Antioxidant properties of raw garlic (*allium sativum*) extract

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Abstract: Garlic has been a favorite additive in food for many years in various cultures. It is known that garlic (*Allium sativum*) possesses antimicrobial, antiprotozoal, antimutagenic, antiplatelet and antihyperlipidemic properties. Allicin, a thiosulfinate extract of garlic, has been presumed to be a very strong antioxidant. High performance liquid chromatography (HPLC) analysis of raw garlic extract was not conclusive to determine allicin's presence. However, using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging methods to determine the antioxidant activity of raw garlic extract shows a color change from deep violet to yellow, indicating antioxidant activity. Thus, raw garlic can be a source of antioxidant based on the results of the DPPH scavenging analysis.

Keywords: Additive, a*llium sativum,* allicin, antioxidant and scavenging

Introduction

Antioxidant compounds in food are found to have a health-protecting factor. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Garlic (*Allium sativum*) has been used in world cuisines as well as in herbal medicine for thousands of years and, at times, has been claimed to help prevent everything from high cholesterol to cancer. No clinical trials have been performed with allicin and it was never developed into a drug or commercial product due to its instability, its inability to be absorbed and its offensive odour.

Allicin is the main biologically active component of freshly crushed garlic (*Allium sativum*) cloves. It is produced by the interaction of the non-protein amino acid alliin with the enzyme alliinase. All of the background research involving allicin has been done on either garlic powder from different manufacturers (Lawson *et.al*. 1991) or processing the garlic cloves through many different chemicals in order to obtain allicin. The instability of thiosulphinates leads to rapid decomposition in the oven of a gas chromatograph, even at moderate temperatures, and while indirect quantitation has been reported, the methods suffer from significant limitations.

A number of researchers have since reported on HPLC analysis of alliums and whilst there are significant differences in the techniques employed, HPLC has been shown to provide a reliable method of measuring what is actually present in garlic tissue and of avoiding the problems of erroneous compounds associated with GC. In this research, the goal was to obtain a crude extract of garlic juices that contained allicin, and to visually observe the contents of this juice as well as the scavenging activity of allicin.

Methods and Materials

Materials

The garlic cloves were purchased from the Giant Supermarket, distributed by Pacific Sun and was grown in Malaysia.

Methods

Garlic cloves were blended with HPLC Grade water in a ratio of 5 mL of water per 1 gm of garlic (Chowdhury *et al.* 2008). The blended mixture was allowed to stand for 10 min in order to ensure a complete enzymatic reaction of alliin with allinase. The mixture was first filtered with filter paper through a Buckner funnel, then centrifuged at 3,500 rpm for 30 seconds, and finally filtered again with a syringe filter, to ensure that no garlic particles would obstruct the column. HPLC mobile phase, in this analysis, was set with Methanol and water, at a 60:40 ratio of water to methanol. UV wavelength was set at 254 nm. Initial volume of 20 micro liters, flow of 1 microliter per min and maximum pressure of 350 Barr at ambient temperature was set as the conditions for the HPLC analysis. The HPLC conditions were set to be as best to accommodate the available chemicals/materials as well as allicin's stability.

DPPH scavenging

Garlic cloves were crushed with mortar and pestle, with addition of water, at the same ratio of water to garlic as with HPLC analysis stated above. It was filtered, then centrifuged and finally pushed through the syringe filter to prevent particles of garlic from affecting the study. The solution was allowed 10 min to complete the enzyme reaction.

DPPH and Trolox solutions were prepared daily, a 1:1 ratio of garlic sample was mixed with DPPH; where within seconds the scavenging activity was visible to the naked eye. Fifteen min after 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was added, the sample was placed into a spectrophotometer and absorbance was evaluated between 400 and 750 nm (Prakash *et al*., ;Marksen *et al*., 2007; Molyneux, 2004). Trolox solution was also mixed with DPPH, at a 1:1 ratio, in order to produce the positive control for these samples. DPPH solution alone was used as a negative control.

Results and Discussion

Garlic (*Allium sativum*) has been used as a remedy and health-promoter for 5,000 years. The chemistry of garlic is extremely complex, but research has shown that it is the unusual organosulfur compounds relatively unique to garlic that promote its broad range of lipid-lowering, antithrombotic, anti-blood coagulation, anti-hypertension, anticancer, antioxidant, and antimicrobial effects. The most well-known and widely studied garlic compound is allicin. When fresh garlic cloves are crushed or chopped, or garlic powder that has been carefully dried is added to water, allicin is produced in seconds. Allicin and other thiosulfinates are somewhat unstable, but dilution and dissolving in water can greatly improve their stability. Allicin can decompose into a broad range of compounds, including *S*-allylmercaptocysteine, allylmercaptan, diallyl disulfide, allylmethyl disulfide, vinyldithiins, ajoene, and possibly allylsulfinic and allylsulfonic acid.

Analysis of allicin has been difficult because of its instability. Direct GC determination has not been achieved because allicin undergoes rapid decomposition in the oven of a GC, even at moderate temperatures. It had been attempted once during this investigation, and the GC-MS was not able to detect anything, at 0.1µL of sample to 0.9 µL of Heptane. The most promising method for analysis of allicin has been found to be reversed-phase HPLC (Lawson *et al.*, 1991).

The area under the peak of the HPLC graph is proportional to the amount of a certain chemical which has passed the detector while the retention time is indicative of the size of the certain molecule. In this study, our results (Figure 2) are in general agreement with the results published by Rabinkov *et al*. (1997) as shown in Figure 1. Possible reasons for differences in the peak area could be due to the difference in mobile phases, the HPLC conditions and the type of column used, as well as the possibility that because of allicin's instability, even the ambient temperature and pressure causes it to further decompose.

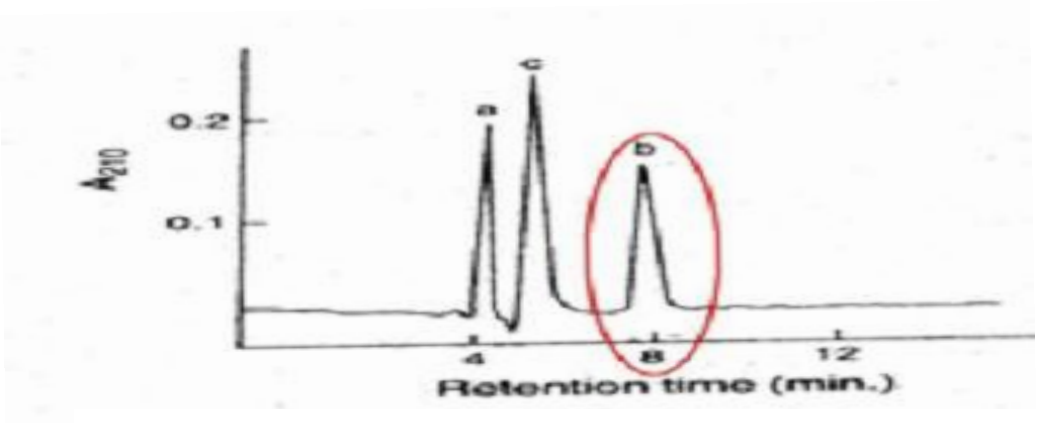

Figure 1. Published article (Rabinkov *et al.*, 1997), graph and specifications of allicin HPLC method

In this study, the garlic powders were blended in Ultrashear homogenizer for one min using 10 mL of water per gram of garlic. The homogenate was allowed to stand at room temperature for five min, filtered and injected directly into the HPLC. Elsewhere, Rosen *et al*. (2001) found that isocratically using the mobile phase of ACN/H_2O at 30:70 and with quantitation at 195 nm can be used to determine the presence of allicin. Though various methods have been shown to be able to be use in extracting allicin, none of them are applicable to the general public. The extraction method used in this study is considered to be suitable for application by the general public with minimum facilities. Garlic itself has many complex substances that are effective against diseases.

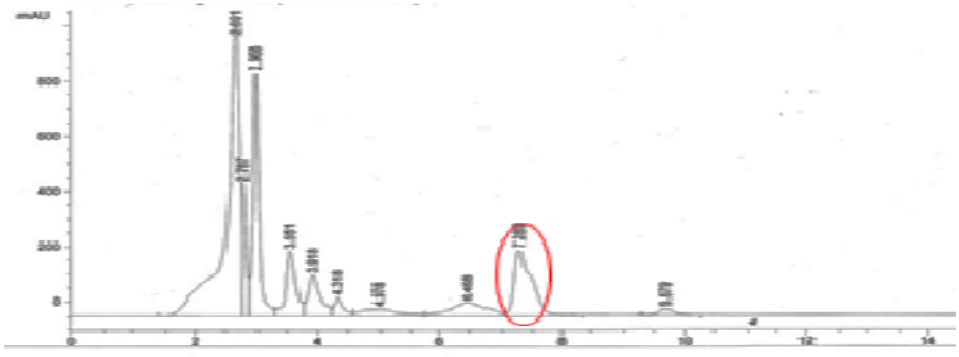

Figure 2. HPLC graph and peaks of retention times

Parasad *et al*. (1995) report that the antioxidant activities of Allicin through HPLC should be complemented with other chemicals to observe its scavenging activities. In this study, the scavenging activities were determines by DPPH testing, which was found to be a rapid, easy and economical method to measure antioxidant activities. Antioxidant activity of the sample can be

merely viewed by the naked eye. When DPPH was placed into a vial of the sample, within seconds, the deep purple colour of DPPH started disappearing, and within less than ten min, the sample and DPPH mixture turned a pale yellow, almost clear colour. When the samples were analysed using the spectrophotometer, it was graphically visible that at 520 nm, the absorbance of DPPH was centred in the negative control, and completely absent at the positive control of Trolox, as well as the sample itself in Figure 3. DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to assess antioxidant activity of foods. DPPH process can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of a sample (Parasad *et al*., 1995). Hence, from the results obtained in this study, the DPPH test is a reliable method to determine the antioxidant ability of raw extract from garlic.

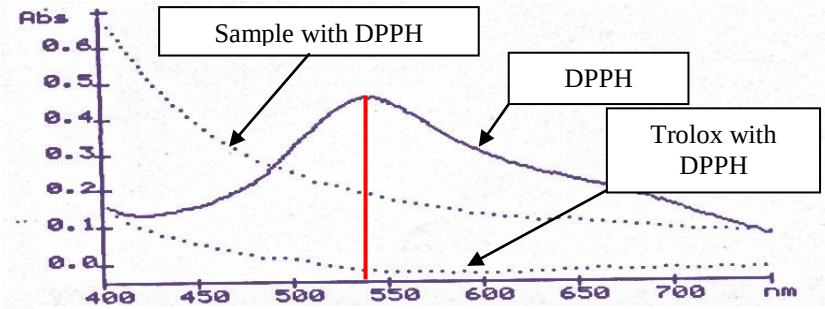

Figure 3. Spectrophotometric analysis of garlic antioxidant activity (against Methanol).

Conclusion

The DPPH test informed of the antioxidant ability of garlic as a whole and the HPLC analysis also give conclusive results and these studies should be furthered due to some research that has shown the inability of bacteria to develop resistance to an antimicrobial agent such as allicin. This research can be beneficial to inform the general public that garlic is an antioxidant, which can quickly act to remove the oxygen free radicals. This may improve the life span as well as the quality of life of people.

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