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Biological Functions and Bioactive Compounds of Consumed Dried Garlic Sheet Versus Fresh Garlic Cloves

Hesham A. Eissa[1], Shreef G. N. Gabrial[2], Nadir. A. S.[1], Mostafa T. Ramadan[1], Atef A. Abou-Zaid[1] and Wafaa A. Ibrahim[1]

[1]Food Technology Department, Food Industries and Nutrition Research Institute, National Research Centre, Cairo, Egypt.
[2]Nutrition and Food Science Department, Food Industries and Nutrition Research Institute, National Research Centre, Cairo, Egypt.

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ABSTRACT

Objective: This study was carried out to evaluate the effects of the drying process on garlic during the production dried garlic sheet, regarding the concentration and activity of its bioactive compounds, and its biological functions represented by the anti-hyperlipidemic and antihypertensive activity. **Methods:** Fresh garlic cloves were dried into sheet according to the process shown in the flow diagram. Blood samples withdrawn from study subjects were analyzed to determine the effect of bioactive compounds in garlic samples on the biochemical parameters. **Results:** Total polyphenol concentration and antioxidant activity measured with the DPPH method were higher in the dried garlic sheets compared with fresh garlic cloves samples. In addition, carotenoids, chlorophyls A, chlorophyls B and chlorophyls AB concentrations were higher in dried garlic sheet than in fresh garlic cloves. However, vitamin C was at a lower level in dried garlic sheet than in fresh garlic cloves, furthermore dried garlic sheet had a significant hypolipidemic effect as compared to fresh garlic cloves, except for the HDL level. As regards the blood pressure, patients that consumed dried garlic sheet had a significant reduction in systolic as well as diastolic blood pressure. **Conclusions:** All selective bioactive compounds (total polyphenol, carotenoids, chlorophyll (A, B and AB) and antioxidant activity) were increased in dried garlic sheet powder as compared to fresh garlic cloves, except vitamin C content. It is worth adding that the current results suggest that the biological functions of dried garlic sheet powder exerted a beneficial effect in maintaining cholesterol, LDL, triglycerides and blood pressure in an acceptable range while it had a lesser effect on HDL.

Keywords: garlic sheet; drying; bioactive; biological; hypolipidemic; hypotensive.

1. Introduction

Garlic is the second most common allium plant grown in the world after shallots. Garlic has been consumed and used widely in the food and pharmaceutical industries as a fresh form or in as dried garlic powder form. Garlic is usually added for its flavour, aroma, and spicy taste in the food processing industry (Pokorný *et al.,* 2001). The properties of garlic have been widely studied as a therapeutic ingredient such as antibacterial, antiviral, anti-fungal, anti-thrombotic, antibiotic, anticancer, antioxidant, immunomodulatory, anti-inflammatory, and hypoglycemic effect (Batiha *et al.,* 2020).

Organosulfur and phenolic compounds as antioxidants in garlic play an important role in preventing cell and organ damage from the oxidation process (Abdel-Gawad *et al.,* 2014; (Sharma *et al.,* 2012 and Collin, 2019).

The antioxidant activity of bioactive compounds in vegetables and fruits, including garlic, can be affected by some factors such as processing and storage, presence of food additives, and interactions with other nutrients (Pedraza-Chaverrí *et al.,* 2007). For example, thermal processes can decrease or

Corresponding Author: Hesham A. Eissa, Food Technology Department, Food Industries and Nutrition Research Institute, National Research Centre, Cairo, Egypt.
E-mail: heshamin62@gmail.com

even increase antioxidant activity depending on whether the polyphenol antioxidant compounds are degraded or conversely the of antioxidant products formation as a result of the release of aglycones and Maillard reaction formed during the thermal process and storage (Yilmaz and Toledo, 2005; Indiarto *et al.,* 2019). The drying treatment of garlic has a positive biological impact on the presence of the organosulfur compounds it contains. The drying process increased the concentration of bioactive compounds in plants and certain foods (such as fruits, vegetables, nuts, oils, and whole grains).

Garlic (*Allium sativum* L. fam. Alliaceae) is a common ingredient in food recipes and added spices (Aviello et al.; 2009). Many reports within the literature indicate that A. sativum has various functions varying from Pharmacological effects like antihypertensive and antihypercholesterolemia, cardioprotective, anticoagulant, hypoglycemic, antibacterial, antineoplastic.

Cardiovascular disease (CVD), consisting of coronary heart condition, stroke, coronary failure, peripheral artery disease, and a spread of other cardiovascular diseases, is that the leading cause of mortality worldwide and contributes significantly to reduced quality of life (Gregory *et al.,* 2020).

CVD risk factors include many factors. Elevated serum cholesterol is a crucial risk factor for the development of heart and cerebrovascular diseases. Similarly, an increased platelet response to drugs clearly poses a risk of thromboembolism within the arterial circulation. It is worth noting that elevated levels (LDL) are associated with a statistically increased frequency of developing heart disease (George *et al.,* 2019).

Uncontrolled hypertension remains the leading cause of death worldwide, accounting for 10.4 million deaths annually during a review of global data; an estimated 1.39 billion people had hypertension in 2010 (Thomas *et al.,* 2020).

A great deal of interest in garlic's role in reducing cardiovascular risk has risen recently, when the cardiovascular protective and anti-atherosclerotic effects of garlic were extensively studied. The biologically active constituents of garlic have been shown to have a wide range of cardioprotective effects, including the lowering total and LDL cholesterol, raising HDL cholesterol, lowering arterial blood pressure in patients with mild to moderate hypertension, improving fibrinolysis, and stopping platelet aggregation (Christie *et al.,* 2001).

Due to the smell and taste of fresh garlic cloves, many people would not like to take garlic directly into their diet for a long period, an option to choose dehydrated garlic sheet in the form of garlic powder can be favorable. Hence, a preparation of dried garlic was prepared to examine the possible efficacy in comparison to fresh garlic.

As dehydrated garlic lasts longer than fresh garlic cloves that with time begin to sprout. Also dehydrated garlic is convenient to use and doesn't require peeling and chopping. Dried garlic improves the flavour and taste of any prepared meal and simply needs to be lightly toasted with oil or butter or lightly browned on its own for a little period of time.

The main aim of this research is to summarize the effects of the drying process of garlic as dried garlic sheet on the concentration and activity of its bioactive compounds, its biological functions as represented by the anti-hyperlipidemic and antihypertensive activity when compared to fresh garlic cloves.

2. Material and Methods

2.1. Plant Material

Egyptian garlic was grown from air bulbs on the experimental farm in the institute of agronomy crops at agricultural Research Institute, Giza, Egypt. During the growing season, no chemical protection was applied due to the absence of signs of pests and disease. Fresh samples of whole garlic plants, including bulbs, were harvested in May, June, and July (mature plants). The plants were thoroughly cleaned. Samples were stored in a suitable dried room at food technology Lab (NRC, Giza, Egypt). Some of the fresh samples were used to prepare dried garlic sheet, to be analyzed for physical and chemical analysis, as described below. Dried garlic sheets were prepared in duplicate.

2.2. Preparation of dried garlic sheet

The basic technique for preparing garlic puree is to peel and then remove the husk from the garlic cloves. The garlic cloves are then pureed as required before drying. Figure 1 describes the 'cold break method' of the garlic cloves that are first pureed, blended for 5 minutes and then pureed again. Common drying methods used for drying garlic sheet include oven-drying (including convection / fan forced)

(Raab and Oehler 1999). The garlic puree was spread into thin layers (1.8 mm) and dried in an air ventilation convection oven at 40 °C for overnight, as shown in the flow chart for dried garlic sheet making process. The dried garlic sheet can then be treated by adding suitable additives such as sodium metabisulphite (SO_2) with 0. 0 5 % to improve and maintain of gold garlic sheet (Che Man *et al.,* (1992), Perera, (2005) and Vijayanand *et al.* (2000). Che Man and Sin (1997) proposed that extended boiling times can destroy the enzyme that causes enzymatic browning. Chan and Calvetto (1978) stated that reducing the sugars involved in the browning process can also be effective in improving the end product.

Flow Chart for dried garlic sheet making process

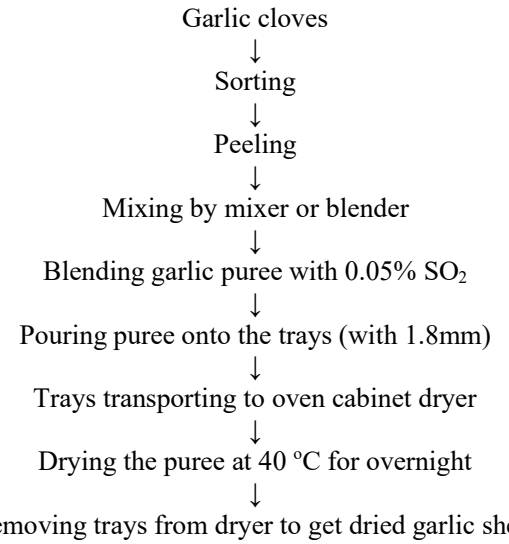

Fig. 1: Process flow diagram of the dried garlic sheet making process.

2.3. Micronutrient or Bioactive compounds (Vitamin C, Total Polyphenol Content, Carotenoids, chlorophyll (A, B and AB) and Antioxidant Activity Concentration determination

2.3.1. Vitamin C Content determination

Vitamin C was analyzed using the A.O.A.C. method (2006). The titrant was prepared with 50 mg of 2, 6-Dichloroindophenol Na salt and 42 mg of sodium bicarbonate (NaHCO3) in 50 mL of water. The solution was diluted to 200 mL with distilled water. The extracting solution was prepared with 15 g of metaphosphoric acid and 40 mL of acetic acid and then diluted to 500 mL with distilled water. Solutions were stored in amber bottles at 4EC. A 100 mL aliquot of WGJ, CJ and their blends were added to 100 mL of the extracting solution and then filtered using a No.1 filter paper (Whatman, Maidstone, England). The solution was then titrated with the titrant until the solution turned bright pink for at least 5 sec. A standard curve was created using pure ascorbic acid (Sigma Aldrich, St. Louis, MO). Vitamin C retention was calculated using Eq.1.

Retention % = (Ascorbic acid (mg)/ 100 mL juice after treatment) / (Ascorbic acid (mg)/ 100 mL juice before treatment) ×100----------(Eq.1).

2.3.2. Total phenolic content determination

The total phenolic contents were determined according to the Folin-Ciocalteu procedure by Zilic *et al.* (2012). Briefly, the extract (100 µL) was transferred into a test tube and the volume was adjusted to 3.5 mL with distilled water and oxidized with the addition of 250 µL of Folin-Ciocalteu reagent. After 5 min, the mixture was neutralized with 1.25 mL of 20% aqueous sodium carbonate (Na_2CO_3) solution. After 40 min, the absorbance was measured at 725 nm against the solvent blank. The total phenolic contents were determined by means of a calibration curve prepared with gallic acid and expressed as milligrams of gallic acid equivalent (mg GAE) per g of sample. Additional dilution was done if the absorbance value measured was over the linear range of the standard curve. The calibration curve was

prepared with gallic acid (GA) (Sigma-Aldrich, Germany), and the results were expressed as mg GA/100 g of dry weight (d.w). Three replicates of extract were analyzed.

2.3.3. Determination of radical DPPH scavenging activity

The free radical scavenging capacity of extracts was determined using the stable DPPH* according to Hwang and Thi (2014). The final concentration was 200 µM for DPPH* and the final reaction volume was 3.0 mL. The absorbance was measured at 517 nm against a blank of pure methanol after 60 min of incubation in a dark condition.

The percent inhibition of the DPPH free radical was calculated by the following Eq. 2:

$$\text{Inhibition (\%)} = (A_{control}\text{-}A_{sample} / A_{control}) \times 100 \quad \text{----------(Eq.2).}$$

Where, $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound). A_{sample} is the absorbance with the test compound.

2.3.4. Carotenoids, Chlorophyll a and b Content Determination

Carotenoids and chlorophyll a and b concentrations were measured according to Lichtenthaler and Wellburn (1983). A 0.1 g sample was weighted on an analytical scale and ground with sand and magnesium carbonate in a mortar. The extraction of carotenoids and chlorophyll a and b was carried out with 25 mL of 80% acetone. The solution was transferred into a centrifuge tube, covered with aluminum foil, and set aside in the dark for 0.5 h and then centrifuged for 10 min. The absorbance of the resulting extracts was measured at wavelengths of 470 nm, 646 nm and 663 nm using a 4054-UV/Visible spectrophotometer, (LKB-Biochrom Comp., London, England).

3. Subjects and Methods

3.1. Study subjects

A total of 44 eligible volunteers moderately hyperlipidemic males aged 40–60 years old were recruited into the study for 12 weeks. Exclusion criteria included extreme dietary habits such as vegetarianism, severely low fat intake and extreme levels of physical activity. The volunteers were randomly assigned to one of two groups as follows:

Group *a* (n = 22) consumed about 900 mg fresh garlic cloves per day for 12 weeks.
Group *b* (n = 22) consumed about 900 mg dried garlic sheet per day for 12 weeks.

3.2. Methods

This was a randomized, parallel treatment study carried out at the Nutrition and Food Science Department, National Research Centre, Dokki, Cairo, Egypt.

Entry into the study after 8 weeks of diet stabilization required a mean low-density lipoprotein cholesterol level on 2 visits of 160 mg/dL or lower and a triglyceride level of 350 mg/dL) or lower. The volunteers received 300 mg of dried garlic sheet powder 3 times per day, consumed with meals (total 900 mg/d). This is equivalent to approximately 2.7 g or approximately 1 clove of fresh garlic per day. The main outcome measures included levels of total cholesterol, triglycerides, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol after 12 weeks of treatment.

3.3. Blood sampling and biochemical analysis

Blood samples were withdrawn after 12 hours of overnight fasting to test the biochemical measurements at zero weeks and after 12 weeks of the intervention period. Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were determined enzymatically using commercially available kits by Stanbio (USA) as described by Allain *et al.* (1974), Lopes-Virella *et al.* (1977) and Buccolo and David, (1973) respectively. Serum low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) were subsequently estimated using the Friedewald *et al.* (1972) formula:

LDL = TC - HDL - TG/5 (mg/dL)
VLDL = TG ÷ 5 (mg/dL)

TC/ HDL- C ratio was also calculated.

The mean blood pressure was measured from the right arm using a mercury sphingo-manometer while the patient is sitting.

4. Statistical analysis

SPSS software version 21.0.0 (SPSS Inc., IBM) was used to analyze the obtained results. Mean and SD were calculated for each variable. A paired t-test was used to assess variations in the mean between the groups from the baseline to 12 weeks of intake for the determination of absolute change. A p-value ≤ 0.05 is considered to be statistically significant.

3. Results and Discussion

3.1. Selected Bioactive Compounds content of fresh garlic cloves and dried garlic sheet

Total phenolic contents increased significantly in dried garlic sheet sample (187.38 µg/100gm) compared with fresh garlic cloves (133.43 µg/100gm), as seen in Table 1. These results are in close agreement with the findings of Salim *et al.,* (2012) and Al- Farsi and Lee (2008), who reported that date fruit contains 240mg/100g of total phenolic contents. Ruiz *et al.,* (2006) reported that apricot contains 160mg/100g phenolics.

The total phenolic content of fresh garlic cloves and dried garlic sheets and the effects of the drying are displayed in table 1. The total phenolic content of fresh garlic cloves was (133.43 µg/100gm) GA/100 g d.w. and in dried garlic sheets (187.38µg/100gm) GA/100 g d.w., which is within the range of values found in other studies of garlic (Chen *et al.,* (2013), Piatkowska *et al.,* (2015) and Gorinstein *et al.,* (2008). The results show that dried garlic sheets had a higher total phenolic content than in fresh garlic cloves. The increase in total phenolic content in dried samples may occur because the drying treatments accelerate bound phenolic compounds as part of the breakdown of cellular constituents (Chang *et al.,* 2006). In this study, the increase could be explained by the degradation of complex phenolic tannins by heat and enzymatic or non-enzymatic oxidation, which causes more phenolics to be extracted. In addition, the increase in phenolic content could be explained by the formation of Maillard reaction products, which would cause new phenolic compounds to form from precursors during thermal treatments (Que *et al.,* 2008; Sultana *et al.,* 2012). The effects of drying treatments on the phenolic compounds of foods have been studied before. Some previous studies showed that heat treatment is very effective for increasing the total phenolic content in different foods, such as dried apricots (Sultana *et al.,* 2012) and tomatoes (Chang *et al.,* 2006). Thus, the effect of drying treatments on phenolic compounds from different materials may not be the same.

Table 1: Bioactive compounds content in fresh garlic cloves and dried garlic sheet.

Nutrients	Fresh Garlic cloves	Dried Garlic sheet
Viamin C (mg/100gm)	7.683	3.312
Total polyphenols (µg/100gm)	133.43	187.38
Carotenoids (µg/100gm)	0.0169	0.0424
Chlorophyls A (µg/100gm)	0.340	0.399
Chlorophyls B (µg/100gm)	0.640	0.763
Chlorophyls AB (µg/100gm)	0.98	1.162
Antioxidant activity-DPPH (%)	37.3376	75.4482

The level of bioactive compounds in whole garlic cloves and dried garlic sheet is shown in table (1). Fresh garlic cloves had the highest concentration of vitamin C (7.683mg/100g). The vitamin C level dropped significantly in the dried garlic sheet (3.312 mg/100g), as seen in Table 1. It was lower in dried garlic sheet than in fresh garlic cloves which was due to high temperature drying of garlic sheet. Piatkowska *et al.* (2015) reported a significantly higher concentration of vitamin C in mature garlic cultivars.

Total polyphenol concentration and antioxidant activity measured with the DPPH method were significantly higher in the dried garlic sheets (187.38 µg/100gm and 75.34%) compared with fresh garlic cloves samples (133.43 µg/100gm and 37.34%), as seen in table 1.

Similar results were found in Chen *et al.,* (2013), Piatkowska *et al.* (2015), and Gorinstein *et al.* (2008) of garlic, which was very close compared with data obtained in our study.

Carotenoids, chlorophyls A, chlorophyls B, and chlorophyls AB concentrations in dried garlic sheet were 0.042, 0.399, 0.762, and 1.162 µg/100gm, respectively, higher than 0.017, 0.340, 0.640, and 0.98 µg/100gm in fresh garlic cloves, as shown in table 1. Similar findings were reported by Dyduch and Najda (2019).

Table (1) shows the changes in antioxidant capacity of fresh garlic cloves and dried garlic sheet samples as affected by drying method. The antioxidant capacity of fresh sample was found to be 187.38 µg/100gm. After drying treatments, this value reached 75.45% in dried garlic sheet samples. Dried garlic sheet had higher values than fresh garlic cloves sample. Additionally, there was not a significant ($P > 0.05$) difference between the antioxidant capacities of fresh garlic cloves sample and dried garlic sheet samples. The results show that higher total phenolic content and antioxidant capacity were obtained with thermal methods for garlic. Que *et al.* (2008) reported that hot air dried pumpkin flour contained higher total phenolic content and antioxidant activity than fresh dried sample, possibly due to production of Maillard products. The increase in antioxidant capacity as a result of drying might be caused by the formation of new antioxidant compounds (Albanese *et al.,* 2013). In this study, the antioxidant capacity of samples increases in parallel with the total phenolic content.

3.2. The hypolipidemic and hypotensive effects of fresh garlic cloves and dried garlic sheet

Table (2) shows the hypolipidemic effect of fresh garlic cloves, there was a significant lowering of tested blood lipids, except for the HDL level. As regards the blood pressure, there was a significant reduction in systolic as well as diastolic blood pressure in this group of patients.

Table 2: The hypolipidemic and hypotensive effect of fresh garlic cloves in Group ***a***

	Week 0	Week 12	P-value
	Mean ± SD	Mean ± SD	
Total cholesterol (mg/dL)	250.0 ± 17.1	239.5 ± 15.5	0.003
LDL cholesterol (mg/dL)	170.6 ± 13.9	155.8 ± 11.2	0.0001
HDL cholesterol (mg/dL)	47.2± 4.4	49.2 ± 5.3	0.0574
Triglycerides (mg/dL)	229.7± 23.2	225.2 ± 26.5	0.0077
SBP (mmHg)	150.3 ± 17.4	141.0 ± 12.1	0.0046
DBP (mmHg)	97.8± 8.9	94.1± 6.4	0.0277

Group a = these patients received fresh garlic cloves in their diet.
* n=44, SBP= Systolic blood pressure, DBP= Diastolic blood pressure, HDL=high density lipoprotein, LDL=low density lipoprotein, TC=serum total cholesterol, TG=triglyceride

Table (3) shows the hypolipidemic effect of dried garlic sheet. There was a significant reduction in the tested lipid parameters except for the HDL level. As regards the blood pressure change, there was a significant reduction in systolic as well as diastolic blood pressure in this group of patients.

The different bioactive components in the dried garlic especially phenolic acids were probably associated with the currently examined hypotensive action. As analysis exposed that, dried garlic sheet powder contains polyphenols in large quantities; this was explained by Juhel *et al.* (2000) a mechanism in which polyphenols can reduce fat capturing and assimilation in gastric tissue. They proposed that polyphenols having a high amount of catechin inhibit the emulsification of lipids in duodenal and gastric media during pancreatic and gastric lipase activity. A further explanation is that important intracellular enzymes essential in the excretion and synthesis of ApoB lipoproteins are also controlled by polyphenols (Wilcox *et al.,* 2001).

The current analysis of the dry garlic sheet showed it as a significant source of carotenoids. These considerably enhance the serum concentration of b-carotenoid. Carotenoid and dietary lipid absorption follow the same steps: discharge from the food milieu, solubilization in miscellaneous micelles, excretion into the lymphatic system, and packing into chylomicrons (Erdman *et al.,* 1993). Therefore, it can be assumed that the existence of enhanced concentrations of carotenoids released from the garlic compete with dietary lipids for assimilation and transport in lipoproteins, subsequently causing the lowering of cholesterol levels.

Table 3: The hypolipidemic and hypotensive effect of dried garlic sheet in Group ***b***

	Week 0	Week 12	P-value
	Mean ± SD	Mean ± SD	
Total cholesterol (mg/dL)	262.6 ± 35.3	248.2 ± 24.6	0.0290
LDL cholesterol (mg/dL)	172.7 ± 17.2	161.6 ± 16	0.0024
HDL cholesterol (mg/dL)	47.5 ± 4.7	49.3 ± 4.3	0.0643
Triglycerides (mg/dL)	236.1 ± 18.2	228.4 ± 16.3	0.0421
SBP (mmHg)	149.8 ± 16.2	140.3 ± 10.8	0.0017
DBP (mmHg)	98.6 ± 7.6	95.1 ± 5.9	0.0179

Group ***b*** these patients received dried garlic sheet in their diet.
* n=44, SBP= Systolic blood pressure, DBP= Diastolic blood pressure, HDL=high density lipoprotein, LDL=low density lipoprotein, TC=serum total cholesterol, TG=triglyceride

The current results show that the total antioxidant activities measured with the DPPH method were significantly higher in the dried garlic sheets. This high antioxidant power of garlic may be a result of its high content of sulfur compounds (Jang *et al.,* 2017).

As regards the cholesterol lowering property of garlic, Batiha, *et al.,* (2020) suggested that some constituents of garlic may act as inhibitors for some enzymes such as hydroxyl methyl glutaryl CoA reductase, which participates in cholesterol synthesis.

In addition, this rich antioxidant content may confer beneficial effects in the protection of cellular structures against peroxidation (Colín-González *et al.,* 2012).

It is worth adding that the current results suggest that the dried garlic sheet powder exerted a beneficial effect in maintaining cholesterol, LDL, triglycerides and blood pressure in an acceptable range, while it had a lesser effect on HDL.

4. Conclusion

All selective bioactive compounds (total polyphenol, carotenoids, chlorophyll (A, B and AB) and antioxidant activity) were increased in dried garlic sheet powder compared with it in fresh garlic cloves, except vitamin C content.

It is worth adding that the current results indicate that the biological functions of dried garlic sheet powder excreted a beneficial effect in maintaining cholesterol, LDL, triglycerides and blood pressure in an acceptable range while it had a lesser effect on HDL.

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