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Effect of freezing and drying on the bioactive compounds and antioxidant potential of garlic

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Abstract

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Garlic (*Allium sativum.* L.) is a culinary and medicinal plant containing various bioactive molecules. The preservation process of garlic is important because it considerably influences the composition of the final product, as well as its biological activity. The choice of a preservation method for garlic is a compromise between convenience and quality. Few studies compare the effects of preservation methods on the bioactive potential of this condiment; hence the interest of this study which aims to evaluate the effect of freezing and drying on the bioactive compounds of garlic. Three samples of local garlic were prepared "fresh, frozen at -20°C, and dried in an oven at 50°C". According to the ANOVA statistical analysis ($p < 0.05$), the contents of proteins, reducing sugars, polyphenols, flavonoids, vitamin C, haven't shown significant difference between frozen and fresh garlic. Contrast to this, drying significantly reduced the content of protein (59.6%), reducing sugars (72.61%), polyphenols (58.6%), flavonoids (38.13%) and vitamin C (76.9%). Peroxidase activity persisted after freezing, but decrease after drying (68% loss) compared to the initial activity. The percentage of DPPH radical scavenging was higher for fresh (37.78%) and frozen garlic (43.46%) extracts at the lowest concentration, in contrast to dried garlic with 20.91% at the highest concentration. The results of this study showed that freezing seems to extend the shelf life of garlic because it preserves most of the biochemical and bioactive substances.

Keywords: Garlic; conservation; freezing; drying; bioactive compounds; food quality

Introduction

Functional foods have enormous health benefits, among these functional foods garlic or *Allium sativum,* which is one of the most known and used popular condiments. It has been consumed and used as natural remedy; its consumption is associated with a reduced risk of hypertension, atherosclerosis and vascular accidents (Qidwai & Ashfaq, 2013; Ried, 2020; Panyod et al., 2022). Garlic has antimicrobial, antioxidant, and immunomodulatory properties (Hashemi et al., 2019; Ansary et al., 2020). The benefits of garlic are attributed to its richness in bioactive compounds, like organosulfur compounds, polyphenols, flavonoids, high amounts of vitamins, minerals and enzymes (Shang et al., 2019). Nowadays, the preservation of garlic is unavoidable, because the fresh product is not available all year round. But its preservation is a compromise between organoleptic quality and its therapeutic virtues. Indeed, the activity of endogenous enzymes as well as other non-enzymatic ones can reduce the shelf life of garlic (Kodera et al., 2020). Drying and freezing are two domestic preservation processes that are also used by the food industry to inactivate spoilage enzymes and ensure food stability (Sridhar et al., 2021). Many studies are devoted to dried garlic, but few compare the biochemical quality of the final products from the two drying and freezing methods.

The objective of this work was to evaluate the stability

of bioactive compounds and the antioxidant potential of prepared frozen and dried garlic compared to fresh using standard and validated biochemical methods.

Materials and Methods

Chemicals reagents

Ortho-dianisidine, 2.2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu phenol reagent, gallic acid and bovine serum albumin (BSA) were purchased from Sigma-Aldrich; hydrogen peroxide was purchased from Merck.

Sample collection and preparation

The plant material used for our study consisted of "*Allium sativum",* precisely garlic cloves obtained from the local market in the region of Tizi-Ouzou (Algeria) collected in May 2021. Garlic sampling was carried out randomly and transported to the laboratory without damaging the fresh cloves.

Samples were divided into three groups: fresh, frozen and dried. Before the extraction step, 250 g of fresh peeled garlic cloves were cut into thin slices of 2 mm thicknesses, one part was subjected to freezing at -20°C for six weeks. On the other hand, another part of fresh garlic slices was submitted to drying in an oven for 48 hours at 50°C. After drying, samples obtained were ground using a mortar. The garlic powder obtained was stored in air tight, at room temperature (25°C) and dark. Untreated garlic slices (fresh) were also prepared to serve as a control. Each pretreatment was repeated at least for three times; the preparation steps of different extracts are summarized in the diagram of Figure 1.

Preparation of extracts

Different garlic simple extracts were prepared according to the protocol described by Diao et al. (2019) with some modifications.

*Dried, frozen and fresh garlic:*5 g of dried garlic powder and 5 g of crushed frozen garlic slices were mixed separately with 30 ml of Tris-HCL buffer (50 mM, pH 7.3) with $CaCl_2$ (0.5 M) and DTT (5 mM) and allowing to stand for 1 h in ice. 5 g of freshly crushed samples were prepared in the same condition to dried and frozen garlic. Each treatment was repeated at least for three times.

Garlic mixtures were centrifuged (4000 *g*, 4°C, 30 min), the obtained supernatants (fresh, dried and frozen) are filtered with a 0.22 µm membrane and stored in freezer at -20°C until use for further analysis (Figure 1).

Biochemical analysis determining the content of protein, reducing sugar, phenolic content, Vitamin C content, peroxidase and DPPH scavenging activity, were carried out based on standard protocols.

Total protein concentration

Total protein concentration in different extracts was determined by the Lowry method (1951). The reaction medium contains 3 ml of solution C and 20 µl of the extract; let it stand for 10 min in dark, at room temperature, then add 0.3 ml of Folin–Ciocalteu reagent diluted to half. After 15 min, absorbance is measured at 750 nm. Concentrations are expressed in grams per 100 g of fresh matter (g/100 g) using the regression equation obtained with bovine serum albumin (BSA).

Total reducing sugar

Sugar content was assessed using the method of Miller (1959). First, 1 ml DNS reagent was mixed to 0.5 ml garlic extract, 100 µl of 10 % sodium hydroxide solutions (w/v) was added to the mixture with vortexing the all. Samples were incubated at 100°C for 15 min and then cooled rapidly, and the absorbance was measured at 540 nm. Results are expressed as g/100 g fresh matter relative to the glucose calibration curve.

Total phenolics and flavonoid content

Total phenolic content was determined following Chen et al. (2007), using gallic acid as a standard. 500 µL of sample was added to 2 ml of 2% Na_2CO_3 solution. After 2 min, 2.5 mL of 50% of Folin–Ciocalteu reagent was added. The final mixture was shaken and then incubated for 30 min at room temperature, in the dark. The absorbance of all samples was measured at 760 nm. The results are expressed as mg gallic acid equivalents (GAE) per g of fresh material (FM), using a standard gallic acid curve. The flavonoid content was determined colorimetrically using aluminium trichloride (Dia et al., 2019).

Vitamin C content

Vitamin C content was determined using the method of Jagota & Dani (1982). After shaking; the tubes were placed in ice for 5 min and then centrifuged for 5 min at 3000 rpm. The extract was diluted to 2.0 ml with distilled water, and then 0.2 ml of Folin-Ciocalteu reagent was added with vigorous mixing. After 10 min of incubation, the absorbance was measured at 760 nm. The results are expressed as mg/100 g fresh matter with reference to the calibration curve of ascorbic acid.

Peroxidase assay

Peroxidase activity was assayed according to Bradley et al. (1982) modified by Bedouhene et al. (2020). A standard assay solution contained 15 mM o-dianisidine, 10 mM H_2O_2 in sodium-phosphate buffer (pH 6.5) was prepared.

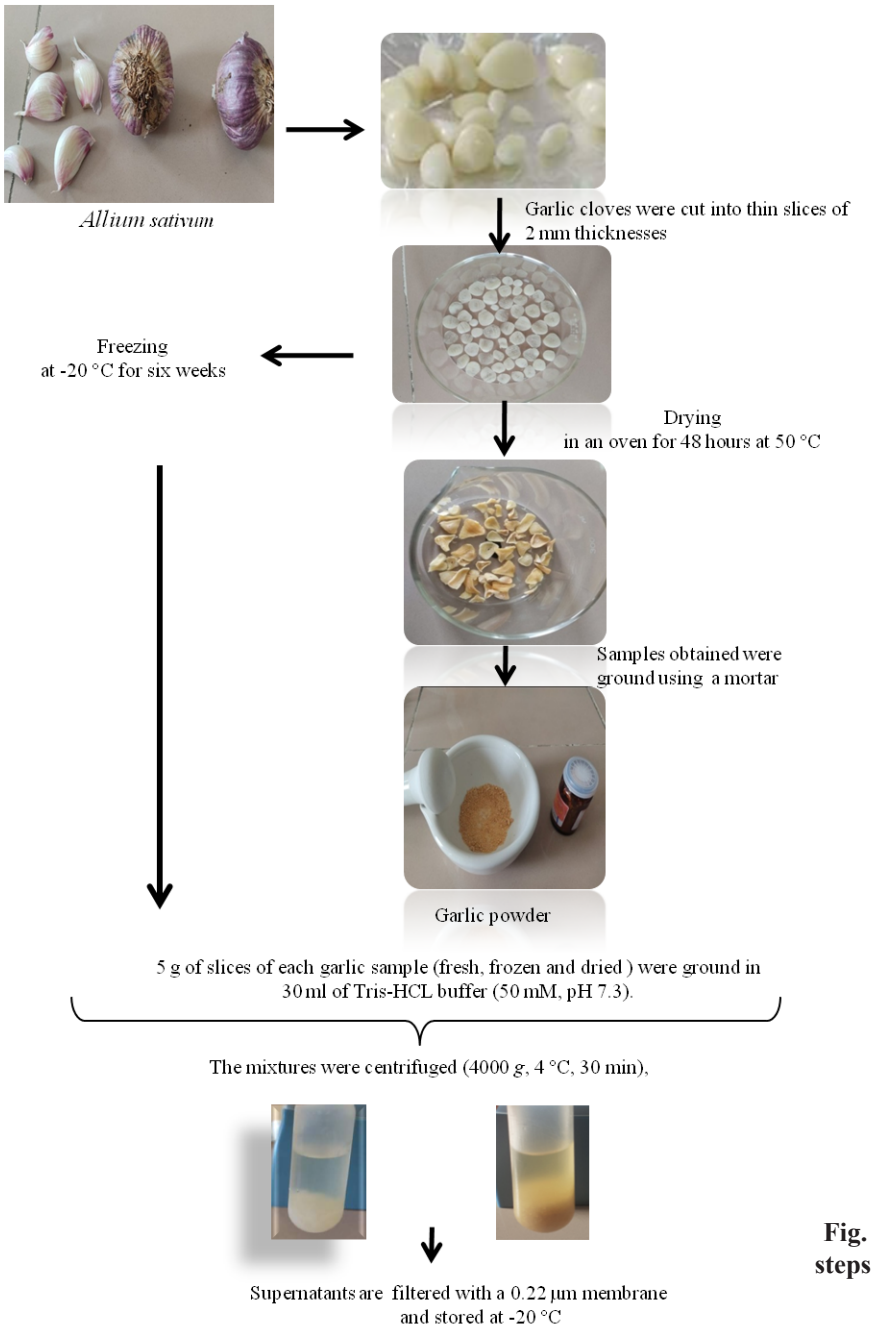


Fig. 1. The preparation steps of frozen and dried garlic extracts

Twenty-five microliters of the crude extract (containing peroxidase enzyme) were added to the standards solution in total volume of 1 mL. The oxidation of ortho-dianisidine, in the presence of H_2O_2 and peroxidase from the extract, is monitored by the change in absorbance at 460 nm at 25°C. One enzyme unit (U) is the amount of enzyme producing a change in absorbance of 0.001/min. Readings were taken every 1 min for 10 min.

DPPH free radical scavenging activity

DPPH scavenging was measured according to Brand-Williams et al. (1995). A 3 ml methanolic solution of DPPH (0.1 M) was mixed with 1 ml of sample. Extracts (5, 10, 20, 40, 80, and 100 µg/ml) were prepared and ascorbic acid (0.2 mg/ml) as reference. The reaction mixture was incubated 30 min in the dark at room temperature and then filtered through a 0.22 µm membrane. The absorbance was mea-

sured at 517 nm. The percentage of DPPH scavenging was determined according to the following formula:

$$\text{DPPH scavenging (\%)} = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$$

$Abs_{control}$: Absorbance of DPPH without simple

Abs_{sample}: Absorbance of DPPH with simple

Statistical analysis

The experimental data were expressed as Mean ± Standard deviation (SD) of three experiments (n = 3). The analysis was performed with one-way ANOVA using Graph-Pad Prism version 5.0. $p < 0.05$ was considered significant.

Results and Discussion

Effect of freezing and drying on biochemical content and bioactive compounds of garlic

Freezing and drying treatments on garlic had different effects on protein content, reducing sugars, and antioxidant molecules, including vitamin C, total polyphenols, and flavonoids as shown in Table 1. Fresh garlic extract remains the richest in biomolecules, followed by frozen garlic extract, relatively stable, in contrast to dried garlic extract; a decrease in its biochemical compounds was recorded. In fact, the technological processes are dependent on their type and potency as well as the most common methods that include heat treatment, affects characteristics, activity and bioavailability of bioactive compounds in fruits and vegetables (Locatelli et al., 2015). Several studies have shown that heat treatment of garlic, caused changes in their concentrations, activities and structures, losing of vitamins and other active compounds, and reduction of natural antioxidants (Najman et al., 2020).

Total protein content

Protein content in fresh garlic extract is 2.58 ± 0.11 g/100 g FM for a concentration of 4.3 mg/ml (Table 1), this result correlates with that obtained by Petropoulos et al. (2018). This biochemical variability would depend on the garlic variety, soil and storage conditions. Both of fresh garlic and frozen garlic contain similar content of proteins. While dried garlic extract contains less protein with a content of 1.04 ± 0.03 g/100 g FM, drying caused a decrease of 59.6% compared to fresh garlic extract. The low protein content of dried garlic is due to the effect of heat treatment on garlic by denaturing the proteins; some proteins are completely degraded at 20°C such as alliinase (Mercier-Fichaux, 2016).

Reducing sugars content

The content of reducing sugars in fresh garlic extract is 1.14 ± 0.04 g/100 g FM (Table 1), this content is close to that found by Lisciani et al. (2017) which varies between 2.12 g and 3.27 g. It was slightly low in frozen garlic extract (1.11 ± 0.04 g) and very low in dried garlic extract (0.31 ± 0.01g). According to our results, freezing would result in a slight decrease in reducing sugar content, whereas drying would decrease it significantly (72.62%).

The significant decrease in protein and sugars in the dried garlic extract would be related to the effect of temperature on biochemical reactions during drying, especially the Maillard reaction (Yuan et al., 2022). It should be noted that the decrease of water during drying would affect the activity of garlic (Gong et al., 2022).

Vitamin C content

In this study, we used a reliable and inexpensive spectrophotometric method. According to Table 1, we have an interesting vitamin C level for the fresh garlic extract (66.7 ± 1.93 mg/100 g of FM), much higher than that of Aydoğmuş et al. (2002), a richness that would be due to the climatic condition. In the frozen extract its concentration is almost similar (59.3 ± 1.93 mg), but markedly decreased for the dried extract (15.38 ± 0.94 mg). This loss of vitamin C may be due to its degradation by oxidases, heat treatment and also water activity. Its stability is all the lower as the drying time is long, a total degradation of vitamin C takes place after 8 days at 45°C (Hsu et al., 2012).

Table 1. Biochemical content and bioactive compounds of garlic extracts, freezing at -20 °C and oven drying at 50°C (48 hours) compared with fresh sample

Extracts	Proteins (g/100 FM)	Reducing Sugar (g/100g FM)	Total phenolic (mg GAE/ 100g FM)	Total flavonoid (mg QE/ 100g FM)	Vitamin C (mg/100g FM)
Fresh garlic	2.58 ± 0.11	1.14 ± 0.04	94.00 ± 3.33	7.21 ± 0.28	66.7 ± 1.93
Frozen garlic	2.58 ± 0.15	1.10 ± 0.04	92.33 ± 3.11	6.79 ± 0.23	59.3 ± 1.93
Dried garlic	1.04 ± 0.03[a]	0.31 ± 0.01[b]	38.33 ± 4.49[c]	4.46 ± 0.37[d]	15.38 ± 0.94[e]

[(a,b,c,d,e)] Values are expressed as Mean ± Standard deviation; GAE: Gallic Acid Equivalents; QE: Quercetin equivalents; FM: Fresh Matter. Differences were considered significant at $P < 0.05$.

Total phenolic and flavonoid

Total phenolic and flavonoids in fresh garlic extract are 94 ± 3.33 mg GAE/100 g of FM and 7.21±0.28 mg QE/100 g of FM, respectively (Table 1). These values are lower than those obtained with other extraction systems (Januarti, 2020). These variations in polyphenol and flavonoid contents are thought to be due to extraction conditions, including solvent, geographic origin and genetic varieties (Senani et al., 2018; Aryal et al., 2019; Maina et al., 2021). Frozen garlic extract has 92.33 ± 3.11 mg GAE /100 g of FM of phenolic compounds and 6.79 ± 0.23 mg QE/100 g of FM of flavonoids, compared to fresh garlic extract these contents are slightly low which means that freezing does not show a remarkable effect on the contents of these compounds. The dried garlic extract contains only 38.88 ± 4.49 mg GAE/100 g of FM of total phenolic and 4.46 ± 0.37 mg QE/100 g of FM of flavonoids (Table 1), these contents are clearly lower compared to fresh garlic extract. These values are similar to those of Çubukçu et al. (2019) showed stability of frozen garlic and alteration of polyphenol content during drying, which is believed to be due to enzymatic oxidation of polyphenols.

Activity of peroxidase

Peroxidase (POD) activity was measured in different extracts by using O-dianisidine as chromogenic agent and hydrogen peroxide (H_2O_2) as substrate (Figure 2).

POD (oxidoreductase EC1.11.1.7) is an enzyme related to plant defense and plays an essential role in resistance to membrane damage, mainly through the enzymatic degradation of H_2O_2.

The obtained results POD activities in different extracts are shown in Figure 3. The kinetics of peroxidase activity is similar for fresh and frozen garlic extract, while dried garlic extract had low activity (Figure 3a). Fresh garlic extract having the highest peroxidase activity (1823 ± 1.11 U/min/g of FM), followed by frozen garlic extract (1693 ± 1.33 U/min/g of FM) almost completely preserved, low activity was recorded for the dried garlic extract which is (587 ± 1.33 U/min/g of FM) (Figure 3b), this indicates that freezing at

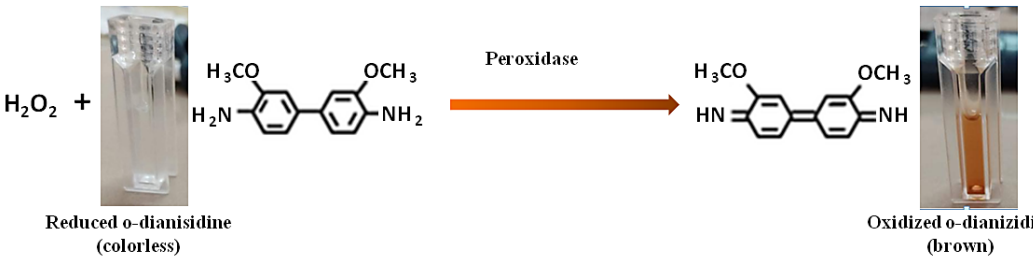
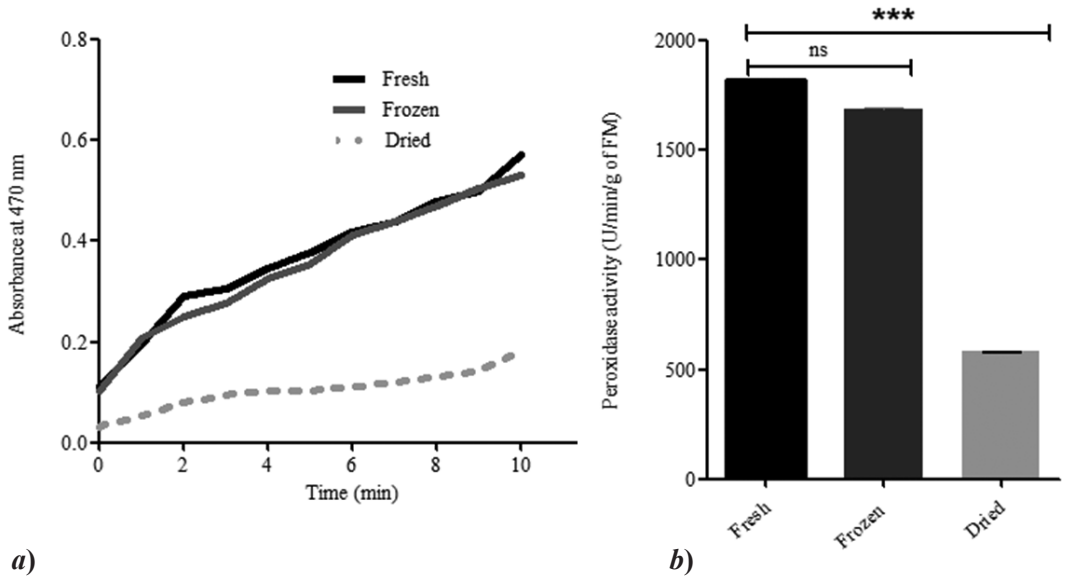


Fig. 2. Oxidation of O-dianisidine by H_2O_2 and peroxidase, color change from colorless to brown



a) *b*)

Fig. 3. The kinetics of Peroxidase absorbance (a) and activity (b) of fresh, freezing at -20°C and oven drying (50°C, 48 h) garlic extracts. Data represent mean values ± standard deviation of three essays. Differences were considered significant at *P < 0.05*

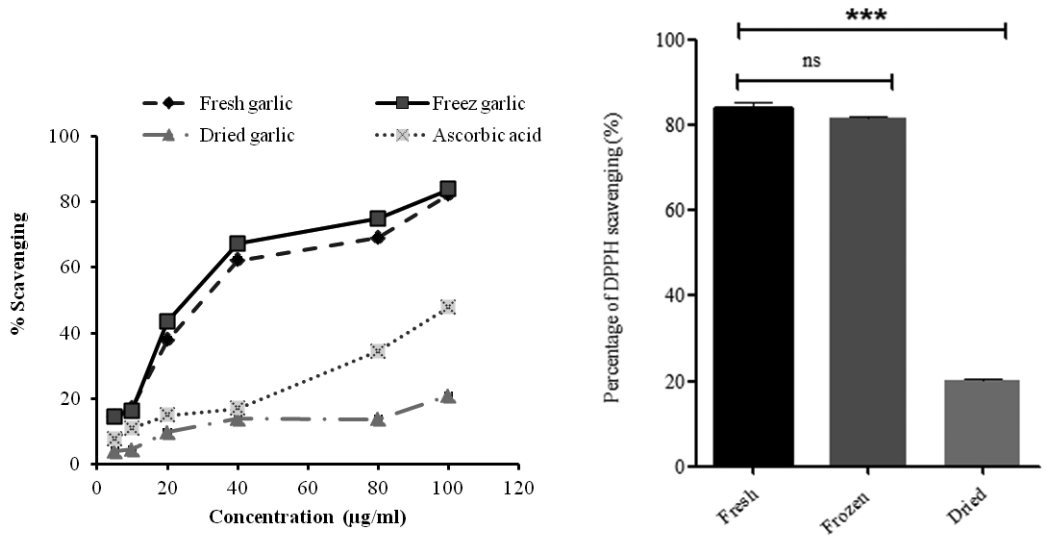


Fig. 4. The kinetics and percentage of DPPH scavenging (%) of garlic extracts, freezing at -20°C, oven drying (50°C, 48 h) compared with fresh sample and Ascorbic acid as a control. Differences were considered significant at *P < 0.05*

20°C does not significantly affect peroxidase activity. The loss of activity of the dried garlic extract (68 %) may be due to the inactivation of the labile peroxidase isoforms by heat. Indeed, the residual activity (32 %) is due to heat-resistant isoforms, which require temperatures above 40°C to be inactivated (Senani et al., 2023; Agüero et al., 2008).

DPPH scavenging activity

All three extracts of fresh, frozen or dried showed a dose dependent DPPH scavenging activity (Figure 4). Fresh and freeze garlic extracts displayed the best value from 37.78 ± 0.006% and 43.46 ± 0.007% respectively at the lowest concentration (20 µg/ml) to 82.24 ± 0.004% and 83.74 ± 0.002% respectively at the highest concentration (100 µg /ml) of extracts. They were followed by ascorbic acid, which represents the positive control of this test and the lowest percentage of inhibition was observed with dried garlic from 20.91 ± 0.001 % at the highest concentration (100 µg/ml).

Çubukçu et al. (2019) showed that heating reduced strongly the phenolic content and antioxidant activity of garlic. Similarly, Park et al. (2009) noted that the DPPH radical scavenging activity and phenolic content of fresh garlic extract were higher than those of heat-treated extract. A significantly positive correlation was found between the phenolic content and the antioxidant activity of garlic. This suggests that the degradation of antioxidants, particularly phenolics, resulted in a decrease in antioxidant activity. The antioxidant activity of phenolics depends on their ability to release electrons and the antioxidant power of flavonoids depends primarily on their ability to scavenge free radicals (Minatel et al., 2017). Phenolic compounds have been reported to be very potent antioxidants (Blamo et al., 2021). Freezing will limit the oxidation process and overcome the difficulties associated with growing seasons and deterioration of garlic during storage.

Conclusion

In this study, we report that the majority of biochemical and bioactive compounds of frozen garlic are well preserved contrary to dried garlic. Biochemical analysis also showed that garlic cloves are rich in peroxidase and that its activity persists after freezing, but very little after drying. The sum of these results clearly indicates that freezing is the method that allows the preservation of garlic without obvious degradation of the majority of the biochemical and bioactive compounds it contains, and thus preserves its nutritional quality.

Freezing and drying overcome the difficulties associated with growing seasons and deterioration of garlic during storage. Both freezing and drying can help maintain the bioactive compounds in garlic. However, heat treatment tends to reduce or destroy the bioactive compounds, which are sensitive to heat. Despite these results, dried garlic remains interesting from a food point of view because it retains its aromatic properties and flavor.

This study reminds us of the importance of choosing the right food preservation methods. It would be interesting to investigate other potential activities associated with dried garlic as its commercialization and consumption increases due to its convenience.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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