EFFECT OF PROCESSING AND PRESERVATION METHODS ON TOTAL PHENOLIC CONTENTS AND ANTIOXIDANT ACTIVITIES OF GARLIC (*ALLIUM SATIVUM*)

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Garlic (*Allium sativum L.*) is one of the most commonly produced vegetables worldwide. It has been consumed as a species and is considered important medicine for treating and preventing many diseases due to its content of various bioactive phytomolecules. This study aimed to assess the effect of different processing and preservation methods, including pickling, frying, freezing, and drying, on total phenolics and antioxidant activities of ethanolic extracts of garlic. Total phenolics, flavonoids, and antioxidant activities of dehydrated, pickled, and fried garlic were measured directly after treatment, and frozen garlic was assessed after 1 and 3 months of storage. Results showed that the total flavonoids and phenolics contents, and antioxidant activities of all treated samples significantly decreased compared with fresh sample. Phenolic contents strongly correlated with DPPH radical scavenging activities (r=0.956) at a concentration of 40 mg/mL for all treatments. The reducing power activity result of fresh garlic sample at a concentration of 20 mg/mL was 213.9% (30µg vitamin c equivalent); other treatments were shown to have significantly lower reducing power activities than fresh samples. Flavonoid contents showed a strong correlation with reducing power activities (r=0.759) at a concentration of 40 mg/mL for all treatments, while in phenolic contents, the correlation (r) was 0.94 at the same concentration. It can be concluded that fresh garlic showed the highest reducing power activities compared to the other treatments, while lowest activity was for dehydrated garlic. In addition, freezing process resulted in the highest preservation of the total flavonoid and phenolic contents and antioxidant activities.

Keywords: DPPH, Garlic, Phenolics, Flavonoids, Preservation methods, Antioxidant activity.

INTRODUCTION

Garlic (Allium sativum L.) is among the most widely cultivated vegetables in the world. It has been consumed for its flavor and spices and as an important medicine for treating many diseases, primarily; cardiovascular diseases and some skin problems [1], atherosclerosis and cancer [2-5]. Studies showed that garlic had been widely used as reactive oxygen species (ROS) scavenger to protect humans from oxidative stress [6,7]. The garlic extracts also have been shown to have high antioxidant activity and protective benefits against oxidative DNA damage [8]. They are useful in preventing endothelial dysfunction [9].

Garlic contains approximately 65% water, 28% carbohydrates, 2.3% organosulfur compounds, 2% proteins, 1.2% free amino acids, and 1.5% fiber [10]. The beneficial health effect of garlic can be attributed to its content of antioxidants such as vitamin C and selenium [11], other biologically active phytomolecules, including allyl thiosulfinates, organosulfur compounds, and especially phenolic acids and flavonoids [12,13].

According to studies, the total phenolics content of several garlic cultivars ranged from 3.4 to 10.8 mg gallic acid equivalent/100g (GAE/100g DMB) [14]. The variation in garlic's phenolics and flavonoid content is greatly related to genetic, agronomic, and environmental factors [15]. Additionally, research revealed that garlic's phenolic and flavonoid components are significant for their antioxidant capacity as well as sensory attributes like flavor and aroma and may be utilized as a marker to assess the nutritional and functional characteristics of garlic [16]. Garlic, like other vegetables, is usually not consumed but can be subjected to different processing and preservation methods. At home, it is usually made based on flavor and taste preferences rather than nutrient content [17]. Studies revealed that various vegetable preparation procedures, processing, and preservation techniques, such as frying, pickling, freezing, drying, and pressure cooking, may result in leaching loss or a reduction of their phytochemicals, nutritive value, and antioxidant capacity [18]. Qiu et al., found that processing fresh garlic maximizes its antioxidant, antiobesity, anticancer, anti-inflammatory, anti-allergic, immunostimulatory, hepatoprotective, cardioprotective, and beneficial effects on memory/nervous systems [19].

Studies concluded that the effect of pickling on the antioxidant capacity and the total phenolic content of vegetables is not consistent and is affected by different factors like the kind of vegetable, microorganisms, cultivation medium, temperature, time, and pH [20]. It was discovered that the preparation method of pickles has a significant impact on the overall effect of pickling on antioxidant activities in vegetables, with pasteurization having a more negative impact. In this case, the loss of antioxidant activities could be attributable to the thermal degradation of phytochemicals, loss of antioxidant enzyme activity, and enzymatic degradation of phenolic compounds [21]. Also, it was found that frying makes the flavor quality of vegetables better by forming aroma compounds compared to boiling. The chemical composition and total phenolic content of vegetables are affected differently by various frying techniques, including deep, roasting, and shallow frying [17]. However, frying preserves the content of minerals, proteins, and carbohydrates more than other heat treatment techniques like boiling [22]. It has been proven that garlic contains a large amount of antioxidants.

Garlic dried by freeze-drying method provides an exceptional quality because most enzymatic reactions and spoilage deterioration are stopped. Moreover, freeze-dried garlic has a porous crust with superior rehydration capability. However, the main disadvantage of the freeze-drying method is that it is one of the most expensive processes for manufacturing dried food products due to high capital and operating costs [23]. A study conducted to determine the effect of drying conditions on characteristics of *Allium resum* (rosy garlic), showed that convectional air-drying results in a significant reduction of total phenolic and flavonoid content. Similar results were found for other vegetables [24,26]. This study is conducted to evaluate the effect of the different processing and preservation methods of garlic on the total phenolic content, antioxidant capacity, and reducing power.

MATERIALS AND METHODS

Chemicals

Gallic acid, ferric chloride ($FeCl_3$), aluminum trichloride ($AlCl_3$), *L*-ascorbic acid, and quercetin were obtained from Sigma-Aldrich (Steinheim, Germany). Trichloroacetic acid, and Folin–Ciocalteu reagent were bought from AppliChem GmbH (Darmstadt, Germany). Potassium dihydrogen phosphate ($KH_2PO_4.2H_2O$) was purchased from Fluka-Garantie (Buchs, Switzerland). Sodium hydroxide (NaOH, HPLC grade) was purchased from LABCHEM laboratory chemicals (Zelienople, USA). 1,1-diphenyl-2-picrylhydazyl (DPPH) was purchased from ICN Biomedicals Inc. (South Chillicothe Road Aurora, Ohio). Di-Sodium hydrogen phosphate extra pure ($Na_2HPO_4.2H_2O$), sodium carbonate (Na_2CO_3), and potassium ferricyanide ($K_3[Fe(CN)_6]_6$) were purchased from E. Merck (Darmstadt, Germany). Other chemicals were of reagent grade and purchased from local companies.

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Sample preparation

Fresh garlic, the Baladi type, was obtained from the local market in Amman city, Jordan. After cleaning (removing dust and foreign materials), peeling, and separating non-edible parts, the garlic cloves (2.50 kg) were subjected to the following treatments;

The first part (500 g, control sample) was taken to directly analyze phenolic and antioxidant activities. The rest were preserved by the following methods:

1) Freezing: 500 g of peeled garlic cloves packed in bags and frozen at -18 ℃ in the freezer.
2) Drying: 500 g of peeled garlic cloves were cut into 2-3 mm thick slices manually to increase the surface area, and then slices were dried in an oven at 70 ℃ for 7 h (model UFE500, Memmert, Schwabach, Germany), the dried garlic then ground in a food grinder to obtain garlic powder.
3) Pickling: after peeling and separating defective and smallest cloves, 500 g of garlic cloves were pickled in 7% brine solution at room temperature for 14 days. Green pepper was added to the solution as a starter for the fermentation process and as an indicator for the completion of the fermentation process, indicated by the color changing from green to bright green.
4) Frying: 500 g of peeled garlic cloves sliced and then fried with corn oil in a frying pan over medium-high heat (170 ℃) for about 10 min, and then cloves were removed from the heat when it was golden brown. The fried garlic cloves were drained on a paper towel-lined plate to get rid of absorbed fat and then washed with diethyl ether to get rid of the remaining fat before analysis.

Except for the fresh garlic, the processed samples were chemically analyzed immediately after processing and after being frozen for 1 and 3 months. Moisture content was determined at 105 ℃ for all treated samples and before any analysis.

Moisture content determination

The moisture content (%) of fresh garlic and other treatments was determined in duplicate using a conventional oven at 105 ℃ until a constant weight was achieved (Memmert, Model UFE500, Schwabach, Germany).

Yield determination

The yield (%) of fresh garlic extract and other treatments was determined in duplicate. In brief, 5 mL from each ethanolic extract (10 g/ 50 mL) was transferred to a glass Petri dish and then kept in a conventional oven until a constant weight was achieved at 105 ℃ (Memmert, Model UFE500, Schwabach, Germany).

Extraction of garlic

A 10.0 g of garlic samples from each treatment were homogenized and extracted with 20 mL ethanol using a blender for 15 min. Then, the mixture was filtered with Whatman no.1 paper to obtain extract I. The homogenized residues were again blended with 20 mL ethanol and filtered to get extract II. Then, extracts I and II were combined and filtered, and the volume was completed to 50 mL. The yields of garlic extract (%) were calculated. Total phenolic contents, flavonoid contents, reducing power activity (%), and DPPH radical scavenging activity were then measured in the garlic ethanol extracts that were collected.

Total phenolic content determination

The total phenolic content in each garlic extract was evaluated separately using the Folin-Ciocalteau reagent [27]. In brief, 2.0 mL of each sample (200 mg/mL) was moved into a 10-mL volumetric flask, followed by adding 2.5 mL of distilled water. After that, Folin–Ciocalteu reagent (250 µL) was added and mixed. 0.5 mL of 10% sodium carbonate (10 g/100 mL) was added after 3 min, and the absorbance was then determined using a 760 nm spectrophotometer (model UVD-2900, Labomed, USA). A calibration curve was constructed with gallic acid standing as the standard.

Based on the established calibration curve, the total phenolic compound contents (mg Gallic acid /100g) were calculated using the following regression equation: Y=0.079X-0.019, R^2 = 0.995. Y is the absorbance, and X is the Gallic acid concentration in mg/L. All measurements were done in triplicate.

Determination of total flavonoids content

The content of flavonoids was determined according to the method described by Miliuskas et al. [28]. In brief, 2.0 mL (200 mg/mL) of each treatment was combined with 1 mL of a 2% aluminium trichloride in ethanol. The solution was then diluted with water into 25 mL and left to stand at 20 °C for 40 min before the absorbance was measured at 415 nm (Labomed spectrophotometer, model UVD-2900, Labomed, USA).

Based on the established calibration curve, the total flavonoid contents (mg Rutin /100g) in each treatment extract were calculated using the following regression equation: Y=0.093X - 0.039, R^2 = 0.987. Y is the absorbance, and X is the Rutin concentration in mg/L. All measurements were done in triplicate.

Determination of antioxidant activities

DPPH free radical scavenging assay

The DPPH free radical scavenging assay is the most widely used analysis method in evaluating antioxidant activities in plant materials. It measures the hydrogen or electron-donating ability of the antioxidants that stop oxidative damage by scavenging free radicals [25]. DPPH was used to assess the free radical scavenging activity in each treatment extract using the method of Hatano [29]. In brief, 100, 200, 300, and 400 µL from each treatment previously dissolved in ethanol (10 g/50 mL) was mixed with 2 mL of a DPPH methanolic solution (2.4 mM). The absorbance of each treatment was read at 517 nm (Labomed spectrophotometer, model UVD-2900, Labomed, USA) after 30 min against a blank that was prepared from similar concentrations for each treatment to decrease the impact of the sample's color on the optical density, while the control sample was only DPPH solution.

DPPH radical inhibition activity (%) =

$$\frac{\text{control absorbance} - (\text{sample absorbance} - \text{blank absorbance})}{\text{control absorbance}} \times 100$$

Reducing power activity (%)

The reducing powers of each treatment extract were determined using the method described by Yildirm [30]. Briefly, 50, 100 and 200 mL from each treatment previously dissolved in ethanol (equivalent to 10 g/50 mL) was combined with 2.5 mL potassium ferricyanide (1g/100 mL) and 2.5 mL phosphate buffer (0.2 M, pH 6.6). After being heated to 50 ºC for 30 min, the liquid was then given 2.5 mL of trichloroacetic acid (10 g/100 mL), and then the mixture was centrifuged at 1650 x g for 10 min. Following that, 2.5 mL of the top layer solution and 2.5 mL of ferric chloride (0.1g/100 mL) were combined. At 700 nm, the absorbance of the treatments and a standard of 30 µg ascorbic acid were measured.

$$\text{Reducing power activity (\%)} = \frac{\text{sample absorbance}}{\text{ascorbic acid absorbance}} \text{x } 100$$

Statistical analysis

The SAS software was used for statistical analyses (SAS Institute Inc., Cary, NC, USA). The LSD test was used to determine significant variations among the various treatments. Differences at P<0.05 were regarded as significant. Microsoft Excel was used to calculate regression equations and correlation coefficients (r).

RESULTS AND DISCUSSION

Moisture content and yields of extracts

The moisture contents (%) and yield of ethanolic extracts of the fresh garlic sample and treatments are shown in Table 1. The fresh garlic sample had a moisture content of 64.3 %. This result is almost similar to the result of Odebunmi [31], who reported that the moisture content of garlic is 66.57%. The variation between the two results may be due to the studied garlic cultivar, soil and climacteric conditions variations. The moisture content doesn't change significantly in frozen garlic; however, it was increased in pickled garlic due to the diffusion of water into garlic cloves from the brine solution. The moisture content of fried and dehydrated garlic treatments decreased significantly due to the evaporation of water as a result of heat treatment.

Table 1. The moisture content (%) and yields of extracts (g/100 g) of fresh, frozen, pickled, fried and dehydrated garlic samples *

Treatment	Moisture content (%)	Yield (g/ 100g)
Fresh	64.30 ±0.27[b]	**10.423 ± 0.001[a]**
Frozen 1 month	64.10±0.25[b]	**4.584 ± 0.001[b]**
Frozen 3 months	64.60±0.14[b]	**4.233 ± 0.006[b]**
Pickled	76.95±0.24[a]	**4.244 ± 0.009[b]**
Fried	24.45±0.49[c]	**3.729 ± 0.002[c]**
Dehydrated	6.14±0.11[d]	**2.739 ± 0.001[d]**

*Means of moisture content ± standard deviation. Values with various letter combinations differ significantly ($P< 0.05$).

The soluble ethanol extracts of the fresh garlic sample yielded the highest yield (10.4%), while the dehydrated garlic sample showed the lowest yield (2.7%). We concluded from the results that the preservation techniques significantly reduced the amount of extracted materials. Our results were in agreement with Patricia et al. [32], who stated a significant reduction in the total solid content and yields of processed garlic. The decrease in the yield of the dehydrated garlic sample was attributed to the great losses of water and, consequently, the yield and other bioactive soluble components. The decrease in the yield of the fried garlic sample was also attributed to the water loss, which was partially compensated by the fat used in the frying process. As expected, the frying process resulted in this decrease as a consequence of the degradation and oxidation of bioactive components. The decrease in the yield for the pickled garlic sample was attributed to the chemical changes of bioactive components in the garlic as a result of the addition of salt [32]. Lawson et al. [33] reported that salt-free garlic products preserved the bioactive components better than salted garlic paste or other products. The decrease in the yield in the frozen garlic sample may be attributed to the effect of storage time since there is a breakage in the plant components under the freezing conditions and possible activity of the bioactive components, which results in losses in the yield and total solid content [6]. Generally, the differences in the extract yields of the tested garlic samples may be attributed to the different availability of extractable components resulting from the different processing and preservation methods [34]. Extraction yields are also affected mainly by the extraction procedure used, which may have a different effect depending on the sample. Still, generally, higher extract yields are usually obtained using aqueous organic solvent and refluxing [35].

Total phenolics and total flavonoids contents

Phenolic compounds are secondary aromatic metabolites responsible for the antioxidant properties, sensory, and color of food [36]. The total phenolic compounds content of fresh garlic samples and other treatments were expressed as mg GAE/100g on DMB. Table 2 shows the average phenolic compound content of garlic in different processing and preservation methods. The total phenolic content of the fresh garlic sample was 5.353 mg GAE/100g DMB. This value was notably ($P< 0.05$) higher than other treatments. This result agreed with Bonzin et al. [37], who stated that the total phenolic content of different garlic cultivars ranged between 5.00 and 18.0 mg GAE/100g DMB.

As shown in Table 2, considerable variation was found in phenolic compound content for different treatments. The total phenolic contents of different treatments were in the following decreasing order: fresh > frozen 1month > frozen 3 months > pickled > fried > dehydrated. Among the treatments, frozen garlic samples (1 and 3 months) had the highest total phenolic content, but they were significantly ($P< 0.05$) lower than the value of the total phenolic content of the fresh sample. The decrease in phenolic content of frozen garlic may be related to oxidative enzyme reactions since polyphenol oxidase enzyme is possibly released from the cellular walls of vegetables during frozen storage [38]. The reduction in the total phenolic content after 3 months of frozen storage may be attributed to the tiny ice crystals that may have formed during frozen storage, which could impale the cell wall, thus causing the losses in total phenolic compounds from the cell [39], or related to sever cellular disruption, this may be created by the enzyme polyphenol oxidase, which is connected to the cellular wall, releasing its oxido reductasic ionic forms [40]. Emy et al. [41] reported a significant decrease in the total phenolic content of okra after the freezing process.

Table 2. Flavonoids and phenolic contents, and IC_{50} of DPPH radical scavenging activities of fresh, frozen, pickled, fried and dehydrated garlic extracts*

Garlic Extracts	Phenolics (mg GAE /100g DMB)	Flavenoids (mg RE/100g DMB)	DPPH· IC_{50} (mg/ml)[a]
Fresh garlic	5.353 ± 0.018[a]	6.257 ± 0.822[a]	30
Frozen (1 month)	3.834 ± 0.269[b]	4.922 ± 0.017[b]	45
Frozen (3 months)	2.720 ± 0.237[c]	4.223 ± 0.155[c]	58
Pickled	2.200 ± 0.141[d]	4.182 ± 0.091[c]	60
Fried	0.812 ± 0.212[e]	4.088 ± 0.174[c]	80
Dehydrated	0.619 ± 0.183[e]	2.913 ± 0.243[d]	>100

* Different letters within columns are significantly different ($P<0.05$). [a] Concentration that resulted in a 50% inhibition of the examined radicals.

The fried and dehydrated garlic samples had the lowest total phenolic content. The decrease in the total phenolic content of vegetables after the frying process was reported in the literature [35,42,43]. The decrease in phenolic content in fried vegetables was ascribed to the high frying temperature or the enzymatic oxidation and destruction during the preparation processor to the long period of frying [44].

The total phenolic content losses in dried vegetables could be due to the degradation of phenolic compounds by drying or that hot air drying promoted polyphenol oxidation by the oxygen present in the air of convectional drying [20,45] and to the use of phenolic compounds as reactants in the Maillard reaction during the drying process, or refers to the chemical changes caused by heat treatment to polyphenols' chemical structure, such as their affinity to other chemicals [46]. These changes inhibit or limit their ability to be extracted and determined using the techniques used. The pickled garlic sample exhibited a substantial reduction in total phenolic content compared to the fresh sample. This result agrees with Kübra et al. [47], who informed a decrease in the total phenolic content of some pickled vegetables, including garlic. This decrease in phenolic content may be attributed to the enzymatic degradation of phenolic compounds or the phenolic compounds leaching from garlic cloves into the brine solution [22] due to the action of lactic acid bacteria. Hur et al. [21] stated that the effect of pickling on the total phenolic content of vegetables is variable and affected by different factors like vegetable kind, microorganisms, cultivation medium, time, temperature, and pH.

The total flavonoid content of the fresh garlic sample and other treatments are given in Table 2. The total flavonoid content of the fresh sample was 6.257 mg (RE/100g DMB). This value is significantly ($P< 0.05$) higher than other treatments. This result agrees with Bonzin et al. [37], who stated that the total flavonoid content of different garlic cultivars ranged between 4.16 and 6.99 mg RE/ 100g, DMB.

As shown in Table 2, all treatments showed a dramatic decrease in total flavonoids content, but the total flavonoids losses were found to be relatively lower than losses of total phenolic content; this result may be elucidated by the higher stability of flavonoids compared to other polyphenols [48]. The levels of the flavonoid content of different treatments were in the following decreasing order: fresh > frozen 1 month > frozen 3 months > pickled > fried > dehydrated.

The frozen 1-month garlic sample had the highest total flavonoid contents among other treatments but significantly lower than the total flavonoids content of the fresh sample; this decrease confirmed that the frozen products still have changed during freezing and frozen storage and can undergo a different process which can affect the bio-accessibility of phytochemical compounds of these products compared to fresh ones [49]. While dehydrated garlic sample had the lowest total flavonoids content, and this decrease may be attributed to their polymerization that occurred during air-drying [50] or as a result of harsh conditions during the drying process that may be affected cell wall integrity; this caused the migration of some components including some flavonoids [51,52], or due to increase in the polyphenol oxidase enzyme activity which causes degradation of flavonoids [53].

The fried garlic sample showed a significant decrease in the total flavonoid content after the frying process. This result agrees with Yara et al. [54], who reported that the concentration of quercetin and some flavonoids is reduced by 24% in fried garlic compared with raw garlic. Most of the losses were attributed to the leaching of some flavonoids from the vegetables into cooking oil during prolonged exposure to heat [55].

The pickled garlic sample also showed a decrease in the total flavonoid content, which could be attributed to the leaching of these compounds from vegetables into the brine solution [22].

Antioxidant activities

There is a variety of in vitro methods that may be used to determine the antioxidant capacity of various plants. These tests are based on several aspects of antioxidant activity. However, it is not advised to use only one method to determine the antioxidant activity of different plant extracts due to their complex composition [56]. Concerning this, the antioxidant activities of several treatments of garlic samples were determined by both DPPH free radical-scavenging activity and the reducing power of ferric ions to the ferrous form.

DPPH radical scavenging activity

Results from Figure 1 showed that the quenching of DPPH radical color by garlic sample extracts was in a concentration-dependent manner, and the samples with the higher phenolics and flavonoid content had higher DPPH free radical scavenging activity (%). The fresh sample showed complete color inhibition at 60 mg/mL and 80 mg/mL concentration due to its highest phenolics and flavonoid content, and this showed to have a strong antioxidant capacity. In contrast, other treatments showed no color inhibition at any of the tested concentrations.

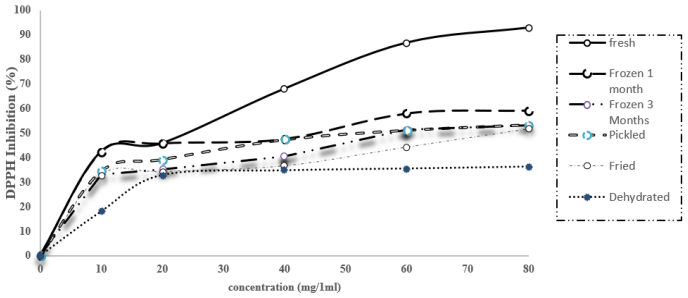

Figure 1. DPPH free radical scavenging activity (%) of fresh, frozen, pickled, fried and dehydrated garlic ethanolic extracts at different concentrations.

Values of DPPH radical scavenging activities for other treatments showed a large variation; frozen samples for 1 month were shown to have DPPH inhibition values ranging from 42.25 to 59.08%, while for frozen for 3 months were 33.13 to 53.26 % at the same tested concentrations. This reflects that freezing was the least harmful preservation technique and could maintain most of the antioxidant properties of garlic. The difference between values of DPPH free radical scavenging activities of frozen 1-month samples and frozen 3 months samples may be explained by the fact that the common consequences of the freezing process are due to cell damage by the growth of large ice crystals resulting from temperature fluctuation during frozen storage, which leads to texture softening and increase the separation between cells and eventually losses in phytochemicals and antioxidant compounds [57]. Many authors reported that any elevation above the designed storage temperature might reduce the quality of frozen foods, and fluctuation in temperature is considered the most factor that adversely affects product quality [58].

The pickled garlic sample showed a significant decrease in DPPH radical-scavenging activities for fresh and frozen samples. This decrease may be attributed to the leaching of antioxidant compounds into the brine solution [22]. The fried garlic sample also showed a significant decrease in DPPH inhibition activity; this decrease may be due to enzymatic oxidation and destruction of polyphenols and other antioxidant compounds during the processing or long frying period [44]. The dehydrated garlic sample had the lowest values for DPPH free radical scavenging activities at a concentration of 10 to 80 mg/mL. DPPH inhibition was the most affected by processing techniques, especially dehydration, which is considered the most damaging preservation technique. The decrease in antioxidant activity of dried garlic may be related to total phenolic content losses [59], because polyphenols are the primary chemicals responsible for the antioxidant activity of plants [45], or due to the development of new molecules with pro-oxidant activity after heat treatment [60].

The DPPH (%) for all treatments displayed a good correlation with the flavonoids content (r= 0.956) at concentration of 40 mg/mL and at concentration of 60 mg/mL and 80 mg/mL, r was = 0.968, while for phenolic content the correlation (r) were 0.674, 0.810 and 0.773, respectively. These results agree with many studies that reported a strong relationship between the amount of available bioactive phenolic compounds in vegetables and their antioxidant activities [61-64]. The IC_{50} values (the concentration needed to inhibit 50% of DPPH free-radical) for treatments were presented in Table 2. The lower the IC_{50} value of the treatments, the higher their antioxidant activity. The IC_{50} values ranged from 30 mg/mL to higher than 100 mg/ mL. The highest IC_{50} was for dehydrated garlic (higher than 100 mg/mL), followed by fried garlic (80 mg/mL) which reflects poor antioxidant activity against DPPH free radicals among all treatments, while the lowest IC_{50} value was for fresh garlic (30 mg/mL) and this reflects its high antioxidant activity against DPPH free radical.

Reducing power activity (%)

The reducing power measures the reducing potential of an antioxidant to reduce Fe^{3+} to Fe^{+2}. Table 3 shows the results of reducing power activity of different treatments, which were in the following decreasing order: fresh > frozen 1 month > frozen 3 months > pickled > fried > dehydrated. The reducing power of vitamin C (30 µg) was measured and considered to be 100%.

The fresh sample showed the highest reducing power activity indicating that the fresh garlic sample had a strong antioxidant activity comparable to ascorbic acid. The fresh sample reducing power values at concentration of 10 mg /mL, 20 mg/mL and 40 mg/mL were 75.1%, 213.90% and 240.35%, respectively, and were different from other treatments.

Table 3. The reducing power activity (%) of fresh, frozen, pickled, fried and dehydrated garlic ethanolic extracts at different concentrations expressed as 30 µg vitamin *C* equivalent*

Treatments	10 mg/mL	20 mg/mL	40 mg/mL
Fresh garlic	75.10± 1.98^{a}	213.90±6.65^{a}	240.35 ±2.76^{a}
Frozen 1 month	44.65±1.20 ^{b}	82.45 ± 1.34^{b}	127.85±16.33^{b}
Frozen 3 months	42.85 ±1.49^{bc}	72.20 ±1.34$^{b\,c}$	87.60 ±3.68^{c}
Pickled	27.45±1.49^{c}	62.60 ±4.10^{c}	82.20± 10.04^{c}
Fried	16.75±1.49^{d}	38.75± 5.02^{d}	60.10± 2.83^{cd}
Dried	11.15± 0.21^{e}	17.85±1.06^{e}	29.55±5.02^{d}

* Mean ± standard deviation. Values in the same column with various letter combinations differ significantly (P<0.05).

The frozen garlic samples significantly decreased in reducing power compared to the fresh garlic sample. The explanation of the reduction in the reducing power activity could be due to enzymatic reactions in frozen products because these reactions are slow in frozen but not completely blocked, and the activity of enzymes is linked to the presence of unfrozen water [65].

The reduction in reducing power activity of processed and preserved garlic samples is attributed to the losses of total phenolics and flavonoids content, as explained before, since not only the level of phenolic compounds contributed to the antioxidant activities of plant materials, but there is a synergy occurring between phenolic compounds, and other plant constituents may influence their antioxidant activity [66]. The reducing power (%) for all treatments exhibited a strong correlation with the flavonoids content (r= 0.759) at a concentration of 10 mg/mL, r=0.661 at a concentration of 20 mg/mL, and r=0.759 at a concentration of 40 mg/ mL. With respect to phenolic contents, the correlation (r) was = 0.967, 0.872, and 0.942, respectively. Reducing power is concentration-dependent; as the concentration of garlic extracts increased, the reducing power activity increased too.

CONCLUSION

The used preservation methods of garlic decreased the total phenolic and flavonoid contents of the tested samples, while the fresh garlic was rich in polyphenols. Also, the total antioxidant activities of all treatments were significantly lower than the fresh sample. Moreover, the fresh garlic showed higher reducing power activities compared with the other treatments, and the lowest activity was for dehydrated garlic. Finally, among all preservation methods, the freezing process resulted in the highest preservation of the total phenolic and flavonoid content and antioxidant activities, while dehydration resulted in the highest adverse effect on them.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declare that they have no conflict of interest, and have not submitted to any other journal in parallel or published previously.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

Not applicable.

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