endogenous levels of reduced glutathione. SAMC also augmented the growth inhibitory and apoptotic effects of SS when co-administered.

Knowles, L. and Milner, J. 1997. *FASEB J*. 11(3): A422/#2445

Experimental carcinogenesis studies indicate that components of garlic (i.e. allyl sulfides) inhibit both the initiation and promotion stages of tumorigenesis for various types of cancer, including colorectal. These researchers previously reported that SAMC inhibits growth, arrests cells in G₂-M, and induces apoptosis in human colon cancer cells. This study concludes that the garlic-derived compound SAMC exerts antiproliferative effects by binding directly to tubulin and disrupting the MT assembly, thus arresting cells in mitosis and triggering JNK1 and capase-3 signaling pathways that lead to apoptosis.

Xiao D, Pinto JT. Et al. *FASEB J*, 2003, 17(4): A1152, # 7511.1.

Xiao D, Pinto JT, et al. 2003. *Cancer Res* 63: 6825-6837

Xiao et al. had previously reported that *S*-allyl mercaptocysteine (SAMC) inhibited growth, arrested cells in G₂/M and induced apoptosis in SW480 and HT29 human colon cancer cells. Upon examination of this mechanism, in this current study, Xiao et al. (2002) concluded that antiproliferative effects exerted by SAMC work to disrupt microtubule assembly, and cells in mitosis are arrested. Jun kinase (JKN1) and caspase-3 are then triggered, which signals pathways leading to apoptosis.

Xiao D, Pinto JT, Palazzo A, Gundersen GG, Shirin H, Weinstein IB. 2002. *International Research Conference on Food, Nutrition and Cancer*. Washington DC, July 11-12. *J Nutr* 132: 3549S.

OTHER CONSTITUENTS IN AGED GARLIC EXTRACT

Oil-soluble and Water-soluble Organosulfur Compounds

Weinberg, D., Manier, M., et al. 1992. *J. High Resolut. Chromatogr.* 15: 641-654.

Weinberg, D., Manier, M., et al. 1993. *J. Agric. Food Chem.* 41: 37-41.

Dimitrov, N. and Bennink, M. 1997. *Modulation of arachidonic acid metabolism by garlic extracts.* Ch. 21. In: *Nutraceuticals: Designer Foods III Garlic, Soy and Licorice.* Paul P. Lanchance (ed.). Food & Nutrition Press, Trumbull, CT, pp. 199-202.

Gwilt, P. et al. 1994. *Cancer Epidemiol. Biomarkers Prev.* 3:155-160.

Ichikawa M, Ide N, Yoshida J, Yamaguchi H, Ono K. 2006. *J. Agric Food Chem.* 54:1535-40.

Vijayaraghavan M, Wanibuchi H, Takada N, Yano Y, Otani S, Yamamoto S, Fukushima S. 2000. *Jpn J Cancer Res.* 91: 780-5.

Greenblatt DJ, Leigh-Pemberton RA, von Moltke LL. *In Vitro Interactions of Water-Soluble Garlic Components with Human Cytochromes P450.* *J. Nutr.* 2006;136 Suppl 3:S806-9.

Wargovich MJ. *Diallylsulfide and Allylmethylsulfide Are Uniquely Effective among Organosulfur Compounds in Inhibiting CYP2E1 Protein in Animal Models.* *J. Nutr.* 2006;136 Suppl 3:S832-4.

Wargovich MJ. 1998. *Recent Advances of the Nutritional Benefits Accompanying the Use of Garlic as a Supplement.* Newport Beach, CA. November 15-17, pp32.

Aged Garlic Extract is rich in water-soluble compounds and contains small amounts of oil-soluble compounds. Weinberg et al. (1992, 1993) developed methodology to detect, identify and quantify nine oil-soluble organosulfur compounds in Aged Garlic Extract: allyl sulfide, allyl disulfide (diallyl disulfide), allyl trisulfide, allyl methyl sulfide, allyl methyl disulfide, allyl methyl trisulfide, methyl disulfide, methyl trisulfide and ethyl 2 propenesulfinate. It was found that the concentration of most of these constituents increased with time. Some compounds nearly tripled in concentration and others increased by an order of magnitude. The results were confirmed by an independent study of the extract from the National Cancer Institute.

Dimitrov et al. (1997) and Gwilt et al. (1994) also quantified various organosulfur compounds in Aged Garlic Extract.

Ichikawa et al. (2006) developed and validated a simple, rapid, and precise analytical method to determine seven organosulfur compounds that are found in garlic: alliin, isoalliin, methiin, cycloalliin, gamma-L-glutamyl-S-methyl-L-cysteine (GSMC), gamma-L-glutamyl-S-(2-propenyl)-L-cysteine (GSAC), and gamma-L-glutamyl-S-(trans-1-propenyl)-L-cysteine (GSPC). The method consisted of using a one-step sample preparation procedure with NP and RP high performance liquid chromatography, with overall recoveries of all seven organosulfur compounds of 97.1-102.3%.

Vijayaraghavan et al. determined the water-soluble components of garlic, S-methylcysteine (SMC) and cysteine provides inhibitory effects on diethylnitrosamine (DEN)-induced hepatocarcinogenesis, promoted with sodium phenobarbitol (NaPB), at the promotion stage. However, only SMC was able to significantly reduce ODC enzyme activity.

Inhibited the Activity of Human Cytochrome-P450 (CYP) Enzymes

Two water-soluble components of aged garlic extract (AGE), S-methyl-L-cysteine and S-allyl-L-cysteine, at 100 µmol/L reduced CYP3A activity to 20-40% of control.

Inhibited the Levels of Hepatic CYP2E1 Protein

Research results indicate that two organosulfur compounds diallylsulfide and allylmethylsulfide, are effective in significantly reducing levels of hepatic CYP2E1 protein.

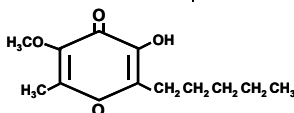
Organosulfur compounds in Alliums (AOSC) are effective in inhibiting carcinogenesis at the initiation stage of esophageal and colon cancers. Inhibitory effects are primarily due to AOSCs ability to reduce CYP2E1 enzyme which activates carcinogens nitrosomethylbenzylamine and azoxymethane responsible for inducing esophageal and colon cancers.

Nishiyama, N., Moriguchi, T. et al. 1996. *Int. Acad. Biomed. Drug Res. Basel. Karger. 11:* 253-258.

Moriguchi, T., Nishiyama, N. et al. 1996. *Phytother. Res. 10:* 468-472.

Matsuura, H. 1997. In: *Nutraceuticals: Designer Foods III Garlic, Soy and Licorice (Lachance, P.A. ed.), Ch 7., pp. 55-69. Food and Nutrition Press, Inc. Trumbell, CT.*

Kokai Tokyo Koho, Japanese Patent H1-252276.



Allixin

Kodera, Y., Matsuura, H. et al. 1989. *Chem. Pharm. Bull., 37:* 1656-1658.

Kodera, Y. et al. 2002. *Chem. Pharm. Bull. 50(3):* 405-407.

Nishino, H., Nishino, A., et al. 1990. *Cancer J. 3:* 20-21.

Yamasaki, T., Lau, B. et al. 1991. *Cancer Lett. 59:* 89-94.

Nishiyama N, Moriguchi T, Katsuki H, Saito H, Matsuura H, Itakura Y. 1996. *116th Annual Meeting of the Pharmaceutical Society of Japan, Kanazawa (Japan), March 27-29. Abst# 27(J1) 10-2. (Japanese)*

Enhanced Nerve Survival

Nishiyama et al. (1996) and Moriguchi et al. (1996) found Aged Garlic Extract's F-4 protein fraction markedly increased the survival of cultured hippocampal neurons, the first clear evidence that Aged Garlic Extract interacts with brain neurons.

Enhanced Growth of Friendly Bacteria *B. bifidum* and *L. acidophilus*

Matsuura (1997) found that the F-4 protein fraction in Aged Garlic Extract enhanced the growth of the beneficial bacteria, *B. bifidum* and *L. acidophilus*. There is an international patent (#H1-252276) for this effect.

Antimicrobial Activity

In 1989, Kodera et al. Isolated a phenolic stress compound from garlic and termed it allixin. Allixin was found to possess weak antimicrobial activity.

Kodera et al. found that allixin, a *de novo* synthesized substance categorized as a phytoalexin, may pose an prohibitory, inhibitory, or post-inhibitory antimicrobial function in garlic. The basis of this conclusion comes from the observation of high accumulation of allixin in necrotic tissue areas after long-term storage.

In vivo Anti-tumor Activity

Nishino et al. found that allixin demonstrated *in vitro* anti-tumor activity and suppressed the promotion of two-stage carcinogenesis *in vivo*. Allixin, inhibited the development of skin cancer induced by the carcinogen, 7,12-dimethylbenz(a)anthracene (DMBA), and the promoter, 12-O-tetradecanoyl (TPA). Nishino et al. suggested that since allixin seems to have no side effects, it might be useful for the prevention of human cancer.

Antimutagenic and Chemopreventive Activity

Allixin showed a dose-related inhibition of histidine+ revertants induced by AFB₁. (AFB₁ is an aflatoxin produced by the molds found on peanuts). Allixin prevented the binding of this carcinogen to calf thymus DNA and reduced the formation of AFB₁-DNA adducts. Allixin also inhibited the formation of carcinogenic metabolites of this aflatoxin and, therefore, the authors suggested that this compound "may thus be useful in the chemoprevention of cancer."

Enhanced Nerve Survival

Nishiyama (1996) has reported the effect of allixin, one of the stress compounds in garlic, on survival of neuron cells using a cell culture system. The neuron cells cannot survive without serum and began to die in 72h. Neuron cells were coincubated with allixin for 48h, and the survival rate and condition of the cells was examined. Results indicate incubation with allixin demonstrated a significant improvement in survival rate in a dose dependent manner, and promoted the branching of cells. In order to reduce the side effects, garlic's derivatives were chemically synthesized and the survival rate of nerve cells was studied. As a result, 2,6-dimethyl-3-hydroxy-4-pyrone (DHP) was found to show similar activity with less toxicity. These two compounds may be useful as external neurotrophics.

Chemistry of AGE

Morihara, N., et al. 2005. *Aged garlic extract inhibits peroxynitrite-induced hemolysis. Redox Report. 10(3): 159-165.*

Amagase H, Rosen R. *Garlic is Effective without Allicin (1) Current Marker Compound for Herbs is Not Marker. ICNPR, July 31-August 4, 2004.*

Ide N, et al. *Antioxidants in processed garlic. 1. Fructosyl arginine identified in aged garlic extract. International Congress Series 1245m 447-448, 2002.*

Ide, N, et al. 2003. *Antioxidants in Processed Garlic: Tetrahydro- β -carboline Derivative in Aged Garlic Extract. American Chemical Society, Ch 22 pg. 250-263.*

Ide N, Ichikawa M, Ryu k, Ogasawara K, Yoshida J, Yoshida S, Sasaoka T, Sumi S, Sumiyoshi H. 2002. *In International Congress Series 1245, The Maillard Reaction in Food Chemistry and Medical Science: Update for The Postgenomic Era. Pp449-50.*

Kodera, Y, et al. 2003. *Garlic Chemistry: Chemical and Biological Properties of Sulfur-Containing Compounds Derived from Garlic. American Chemical Society, Ch. 30, pg. 346-357.*

Thomson M, Ali M. 2003. *Garlic [Allium sativum]: A Review of its Potential Use as an Anti-Cancer Agent, Curr Cancer Drug Targets, Feb 3(1): 67-81.*

Ryu, K, and Ide, N., et al 2003. *Liquid chromatography-mass spectrometry method for fructosylarginine, an antioxidant in the aged garlic extract. American Chemical Society, Ch 23, pg. 264-273.*

Kodera Y, Matsuura H, Sumiyoshi H, Sumi S. 2001. *Joint ACS-ISOFF Symposium on Food Factors for Health Promotion. San Diego, CA April 1-5 Abst #AGFD 108.*

Ichikawa M, Ide N, Ono K. 2006 *J. Agric Food Chem. 54: 4849-54 9*

Morihara, N., et al. found that when AGE was combined with a suspension of erythrocytes, it decreased peroxynitrite-induced hemolysis in a concentration-dependent manner. SAC, found in AGE, was also found to decrease hemolysis. Since peroxynitrite is a strong oxidant, it has been shown to cause vascular or tissue damage. Therefore, AGE and its constituents may be helpful for preventing cardiovascular diseases and may help prevent the damage of membranes in erythrocytes.

Allicin is not active or marker compound in garlic products. AGE uses *S*-allyl cysteine (SAC) instead, since SAC is bioavailable and active in the body. It is reasonable to use such compound for standardization.

Ide, et al. used several in vitro assay systems and high-performance liquid chromatography (HPLC) to determine the antioxidant effects of fructosyl arginine (Fru-Arg), a compound in AGE. The study reported that Fru-Arg forms and is increased during the aging process and plays an important role as an antioxidant.

Ide, et al. used liquid chromatography mass spectrometry (LC-MS) to determine the antioxidant effects of four tetrahydro- β -carboline derivatives. These compounds were found to increase during the aging process and may be an important antioxidant in AGE.

Garlic chemistry has been overviewed by this article. It explains unique characteristics of AGE with a cascade of chemical reactions of garlic. Many unique chemical constituents in AGE were listed and reviewed with pharmacokinetic behavior *in vivo* (in the actual body), which is essential for the true active compounds. AGE has many of these bioavailable compounds in it.

In a review on garlic, SAC and SAMC, present in aged garlic extract, were found to have the highest activity of scavenging free radicals. SAC, in several disease models, was also shown to decrease the growth of both chemically induced and transplantable tumors. This review suggests that garlic consumption may play an important role in the protection from the development of cancer.

Fructosyl-arginine (Fru-Arg) is a unique compound only found in AGE and no other garlic products have this kind of compounds in it. Fruct-Arg has a strong anti-oxidant effect and in this article, state-of-the-art analytical technology, new and sensitive liquid chromatography-mass spectrometry (LC-MS) method has been developed for this unique compound and reported.

The biological activities of water-soluble compounds derived from garlic, such as *S*-allylcysteine (SAC) and *S*-allylmercaptocysteine (SAMC), has become the center of attention because they are odorless and safe. *S*-allyl groups play a significant role in the pharmacological activities of organosulfur compounds derived from garlic based on studies on structure-activity relationships.

Ichikawa et al. (2006) studied the changes in organosulfur compounds in garlic cloves during storage at different temperatures. Results indicated that gamma-glutamyl peptides undergo marked conversion to sulfoxides when garlic cloves are stored at 4 degrees Celsius. They also demonstrated that isoalliin produced enzymatically from γ -L-glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine (GSPC) is chemically converted to cycloalliin and that the cycloalliin content increases when garlic cloves are stored at higher temperatures.

