*Simona Sarafinovska*

# Changes in the Antioxidant Capacity of Garlic in Relation to the Cooking Method

*Garlic (*Allium sativum *L.) is a nutritional plant widely known for its high antioxidant capacity. However, up to date, the knowledge about the impact of temperature on its bioactive properties is very limited. Therefore, we conducted a study to evaluate the difference in antioxidant activity between fresh garlic, commercially available garlic powder, and garlic cooked in four different ways (boiling, frying with and without oil, and baking). The antioxidant capacity was analyzed by a method based upon the radical scavenging effect of the stable free radical DPPH (2,2-diphenyl-2-picryl-hydrazyl). The garlic powder retained the highest antioxidant activity (1.016 mg Eq of ascorbic acid/g), while the samples of boiled garlic and garlic fried with oil were most affected by the cooking method (0.169 and 0.158 mg Eq of ascorbic acid/g, respectively). Baking and frying without oil cause intermediate losses of the radical scavenging activity of the garlic samples (0.314 and 0.382 mg Eq of ascorbic acid/g, respectively). According to the results obtained in our study, fresh garlic and garlic powders are the best way for consummation considering its antioxidant properties. However, to gain better views on the antioxidant activity of garlic, further research should be carried out in the future.*

## Introduction

Nowadays, the use of dietary supplements has increased drastically, with fruit and vegetables being especially marketed as a natural way to improve health. Vegetables and fruits are exceptionally useful in their role as Reactive Oxygen Species (ROS)-scavenging antioxidants. Antioxidants are substances which significantly delay or prevent oxidation of a substrate even at a low concentration compared to those of the substrate (Li *et al*. 2007). Antioxidants found in a variety of foods can inhibit or neutralize the deleterious and harmful effects of ROS, also called free radicals, which are harmful by-products of the aerobic activity of normal cells that occur in the human body on a continuous basis. Antioxidants work by binding with the free radical and providing the extra electron required to make a pair, thus,

*Simona Sarafinovska (1996), Skopje (Macedonia), Nikola Trimpare 31/1-3, eleventh-grade student at Nova International Schools, Skopje, Macedonia*

*MENTORS:*
*Ivan Milenković and Luka Mihajlović, Petnica Science Center*

stabilizing the radical. Free radicals can be formed as a result of exposure to environmental factors such as pollution, pesticides, tobacco smoke, industrial chemicals, and solar radiation. Although they are considered responsible for some beneficial functions within the immune system, those reactive species can attack and damage healthy cells. They have been linked to several diseases and pathological conditions such as: aging, rheumatoid arthritis, diabetes, cardiovascular disease, neurodegenerative conditions (Parkinson's and Alzheimer's diseases) and some types of cancer (Perry *et al*. 2000). Hence, the balance between antioxidants and free radicals is necessary to protect the body against oxidative stress and its adverse effects.

Garlic (*Allium sativum* L.) is a nutritional plant widely known for its medicinal and culinary benefits. Raw garlic is widely used, but this vegetable is also an obligatory part in many cooked dishes (Gorinstein *et al*. 2005). Epidemiologic studies show an inverse correlation between garlic consumption and progression of cardiovascular disease (Williams *et al*. 2005). Some authors reported that garlic helps in regulating plasma lipid levels (Steiner and Li 2001) and increases plasma anticoagulant activity (Ackermann *et al*. 2001). In addition, it has been shown that garlic inhibits enzymes involved in lipid synthesis, prevents lipid peroxidation of oxidised erythrocytes and LDL and increases antioxidant status (Rahman and Lowe 2006). In the past 20 years interest has sparked in the biochemistry behind the healthful properties of garlic, among which are its relatively high antioxidant capacity.

The biological and medicinal functions, such as antimicrobial, hypolipidemic, antioxidant, and antithrombotic properties of garlic, depend on a variety of sulphur-containing compounds, including volatiles such as allicin, nonvolatile water-soluble sulphur compounds such as S-allyl cysteine, and lipid-soluble sulphur compounds such as diallyl sulphide and diallyl disulphide (Koch and Lawson 1996; Kim *et al*., 1997). Recently, many studies reported that antioxidant properties of garlic are mainly attributed to allicin (diallyl thiosulphate), which is formed upon cutting, crushing, or any enzymatic action on the garlic bulb (Li and Xu 2007). Allicin scavenges hydroxyl radicals ($OH^{\bullet}$), and prevents the lipid peroxidation in-vitro in a concentration-dependent manner (Prasad *et al*. 1995; Krest *et al.* 2000). Hence, it is essential that the allicin and other volatile compounds in garlic have to be preserved during processing in order to ensure its medicinal benefits (Abano *et al*. 2011).

It is well known that fruits and vegetables subjected to higher temperature lose a significant part of their bioactivity (Cardelle-Cobas *et al*. 2005; Jiménez-Monreal *et al*. 2009). Additionally, while the antioxidant activity in the liposome system of diallyl sulphide, diallyl disulphide, S-ethyl cysteine, and N-acetyl cysteine derived from garlic was demonstrated by Yin and associates (2002), the same authors reported that this activity was decreased and even lost when the temperature reached 65°C (Yin *et al*. 2002). Up to date, the knowledge about the impact of temperature on the bioactive properties of garlic *in vitro* and *in vivo* is very limited and the results are quite contradictory. Therefore, we decided to determine the effect

of the cooking regime on the antioxidant activity of garlic with a method based upon the use of the stable free radical diphenylpicrylhydrazyl (DPPH).

# Materials and Methods

**Preparation of samples**. Edible portions from fresh garlic (*A. sativum* Linn.) were separated, cleaned, and then divided into six parts, weighing 50 g or 25 g each (using an analytical balance). The samples were then chopped up and stored at room temperature, or stored without being cut.

**Control Sample** (Fresh Garlic). 50 g cleaned and chopped fresh garlic was homogenized in 100 mL ethanol using a blender at high speed, and then filtered using a vacuum filter. The remains in the blender were re-extracted under the same conditions with 100 mL ethanol. Once again, the sample was filtered, and then what is left in the blender was re-extracted under the same conditions with 100 mL ethanol.

**Boiling** (chopped and not chopped). 50 g cleaned and chopped garlic was added to 500 mL of boiling water and cooked in a glass beaker 10 minutes. After cooking, 50 mL of the liquid portion was taken as the sample. The same procedure was followed for the uncut garlic.

**Frying with oil**. 50 g cleaned and chopped garlic was fried in a Teflon frying pan (22 cm) with 10 mL sunflower oil for 8 minutes with occasional stirring. After cooking, the garlic was homogenized in 100 mL ethanol using a blender at high speed, and then filtered using a vacuum filter. The remains in the blender were re-extracted under the same conditions with another 100 mL ethanol and filtered under the same conditions. The filtrate was then once again blended at high speed and filtered under the same conditions.

**Frying without oil**. 25 g cleaned and chopped garlic was fried in a heated Teflon frying pan (22 cm) for 10 minutes with occasional stirring. After cooking, the garlic was homogenized in 50 mL ethanol using a blender at high speed, and then filtered using a vacuum filter. The remains in the blender were re-extracted under the same conditions with another 50 mL ethanol and filtered under the same conditions. The combined filtrate was then once again blended at high speed and filtered under the same conditions.

**Baking**. 25 g cleaned and chopped garlic was cooked in a glass beaker, covered with aluminum foil, 30 minutes at 180°C. After cooking, the garlic was homogenized in 50 mL ethanol using a blender at high speed, and then filtered using a vacuum filter. The remainder in the blender was re-extracted under the same conditions with another 50 mL ethanol and filtered under the same conditions. The combined filtrate was then once again blended at high speed and filtered under the same conditions.

**Garlic powder**. 1 g garlic powder was dissolved in 50 mL ethanol using a magnetic stirrer for 10 minutes. After preparation, all samples were

centrifuged at 5000 rpm 20 minutes at 8°C and the ethanolic and water extracts were stored in the refrigerator until analyzed.

To prepare ascorbic acid stock solution, 50.0 mg ascorbic acid was measured and dissolved in a 50 mL volumetric flask with distilled water. The working standard solutions were prepared by diluting the stock solution in distilled water to obtain final concentrations of: 1, 5, 10, 11, 12, 13, 14, 15, and 30 μg m/L ascorbic acid.

**Preparation of DPPH reagent**. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical solution (0.4 mmol/L) in ethanol was prepared. 47.1 mg DPPH (2,2-diphenyl-1-picrylhydrazyl) was weighed using an analytical balance and dissolved in 300 mL ethanol (Benkeblia 2005).

**Procedure**. The method of Brand-Williams *et al*. (1995) was used with some modifications (Narendhirakannan and Rajeswari 2010; Benkeblia 2005; Othman *et al*. 2011; Lawrence and Lawrence 2011). To 2 mL ethanolic solution of DPPH (0.4 mmol/L) 200 μL of ethanolic or aqueous extract of garlic was added and the mixture was vortexed. The samples were then left to stand for 15 min. The decrease in absorption was measured at the wavelength of 517 nm. The blank solution was prepared in the same manner, with 200 μL of ethanol instead of ethanolic extract or 200 μL of distilled water instead of aqueous extract. Different concentrations of ascorbic acid dissolved in distilled water (1, 5, 10, 11, 12, 13, 14, 15 and 30 μg/mL) were used as the standard.

**Calculation**

$$\text{Percentage of inhibition} = ((A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}) \times 100$$

where $A_{\text{blank}}$ – absorbtion of blank, $A_{\text{sample}}$ – absorbtion of sample.

Absorbance decreases were calculated as DPPH values by comparing with the standard curve created by ascorbic acid (1 μg/mL - 30 μg/mL), and the results were reported as mg ascorbic acid (vitamin C) equivalent per gram of sample (fresh weight).

# Results and Discussion

We evaluated the effect of cooking on the antioxidant properties of garlic with a method which is based upon the use of DPPH. The molecule of DPPH is characterized as a stable free radical because of the delocalization of the valence electrons over the molecule as a whole, which gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 517 nm (Molyneux 2004). When a DPPH solution is mixed with the solution of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color. Representing the DPPH radical by $Z^{\bullet}$ and the donor molecule by AH, the primary reaction is $Z^{\bullet}+AH = ZH+A^{\bullet}$, where ZH is the reduced form and $A^{\bullet}$ is free radical produced (Molyneux 2004).

In the present study, the decrease of the absorbance in the presence of antioxidants can be measured and calculated as the percentage of DPPH inhibition. The values for the percentage of inhibition obtained from different

garlic extracts were compared with the standard curve constructed using ascorbic acid working solutions, and the results were reported as mg ascorbic acid (vitamin C) equivalent per gram of sample (fresh weight).

The decrease in absorption was measured at the wavelength of 517 nm for four dilutions in the ratios of 1:1, 1:2, 1:3, and 1:4 (with ethanol or distilled water for ethanolic or aqueous extracts, respectively). The optimal absorbance was obtained at the ratio of 1:3 for the ethanolic extracts, while the aqueous extracts were measured undiluted.

The linearity of the method was studied in the concentration range from 1 μg/mL up to 30 μg/mL ascorbic acid. The method of least squares was used to calculate a best straight line relationship and the following regression equation was found by plotting the percentage of DPPH inhibition ($y$) versus the concentration of ascorbic acid ($x$) expressed in μg/mL:

$$y = 1.4268x + 0.969; R^2 = 0.9953$$

where $x$ is the ascorbic acid concentration, expressed in μg/mL, while $y$ is the percentage of DPPH inhibition.

The resulting parameters were used to estimate mg ascorbic acid (vitamin C) equivalent per gram of sample (fresh weight).

The $IC_{50}$ value for ascorbic acid found was 34.4 μg/mL, which is in agreement with the value reported previously (34.1 ± 9.3 μg/mL) by Thambidurai *et al*. (2010).

In the present study, we have evaluated the free radical scavenging activity of ethanolic and aqueous extracts of garlic before and after cooking. The results are expressed as mg ascorbic acid equivalent of gram garlic sample and they are presented in Table 1. Among the six extracts tested for the in vitro antioxidant activity using the DPPH method, the highest antioxidant activity was shown by garlic powder, while the lowest one was found in the boiled garlic extract (10 minutes, not chopped) and fried with oil (8 minutes, chopped).

Table 1. Antioxidant activity of the samples presented as percentage of DPPH inhibition and mg equivalent of ascorbic acid per gram of garlic sample

| Method of cooking | Sample concentration (mg/mL)* | DPPH inhibition perc. (mean ± SD) | mg Eq of ascorbic acid/g garlic (mean ± SD) |
|---|---|---|---|
| Fresh | 5.05 | 7.0 ± 1.35 | 0.845± 0.065 |
| Boiled (not chopped, 10 minutes) | 22.73 | 6.4 ± 0.24 | 0.169±0.020 |
| Fried with oil (chopped, 8 minutes) | 7.58 | 2.6 ± 1.07 | 0.158±0.018 |
| Fried without oil (chopped, 10 minutes) | 7.58 | 5.0 ± 1.52 | 0.382±0.058 |
| Powder | 0.61 | 1.8 ± 0.50 | 1.016±0.291 |
| Baked (chopped, 30 minutes at 180°C) | 7.58 | 4.3 ± 1.00 | 0.314±0.011 |

*Concentration of the garlic was calculated from the fresh weight, except for the garlic powder, where the dry weight is considered

The scavenging activity of the garlic powder was found higher when compared to the fresh garlic. This result could be expected and explained considering the relatively high moisture content of fresh garlic (about 70%) (Abano *et al*. 2011). The higher water content of fresh garlic increased the mass of the samples, without adding to the amount of active antioxidant substances; hence, although the powdered sample weighed only 1 g, its concentration of active substances was greater than in fresh samples, as the powdered garlic contained less water.

Boiled and fried garlic partially loses its antioxidant activity. This may be explained in a way that heating at high temperatures can destroy the enzyme, allinase, which is responsible for converting alliin to allicin. A lower result in the boiled uncut garlic may be expected because the it was prepared without chopping, while allicin is formed only when raw garlic is chopped or crushed. Furthermore, with the boiling process, the bioactive lipid-soluble sulphur compounds such as diallyl sulphide and diallyl disulphide which are not present in the aqueous extracts are lost (Ali 1995). Our findings were in agreement with the previous ones that reported the decrease in antioxidant capacity of boiled garlic at 100°C (Gorinstein *et al*. 2006; Jastrzebski *et al*. 2007).

Interestingly, according to our results the baked garlic (chopped, 30 minutes at 180°C) and garlic fried without oil (chopped, 10 minutes) retained more antioxidant activity when compared to boiled garlic. During the baking or frying process, non-enzymatic browning reactions including Maillard reaction, caramelisation, and chemical oxidation of phenols could occur. The antioxidant activity of the products from the Maillard reaction (Osada and Shibamoto 2006) and caramelisation have been reported. According to the previously published reports (Benjakul *et al*. 2005), when browning pigments were formed some of the antioxidant capacity of the heated brown garlic could be regained as a result of certain compounds being created during the browning reactions. The browner garlic expressed higher antioxidant capacity, but at the end of the heating period at 121°C, the antioxidant capacity started to decrease (Benjakul *et al*. 2005).

According to the results obtained in our study, fresh garlic and garlic powders are the best way to consume garlic considering its antioxidant properties. Boiling of the garlic could lead to the greatest losses; while baking and frying cause intermediate losses of its scavenging activity. However, further research about the impact of cooking on chemical efficacy in vivo should be carried out in the future.

# Conclusion

The goal of the study was to determine the differences in antioxidant activity between garlic samples cooked with different methods, seeing as previous studies had concluded the high antioxidant content of the vegetable, yet remained ambiguous about the effects of cooking on this property. The obtained results show that, corresponding to other studies, fresh garlic contains the highest amount of allicin and other compounds with radical-

-scavenging properties; however, garlic powder was also shown to have high antioxidant properties. The samples most affected by the cooking were the chopped boiled garlic and fried garlic, which agrees with other studies' findings. Especially interesting were the results of baked garlic, garlic fried without oil and boiled garlic which was uncut, as these samples retained more of their antioxidant activity, although it was notably lower than that of raw garlic. Thus, future research could be conducted on these specific methods of cooking, but also to add to the conclusiveness of the results obtained in the present study.

## References


Abano E. E., Ma H., Qu W. 2011. Effects of Pretreatments on the Drying Characteristics and Chemical Composition of Garlic Slices in a Convective Hot Air Dryer. *Journal of Agriculture and Food Technology*, **1** (5): 50.

Ackermann R., Mulrow C., Ramirez G., Gardner C., Morbidoni L., Lawrence V. 2001. Garlic shows promise for improving some cardiovascular risk factors. *Archives of Internal Medicine*, **161**: 813.

Ali M. 1995. Mechanism by which garlic (Allium sativum) inhibits cyclooxyganase activity. Effect of raw versus boiled garlic extract on the synthesis of prostanoids. *Prostaglandins Leucotrienes and Essential Fatty Acids*, **53**: 397.

Benjakul S., Visessanguan W., Phongkanpai V., Tanaka M. 2005. Antioxidant activity of caramelisation products and their preventive effect on lipid oxidation in fish mince. *Food Chemistry,* **90**: 231.

Benkeblia N. 2005. Free-Radical Scavenging Capacity and Antioxidant Properties of Some Selected Onions (*Allium cepa* L.) and Garlic (*Allium sativum* L.) Extracts. *Brazilian Archives of Biology and Technology*, **48** (5): 753.

Brand-William W., Cuelier M., Berset M. E. 1995. Use of free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und Technologie*, **28**: 25.

Cardelle-Cobas A., Moreno F. J., Corzo N., Olano A., Villamiel M. 2005. Assessment of initial stages of Maillard reaction in dehydrated onion and garlic samples. *Journal of Agricultural and Food Chemistry*, **53**: 9078.

Gorinstein S., Drzewiecki J., Leontowicz H., Leontowicz M., Najman K., Jastrzebski Z., Zachwieja Z., Barton H., Shtabsky B., Katrich E., Trakhtenberg S. 2005. Comparison of the bioactive compounds and the antioxidant potentials of fresh and cooked Polish, Ukrainian and Israeli garlic. *Journal of Agricultural and Food Chemistry*, **53**: 2726.

Gorinstein S., Leontowicz H., Leontowicz M., Drzewiecki J., Najman K., Katrich E., Barasch D., Yamamoto K., Trakhtenberg S. 2006. Raw and boiled garlic enhances plasma antioxidant activity and improves plasma lipid metabolism in cholesterol fed-rats. *Life Sciences,* **78**: 655.

Jiménez-Monreal A. M., García-Diz L., Martínez-Tomé M., Mariscal M., Murcia M. A. 2009. Influence of cooking methods on antioxidant activity of vegetables. *Journal of Food Sciences,* **74** (3): H97.

Jastrzebski Z., Leontowicz H., Leontowicz M., Namiesnik J., Zachwieja Z., Barton H., Pawelzik E., Arancibia-Avila P., Toledo F., Gorinstein S. 2007. The bioactivity of processed garlic (*Allium Sativum* L.) as shown in vitro and in vivo studies on rats. *Food and Chemical Toxicology,* **45**: 1626.

Kim S. M., Kubota K., Kobayashi A. 1997. Antioxidative activity of sulfur-containing flavor compounds in garlic. *Bioscience, Biotechnology, and Biochemistry*, **61**: 1482.

Krest I, Glodek J., Keusgen M., 2000. Cysteine sulfoxides and alliinase activity of some Alliumn species. *Journal of Agricultural and Food Chemistry*, **48**: 3753.

Koch H. P., Lawson L. D. 1996. *Garlic: the science and therapeutic application of* Allium sativum *L. and related species*. 2nd Ed. Baltimore: Williams & Wilkins

Li H. B., Cheng K. W., Wong C-C., Fan K. W., Chen F., Jiang Y. 2007. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chemistry*, **102** (3): 771.

Li Y., Xu S. 2007. Preparation of Garlic Powder with high allicin content. *Agricultural Sciences in China*, **6** (7): 890.

Lawrence R, Lawrence K. 2011. Antioxidant activity of garlic essential oil (Allium Sativum) grown in north Indian plains. *Asian Pacific Journal of Tropical Biomedicine*, **1** (1): S51.

Molyneux P. 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology*, **26** (2): 211.

Narendhirakannan R. T., Rajeswari K. 2010. In vitro antioxidant properties of three varieties of *Allium sativum* L. extracts. *E-Journal of Chemistry*, **7** (S1): S573.

Osada Y., Shibamoto T. 2006. Antioxidative activity of volatile extracts from Maillard model system. *Food Chemistry,* **98**: 522.

Othman S. F. C., Idid S. Z., Koya M. S., Rehan A. M., Kamarudin K. R. 2011. Antioxidant study of garlic and red onion: a compara-

tive study. *Pertanika Journal of Tropical Agricultural Science*, **34** (2): 253.

Perry R. J., Watson P., Hodges J. R. 2000. The natures and staging of attention dysfunction in early (minimal and Mill.) Alzheimer's disease relationship episodic and semantic memory impairment. *Neuropsycholgia*, **38**: 252.

Prasad K., Laxdal V. A., Yu M., Raney B. L. 1995. Antioxidant activity of allicin, an active principle in garlic. *Molecular and Cellular Biochemistry,* **148**: 183.

Rahman K., Lowe G. M. 2006. Garlic and cardiovascular disease: a critical review. *The Journal of Nutritio*n, **136**: 736S.

Steiner M., Li W. 2001. Aged garlic extract, a modulator of cardiovascular risk factors: a dose-finding study on the effects of AGE on platelet functions. *The Journal of Nutrition*, **131**: 980S.

Thambidurai M., Rajesh P., Balamurugan B., Rajesh V. 2010. *In-vitro* anti-oxidant and anti-microbial study on Cassia auriculata Linn. *International Journal of Pharma and Bio Sciences*, **1** (2): 1.

Williams M. J. A., Sutherland W. H. F., McCormick M. P., Yeoman D. J., de Jong S. A., 2005. Aged garlic extract improves endothelial function in men with coronary artery disease. *Phytotherapy Research*, **19**: 314.

Yin M. C., Hwang S. W., Chan K. C. 2002. Nonenzymatic activity of four organosulfur compounds derived from garlic. *Journal of Agricultural and Food Chemistry*, **50**: 6143.

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*Simona Sarafimovska*

## Promena antioksidativnog kapaciteta belog luka u zavistosti od metoda termičke obrade

Beli luk (*Allium sativum* L.) je nutritivna biljka poznata po visokom sadržaju antioksidanasa. Međutim, saznanja o uticaju temperature na njegove bioaktivne karakteristike su veoma ograničena. Ovo istraživanje sproveli smo sa ciljem da procenimo razliku antioksidativne aktivnosti između svežeg belog luka, komercijalno dostupnog belog luka u prahu, i belog luka spremljenog na četiri različita načina (kuvanjem, prženjem sa i bez ulja, kao i pečenjem). Antioksidativna aktivnost uzoraka analizirana je na osnovu reaktivnosti potencijalnih antioksidanasa sa stabilnim, slobodnim 1,1-difenil-2-pikril-hidrazil radikalom (DPPH). Beli luk u prahu zadržao je najveću antioksidativnu aktivnost (ekvivalentnu 1.016 mg

askorbinske kiseline po gramu), dok su uzorci kuvanog luka i luka prženog u ulju bili najviše pogođeni načinom obrade (aktivnost ekvevalentna 0.169 i 0.158 mg askorbinske kiseline po gramu, respektivno). Pečenje i prženje bez ulja izazvali su umereni gubitak antioksidativne aktivnosti uzoraka (0.314 i 0.382 mg askorbinske kiseline po gramu, respektivno). Prema rezultatima dobijenim u našoj studiji, sveži beli luk i beli luk u prahu su najbolji način konzumacije sa aspekta njegove antioksidativne aktivnosti. Međutim, da bismo dobili više saznanja o antioksidativnoj aktivnosti belog luka, potrebno je sprovesti dalja istraživanja.