Influence of novel freezing and storage technology on nutrient contents, bioactive compounds and antioxidant capacity of black eggplant

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Abstract: The present work aims to study some technological aspects related to the preservation of black eggplant (*Solanum melongena* L.) fruits by freezing process. Also, the influence of such preservation method on the chemical composition, nutritional value, bioactive compounds content and antioxidant capacity of eggplant fruits will also determine. In fresh eggplant fruits, water is by far the most abundant components with 90.21% of the total fruit weight and fiber is particularly abundant (2.98%). Also, fruits has s moderate levels of vitamins (A, B3, B9, C, E and K) and minerals (P, Mg, Ca, Na, Fe, and Zn) but are relatively rich in K. Phenolics was reported the most abundant bioactive compounds, followed by anthocyanins, flavonoids and β-carotene. The high content of bioactive compounds in the eggplant samples was met with a high level of antioxidant capacities, which was determined by several different methods. The freezing method (slow freezing with grilling pretreatment) that was applied in this study led to cause slightly loses of all studied vitamins by the ratio of ranged -0.88 to -10.97%, and significant ($p \leq 0.05$) losses of bioactive compounds ratio of ranged -2.52 and -10.23% as compared with the fresh samples, respectively. In contrary, freezing has been shown to cause significant ($p \leq 0.05$) increase of antioxidant capacities by 4.57 and 11.74% for antioxidant activity and trolox equivalent (TE) assays, as compared with the fresh samples, respectively. Also, prolonging the storage period for six months at -18^{0}C did not have a significant effect on all previous measurements. In conclusion, the use of this new and effective method in eggplant preservation may contribute to increasing the economic importance of this product by making it available in the markets regularly throughout the year, easing of export, and increasing the income of the producing countries.

Keywords: Grilling, nutritional value, vitamins, minerals, β-carotene bleaching assay, DPPH, total bacterial count.

1. Introduction

Vegetables play an important role in human diets, as they support the normal functioning of the different body systems. They provide our cells with vitamins, minerals, fiber, essential oils and phytonutrients. Vegetables contain low amounts of fat and calories (Banerjee *et al.*, 2012). Eggplant (*Solanum melongena* L. Family: S*olanaceae*) also known as aubergine, brinjal, melanzane, berenjena or patlıcan is very popular vegetable grown over 1.7 million hectares world-wide (Gramazio et al., 2014). It is an economically important vegetable crop of tropical and subtropical zones of the world including Asia (47,142,210 tons), Africa (1,814,535 tons), Europe (936,642 tons), the Americas (295,387 tons) and Oceania (4342 tons) (Niño-Medina et al., 2017; Colak et al., 2022). In 2020, Egypt ranks third in the world in eggplant production with an annual production of approximately 1.34 million metric tons (https://www.tridge.com/intelligences/eggplant/EG). Egyptian black fruit are the most commonly marketed type, though purple and white cultivars have gained acceptability in recent years.

Eggplant fruits play an important role in human diets through their contain a considerable amount of carbohydrates, protein, vitamins and good minerals like potassium, calcium, magnesium, sodium, iron (Quamruzzaman et al., 2020). Also, it uses for medicinal purposes due to its composition, which includes has very low calories and phytochemicals that contain phenolic components (caffeine and chlorogenic acid), flavonoids, mainly nasunin as well as dietary fiber that is helpful for our health (Mohamed et al., 2003; Raigon et al., 2008; Quamruzzaman et al., 2020). Also, studies have indicated that anthocyanins and phenolic acids contribute to high antioxidant properties in eggplant (Plazas et al., 2013; Liu et al., 2018). The major phenolic compounds in eggplant fruits are reported to be highly beneficial for human health due to their known biological activities, and they can be potentially used in the treatment of several metabolic and cardiovascular diseases (Plazas et al., 2013). Also, eggplant exhibits potential health benefits in a number of degenerative diseases, cancer, cardiovascular diseases, diabetes, pulmonary disorders, and Alzheimer’s disease (Kwon et al., 2008; Li et al., 2017; Singh et al., 2017; Sharma and Kaushik, 2021). Furthermore, several authors have declared that eggplant enriched in phenolic compounds and free radical scavenging activity have a potential to reduce hyperglycemia-induced vascular complications resulting from oxidative damage (Cao et al., 2020; Sun et al., 2020; Elhassaneen et al., 2022-a). For all of these previous reasons, it is necessary to search for ideal preservation methods for eggplant fruits, through which the important nutritional and medicinal value is preserved, in addition to ensuring that they are available for feeding throughout the year.

Freezing is one of the oldest and most widely used methods of food preservation, which allows preservation of taste, texture, and nutritional value in foods better than any other method. The freezing process is a combination of the beneficial effects of low temperatures at which microorganisms cannot grow, chemical reactions are reduced, and cellular metabolic reactions are delayed (Delgado and Sun, 2000; Gustavo et al., 2005). The process involves lowering the temperature of the product i.e. plant parts or removing heat from it through freezing water in cell juices and tissue spaces that ensure that the frozen products remain at deep temperatures (-18 ^{0}C) without any physical and chemical characteristics changed. °C (Fennema *et al.*, 1973; Arthey, 1993). It is also scientifically proven that lowering the temperature to the level of freezing leads to the inhibition of the activity of microorganisms and the delay of chemical and enzymatic reactions, and thus preventing the products deterioration (cracking the nutrients contained in the product) and their corruption for long periods of time that may extend to years(George, 1993). Competing with new technologies of minimal processing of foods, industrial freezing is the most satisfactory method for preserving quality during long storage periods (Gustavo et al., 2005). Therefore, the present work aims to study some technological aspects related to the preservation of eggplant fruits by freezing process. Also, the influence of the preservation method on the chemical composition, nutritional value, bioactive compounds content and biological activities of eggplant fruits will be in the scope of this investigation.

2. Materials and Methods

2.1. Materials

2.1.1. Eggplant fruit samples

Eggplant (*Solanum melongena*) raw fresh fruits were collected by special arrangements with some farmers from Benha City, Al Qalyubia Governorate, Egypt The samples were verified in strains by the Staff of Agricultural Plant Department, Faculty of Agriculture in Moshtohor, Benha University, Al Qalyubia Governorate, Egypt.

2.1.2. Chemicals

Bioactive compounds standard [gallic acid (GA), Delphinidin 3-O-glucoside equivalent (DOGE), quercetin, α-tocopherol and Butylated hydroxytoluene (BHT)], DDPH (2,2-diphenyl-1-picrylhydrazyl), were purchased from Sigma Chemical Co., St. Louis, MO. All other chemicals, reagents and solvents (Except as otherwise stated), were of analytical grade were purchased from El-Ghomhorya Company for Trading Drug, Chemicals and Medical Instruments, Cairo, Egypt.

2.2. Methods

2.2.1. Preparation of frozen eggplant samples

Such as shown in Figure (1), immediately after raw fresh fruits of eggplant samples arrive at the laboratory, it has been initially cleaned by dry methods to remove dust and coarse surface residues. Fruits were washed with potable water by immersion methods and dried with sterile paper towels. The fruits were then sliced with sharp and thin blades, are mandatory during cutting operations to minimize cell disruption and nutrient leakage. Immediately upon cutting, the slices were subjected to grilling treatment, by placing them between stainless wire nets (so that the grill is above and below the slices) and subjected to grilling heat for 50 seconds, then suddenly cooled to 4 degrees Celsius in preparation for the process of freezing. Samples were taken from the grilled slides for measured the activity of the peroxidase enzyme and the results were negative. Such data proves the efficiency and adequacy of the grilling process to inactivate the enzymes that cause color degradation/browning reaction. The cooled slides were introduced to the slow freezing process at -18°C for 8 hours, after which they were used as samples for the study.

Eggplant fruits, **Raw and fresh slices** **Grilled slices**

Frozen slices

Figure 1. Stages of freezing eggplant

2.2.2. Preparation of eggplant sample extracts

Extract of eggplant samples was prepared for antioxidant capacity studies as follow: 20 g frozen eggplant plus 180 ml water were homogenised and transferred to a beaker, where they were agitated at 200 rpm for 1 hour at room temperature in an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany). Filtration via Whatman No. 1 filter paper separated the extract from the residue. The leftover residue was extracted twice more, and the two extracts were then mixed. A rotary evaporator was used to extract the residual solvent at 45°C under decreased pressure (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany).

2.2.3. Proximate and nutritional analysis of fresh and frozen eggplant samples

Fresh and frozen eggplant samples were analyzed for proximate composition including moisture, protein (T.N.× 6.25, micro - kjeldahl method using semiautomatic apparatus, Velp company, Italy), fat (soxhelt semiautomatic apparatus Velp company, Italy, petroleum ether solvent), ash, fiber and dietary fiber contents were determined using the methods described in the AOAC, (1995). Carbohydrates calculated by differences. Total energy (Kcal/100 g) was calculated according to Insel *et al*, (2002) using the following equation: total energy value (Kcal/100g) = 4 (Protein, % + carbohydrates, %) + 9 (Fat, %). Grams consumed of food as wet weight basis to cover the daily requirements of adult man, in protein (63 g), G.D.R. g, was calculated using the RDA (1989) values. Percent satisfaction of the daily requirement of adult man in protein (P.S.,%) and in energy when consuming the possibly commonly used portions in Egypt i.e. 100 g weight, was also calculated.

2.2.4. Determination of minerals content

Minerals content of eggplant samples were determined according to the method mentioned by Singh *et al.*, (1991) as follow: half gram of defatted sample were transferred into a digested glass tube and 6 ml of tri-acids mixture (nitric acid: perchloric acid : sulfuric acid in the ratio of 20 : 4 : 1 v/v respectively) were added. The tubes content were digested gradually as follow, 30 min at 70 ^{0}C; 30 min at 180 ^{0}C and 30 min at 220 ^{0}C. After digestion, the mixture was cooled, dissolved in distilled water, and the volume was increased to 50 ml in volumetric beaker. After filtration in ashless filter paper, aliquots were analyzed for minerals (Ca, Fe, Mg, P, K, Na and Zn) using of atomic absorption spectrophotometer (Shimadzu Corporation, Tokyo, Japan).

2.2.5. Vitamins determination

Fat soluble vitamins (A and E) were extracted from the eggplant samples according to the methods described by Epler *et al.*,(1993) and Hung *et al.*, (1980) while water soluble vitamins (B group and C) according to Moeslinger *et al.,* (1994), and analyzed by HPLC techniques. Under the chromatographic conditions used in those methods, mean values ±SD of vitamins A, C, E, K, B9 and B3 recoveries were 89.67± 2.11, 87.96 ±2.14, 87.93± 2.94, 84.74 ±1.03, 91.11± 1.05, and 87.01±3.14%, respectively.

2.2.6. Bioactive compounds determination

Total phenolics in fresh and frozen eggplant samples were determined using Folin-Ciocalteu reagent according to Singleton and Rossi, (1965) and Wolfe *et al.*, (2003). Gallic acid (GA) and equivalents are used to express the results (GAE). The total β-carotene was determined by using UV-Vis spectrophotometric (Shimadzu Corporation, Kyoto, Japan) method reported by Biswas et al., (2011). Total flavonoids contents were estimated using colorimetric assay described by Zhisen et al., (1999). Total flavonoid content was measured in mg of quercetin equivalent (QE) per 100g DW. Total content of anthocyanins measured spectrophotometrically (Shimadzu Corporation, Kyoto, Japan) using the method of Sharif et al., (2011). Total anthocuanins content was measured in mg of delphinidin 3-O-glucoside equivalent (DOGE) per 100g DW.

2.2.7. Antioxidant activity determination

2.2.7.1. Antioxidant activity (AA)

Antioxidant activity (AA) of eggplant samples extract and standards (α-tocopherol and BHT) was determined and calculated according to the procedures described by Marco, (1968) and Al-Saikhan *et al.,* (1995).

2.2.7.2. *β*-carotene bleaching (BCB) assay

For *β*-carotene bleaching (BCB) assay, antioxidant activity (AA) against time (every 10 min thereafter for 120 min) for the eggplant samples extract was measured/constructed according to Marco, (1968). The AA was all calculated as percent inhibition (bleaching rates of *β*-carotene in reactant mixture of eggplant extracts) relative to control (bleaching rates of *β*-carotene in reactant mixture of without eggplant extracts) such as described by Al-Saikhan *et al.,* (1995).

2.2.7.3. Trolox equivalent Antioxidant capacity (TEAC) assay

Antioxidant capacity (AC) of eggplant samples extracts and standards (Trolox equivalent, TE) was determined by Trolox equivalent Antioxidant capacity (TEAC) assay according to the procedures mentioned by Nallan et al., (2021) using the specific assay kit (Cell Biolabs, Inc., San Diego, CA, USA).

2.2.7.4. DPPH radical scavenging assay

Free radical scavenging ability of eggplant extracts was tested by DPPH radical scavenging assay as described by Desmarchelier et al. (1997). A solution was prepared, and 2.4 mL of DPPH (2,2-diphenyl-1-picrylhydrazyl, 0.1 mM in methanol) was mixed with 1.6 mL of eggplant extract at different concentrations (12.5–150 μg/mL). The mixture's absorbance was measured spectrophotometrically (Shimadzu Corporation, Kyoto, Japan) at 517 nm (UV-160A; Shimadzu Corporation, Kyoto, Japan). BHT was utilized as a standard. Percentage DPPH radical scavenging activity was calculated by the following equation: DPPH radical scavenging activity (%) =[(A0− A1)/A0]×100. where A_0, absorbance of the control, and A_1, absorbance of the eggplant/BHT. Then inhibition (%) was plotted against concentration, and IC_{50} was calculated from the graph.

2.2.7.5. Total bacterial counts

Total bacterial counts of fresh and frozen eggplant samples were determined by plating suitable dilution in duplicates using nutrient agar medium according to the Difco Manual (1966). This medium consists of beef extract, 3 g/L, Bacto peptone, 5 3 g/L, agar, 15 3 g/L, sodium chloride, 5 g/L, distilled water to 1000 ml and pH, 7. Plates were incubated at 32ºC for 3 days before counting and recording the results.

2.3. Statistical Analysis

All the experiments were carried out in a completely randomized design. All measurements were done in triplicates and presented as mean ± standard deviations (SD). Results were subjected to analysis statistically by ANOVA and means were compared by a Fisher test at $P \leq 0.05$.

3. Results and Discussion

3.1. Proximate composition and nutritional evaluation of eggplant fruit

The results for the proximate composition of and nutritional evaluation of eggplant fruit are presented in Table (1). Water is by far the most abundant components with 90.21% of the total fruit weight. Fiber is particularly abundant (2.98%) compared to other foods and even to other vegetable sources. Proteins and lipids are present at very low levels which recorded 1.15 and 0.21%, respectively. The results are consistent with that reviewed by Zaro *et al.,* (2015) where was the moisture, protein, fat, fiber, carbohydrates were 92.30, 0.98, 0.18, 3.00 and 5.88%, respectively. Also, Raigon et al. (2008) found that protein content varied from 0.41 g/100g to 0.62 g/kg for landrace cultivars and from 0.43 g/kg to 0.59 g/kg for commercial cultivars eggplant. The USDA database also reports that the protein content for fresh eggplant is 0.98% (12.73% in dry basic) (Rodriguez-Jimenez et al., 2018). Some studies noticed that the high nutritional value of eggplant is attributed to the presence of proteins in its fruit. For example, Kandoliya et al. (2015) eggplant protein intake can significantly contribute to the development of hormones responsible for different body functions, including growth and repair, in addition to the maintenance of the body. In another study, San José et al., (2006) identify total available carbohydrates in several verities of eggplant which varied from 2.89 g/100 g to 8.04 g/100 g. Also, available carbohydrates in eggplant contribute to low sugar content in the fruit (Rodriguez et al., (2018). The previous studies with the others reveals that the difference in proximate composition of eggplant fruits could be due to a number of factors, namely the verities/genotypes of eggplant, the stage of development, the origin of the samples, agricultural treatments, (San José et al., 2013; Raigon et al., 2008 and 2010; Sharma et al., 2021). Generally, data of the present study indicate that the eggplant fruit was good source of different nutrients for humans including carbohydrates, fiber and ash. Also, it is low fat calorie foods subsequently more suitable for humans in diet and cardiovascular diseases (CVD). Additionally, eggplant fruit is characterized by a reasonable amount in fiber. Although fiber is indigestible, it plays significant nutritional role through helps to provide bulk to stool and aid in the movement through the digestive tract.

The nutritional evaluation of the eggplant fruits is shown in Table (2). From such date it could be noticed that the total energy was recorded 23.84 Kcal/100g DW, G.D.R. (g) for protein (63 g) was 5469g, G.D.R. (g) for energy (2900 Kcal) was 1583.63 ±20.67, P.S./ 100 g for protein (63g) was 12.53 ±1.11% and P.S./100 g for energy (2900 Kcal) was 12166 ±0.85%. The nutritional evaluation reported was accordance with that observed by Zaro et al., (2015) and Sharma et al., (2021). Such data prove that the eggplant fruits represents a low source of protein. The onsumption of 100 g fresh fruit covers 1.83 % of the daily requirement of the adult person for energy (63 g). Also, eggplant represents extremely low-calorie foods i.e. consumption of 100 g powder cover only 0.82 % of the daily requirement of the adult person for energy (2900 Kcal). This is due to its low fat content. Such data confirm the possibility of successfully using eggplant fruits in nutritional applications for obese and overweight patients, CVD, diabetes etc.

Table 1. Proximate composition and nutritional evaluation of eggplant (per 100 g of fresh and raw fruit)*

Component	Content
Proximate composition	
Moisture	90.21 ± 2.16
Total protein (g)	1.15 ± 0.13
Crude fat (g)	0.21 ± 0.08
Crude Fiber (g)	2.98 ± 0.36
Ash (g)	1.12 ± 0.09
Carbohydrate (g)	4.33 ± 0.78
Nutritional evaluation	

Total Energy (Kcal)	23.84 ± 2.56
G.D.R. (g) for protein (63 g)	5469 ± 127
G.D.R. (g) for energy (2900 Kcal)	12166 ± 215
P.S./100 g (%) for protein (63g)	1.83 ± 0.21
P.S./100 g (%) For energy (2900 Kcal)	0.82 ± 0.07

* Each value represents the mean of three replicates ±SD. G.D.R., Grams consumed of eggplant fruit (fresh weight basis) to cover the daily requirements of adult man (63 g) in protein or energy, P.S. (%) , the percent satisfaction of the daily needs of adult man in energy upon consumption 100 g of eggplant fruit (fresh weight basis).

3.2. Minerals content of eggplant fruit

Several nutritional studies indicate that there is a general public interest in the availability of essential and non-essential elements in the foods consumed daily. Thus, the current study concerned with estimating the mineral content in eggplant fruits (Table 2). Our results showed that the eggplant fruits are rich in different estimated elements. K recorded higher contents followed by P, Mg, Ca, Na, Fe, and Zn. Such data are in accordance with that reported by Sharma and Kaushik, (2021) who found that eggplants are rich sources of biologically essential minerals, such as Na, K, Ca, Mg, P, Fe, Cu, and Zn, for which contents are similar to those reported in tomatoes and higher than those found in carrots, potatoes, or onions. Also, Arivalagan et al., (2012) the mean values of K, Mg, Fe and Zn in five Indian commercial varieties of eggplant were recorded 193, 9.91, 0.342 and 0.135 mg/100 g, respectively. Furthermore, Guillermo et al. (2014) found that Hindu cultivar exhibited the high values for concentrations (mg/100 g) of K (191.18), Ca (59.63), P (33.52), Mg (28.96), Zn (0.78), and Mn (0.44); whereas the Philippine cultivar reported high concentrations of Fe (3.13). Minerals such as K, Ca, P and Mg are said to be major elements because they are in high concentrations of eggplant fruits. However, Na is relatively less in eggplant; thus, it is said to be good for patients with hypertension. As a source of trace elements (Fe and Zn), the situation of eggplant becomes quite practical when considered consumption of daily or several times a week by inserting it into different dishes.). The roles of Zn in the immune system is a consequence of its role in cell growth, division and maturation, cell membrane stabilization, as well as in DNA and RNA synthesis (Osredkar Sustar, 2011 and Nazanin *et al.,* 2013). The importance of Fe, as an integral part of hemoglobin in red blood cells (RBC's), the transfer of oxygen from the lungs to the tissues of all organs in the body. Also, it is necessary for DNA synthesis and plays an important role in the immune system (Nazanin *et al.,* 2014).

Table 2. Minerals content of eggplant (per 100 g of fresh and raw fruit)*

Component	Content
Ca	11.23 ± 3.67
Fe	0.27 ± 0.04
Mg	16.34 ± 2.54
P	28.56 ± 2.45
K	234.67 ± 10.45
Na	2.38 ± 0.23
Zn	0.19 ± 0.03

* Each value represents the mean of three replicates ±SD.

3.3. Vitamins concentrations in Eggplant fruits

The vitamins concentration of eggplant fruits is given in Table (3). Vitamin b3 (Niacin) was the most abundant vitamins, followed by vitamins C, E, K, A and B6 (folate). Such data are accordance with that observed by Zaro et al., (2015) who reviewed that the fruit has moderate levels of most vitamins. In another study, Shabetya et al., (2020) reported that the vitamin C content of eggplant varied from 3.9 to 4.1 mg (per 100 g) in the Belgorod region. Also, Guillermo et al., (2014) found that the vitamin C content in eggplant grown in the Crimea foothill zone was slightly higher than that in eggplant grown in the Belgorod region, which ranged from 4.8 to 4.9 mg/100 g. In general, vitamins are essential for good health and for growth. Eggplant is a good source of vitamins b3 (niacin) and C. Several nutrients such as vitamin C, vitamin E and carotenoids are known to act as antioxidants; however, niacin is one of the neglected antioxidant nutrients that may have an antioxidant action both independently, and also as a component of the glutathione redox cycle (Ilkhani et al., 2014). Also, niacin is a B vitamin that's made and used by your body to turn food into energy. It helps keep your nervous system, brain functions, digestive system, heart and skin healthy (Arun et al., 1999).Vitamin C is an essential dietary nutrient for the biosynthesis of collagen and a co-factor in the biosynthesis of catecholamines, L-carnitine, cholesterol, amino acids, and some peptide hormones. Giuseppe eal., 2013). Also, it is potentially involved also in cancer and cardiovascular diseases prevention. In addition, vitamin C effects on nervous system and chronically ill patients have been also documented (Walingo, 2005).

Table 3. Vitamins content of eggplant (per 100 g of fresh and raw fruit)*

Component	Content
Vitamin A (µg)	1.22 ± 0.11
Vitamin C (mg)	2.20 ± 0.19
Vitamin E (mg)	0.43 ± 0.07
Vitamin K (µg)	26.43 ± 2.17
Vitamin B9 (Folate, µg)	0.81 ± 0.07
Vitamin B3 (Niacin, mg)	4.11 ± 1.23

* Each value represents the mean of three replicates ±SD.

3.4. Bioactive compounds and antioxidant capacity in eggplant fruits

Total phenolics, β-carotene , flavonoids and anthocyanins were determined in eggplant fruits as shown in Table (4). Phenolics was reported the most abundant ones, followed by anthocyanins, flavonoids and β-carotene. The total phenolic content recorded in eggplant fruits samples content are higher than those found different categories of vegetables which studied by El-Mokadem (2010) and Elhassaneen and Abd Elhady, (2014). The high content of bioactive compounds in the eggplant samples was met with a high level of antioxidant activity, which is determined by two different methods. Such data are partially in accordance with that reviewed by Zaro *et al.,* (2015). Also, Ninfali et al. (2005) found the amounts of total phenolic content in Black Beauty and Violetta Lunga eggplant variants was 57.4 and 64.8 mg caffeic acid equivalents (CAE)/ 100 g FW, respectively. Furthermore, Okmen et al. (2009) examined different Turkish eggplant cultivars and found that the total phenolic content varied from 615 mg/kg to 1389 mg/kg. Several studies reported that bioactive compounds determined in the present study for eggplant fruits samples was affected by the environmental factors such as light, water, and temperature, shape, cultivated genotypes and species (Hanson et al., 2006; Guillermo et al., 2014; Kaur et al., 2014). In this context, Guillermo et al., (2014) found that the levels of total phenolics in eggplant fruits cultivars which varied from 1350 mg of chlorogenic acid equivalents (CAE)/100 g DW for the Chinese cultivar to 2049 mg CAE/100 g for the Thai cultivar. Also, Matsuzoe et al., 1999) reported that the violet hues of dark eggplant genotypes (such as characterized by the present study samples) are imparted mainly by delphinidin type anthocyanins.

In general and several years ago, eggplants have received higher interest due to their high levels of bioactive compounds. In a study evaluating the antioxidant capacity of different fresh vegetables, eggplants ranked within the top 10 (Cao et al., 1996). Also, eggplant extracts inhibited the inflammation and radical-mediated pathogenesis, carcinogenesis, and atherosclerosis (Han et al., 2003; Matsubara et al., 2005; Aly et al., 2017). Furthermore, high eggplant intake exerted hepatoprotective and hypolipidemic effects as well as reduced blood glucose levels in experimental animals Sudheesh etal., 1997; Derivi et al., 2002; Akanitapichat et al., 2010; Zaro *et al.*, (2015). Our previous studies, along with others, have proven that bioactive compounds (Phenolics, flavonoids and β-carotene) which was spotted in this study inside eggplant fruits play an significant roles in preventing and/or treating many diseases such as diabetes, atherosclerosis, cancer, obesity, bone and aging (Elhassaneen *et al.*, 2016 a-c, 2019, 2022 a and b; Elsemelawy et al., 2021). All of the previous effects of these compounds are due mainly to their magical antioxidant activities. Also, anthocyanin rich foods such eggplant are known to be highly effective against various health problems, such as diabetes, neuronal disorders, cardio-vascular disorders, and cancer (Yousuf et al., 2016). Furthermore, supplementation of diets with anthocyanins in experimental animals has been shown to exhibit prevention/ treatment effects against cancer and CVD (Mazza, 2007; Lin et al., 2017).

Table 4. Total content of bioactive compounds and antioxidant capacity in eggplant (per 100 g of fresh and raw fruit)*

Component	Content
Bioactive compounds:	
Phenolics (mg GAE/100 g FW)	1875.02 ± 67.75
β-carotene (µg/100g)	33.14 ± 3.68
Flavonoids (mg QE/100g FW)	311.21 ± 19.43
Anthocyanins (mg DOGE/100g FW)	1383.07 ± 74.39
Antioxidant activities:	
Antioxidant activity, AA (% , α-tocopherol standard, 50 mg/L)	87.56 ± 1.97
Trolox equivalent (mM TE/g dw)	209.56 ± 14.56

Each value represents the mean of three replicates ±SD. GAE, gallic acid equivalent, QE, quercetin equivalent, DOGE, delphinidin 3-*O*-glucoside equivalent.

3.5. Effect of freezing process on proximate composition and nutritional evaluation of eggplant fruits

Effect of freezing process on proximate composition and nutritional evaluation of eggplant was studied as shown in Tables (5). Freezing has been shown to cause slightly increases of moisture and ash content in eggplant fruits by the ratio of 1.40 and 8.04% and losses of the total protein, crude fat, crude fiber and carbohydrates by -3.82, -10.71, -3.90 and -27.06% as compared with the fresh samples. As for the nutritional value of the frozen eggplant, it has slightly changed as a result of the changes that occurred in the chemical composition as a result of the freezing process. According to the results of the current study, the slight changes that occurred in the chemical composition of eggplant as a result of freezing may be due to the increase in the percentage of moisture trapped inside the frozen parts as a result of treatment with pretreatments (grilling), and the consequent deficiency in the rest of the nutrients. Such data are partially in accordance with that found by Gamila (2102) who study the effect of freezing on the chemical composition of artichoke. Also, Grafe and Mirko, (2014) found that the chemical changes which take place during actual freezing are limited,as compared to the storage changes. However, if hydrolysis, oxidation, and action of the enzymes are not controlled during the freezing of foods, then storage changes are of greater magnitude. Furthermore, for any type of food preservation method, the retention of nutritional components is a concern, but freezing is probably the least destructive when properly done (Sebranek, 1996). To keep high nutritional quality in frozen vegetables, it is essential to follow directions contained for each and every step of the freezing process (Schafer and Munson, 1990). In another study, Bender, (1981) reported that the freezing process itself has no effect on nutrients, but during pre-treatment (prior to freezing) water-soluble nutrients may be leached-out during the process. Finally, many studies have proven that the changes that occur in the chemical composition of foodstuffs as a result of freezing may be due to the applied freezing method, and whether it was slow or fast freezing. On this context, Zaro et al., (2015) reviewed that rapid freezing is reducing intracellular water and minimizing the formation of large ice crystals and drip losses after thawing, which would result in highest grade products. Also, high-pressure freezing minimized fruit structural damage.

Table 5. Effect of freezing process on proximate composition and nutritional evaluation of eggplant (per 100 g of fresh and raw fruit)*

Component	Fresh samples	Freezing storage (Month)			
		0	2	4	6
Proximate composition					
Moisture	90.21 ± 2.16 a	91.47 ± 1.67a	91.43 ± 0.97 a	91.39 ± 1.12 a	91.14 ± 2.07 a
Total protein (g)	1.15 ± 0.13 a	1.11 ± 0.14 a	1.10 ± 0.09 a	1.11 ± 0.10 a	1.12 ± 0.07 a
Crude fat (g)	0.21 ± 0.08 a	0.19 ± 0.05 a	0.19 ± 0.04 a	0.20 ± 0.01 a	0.20 ± 0.04 a
Crude Fiber (g)	2.98 ± 0.36 a	2.86 ± 0.65 a	2.90 ± 0.53 a	2.91 ± 0.45 a	2.93 ± 0.61 a
Ash (g)	1.12 ± 0.09 b	1.21 ± 0.08 a	1.22 ± 0.11 a	1.23 ± 0.07 a	1.24 ± 0.12 a
Carbohydrate (g)	4.33 ± 0.78 a	3.16 ± 0.63 b	3.17 ± 0.69 b	3.17 ± 0.57 b	3.37 ± 0.49 **b**
Nutritional evaluation					
Total Energy (Kcal)	23.84 ± 2.56 a	18.77 ± 1.23 b	18.76 ± 1.11 b	18.86 ± 1.43 b	19.72 ± 0.89 b
G.D.R. (g) for protein (63 g)	5469 ± 127 a	5686 ± 103 a	5727 ± 176 a	5676 ± 134 a	5625 ± 151 a
G.D.R. (g) for energy (2900 Kcal)	12166± 215 b	15453 ± 197 a	15462 ± 203 a	15376 ± 184 a	147034 ± 127 ab
P.S./100 g (%) for protein (63g)	1.83 ± 0.21 a	1.76 ± 0.23 a	1.75 ± 0.19 a	1.76 ± 0.32 a	1.78 ± 0.26 a
P.S./100 g (%) For energy (2900 Kcal)	0.82 ± 0.07 a	0.65 ± 0.10 b	0.65 ± 0.11 b	0.65 ± 0.12 b	0.68 ± 0.09 b

* Each value represents the mean of three replicates ±SD. G.D.R., Grams consumed of eggplant fruit (fresh weight basis) to cover the daily requirements of adult man (63 g) in protein or energy, P.S. (%) , the percent satisfaction of the daily needs of adult man in energy upon consumption 100 g of eggplant fruit (fresh weight basis).

3.6. Effect of freezing process on vitamins content of eggplant fruits

Effect of freezing process on vitamins content of eggplant was studied as shown in Tables (6) and Figure (2). Freezing has been shown to cause slightly loses of all studied vitamins in eggplant fruits by the ratio of ranged -0.88 to -.10.97%. All the vitamins also continue to degrade during prolonged storage of frozen products by the rates -1.49 and -22.20% after 6 months of storage. The most effective vitamins were vitamin C and the least was vitamin K, which recorded a decrease rate of -22.20% and -1.49% at the end of the experiment period (6 months). Vitamin C is a water soluble vitamin that is an important antioxidant in the human body, which can prevent cancer and other diseases (Martins and Silva, 2004). Due to its high sensitivity, it is widely considered as an appropriate marker for monitoring changes during processes including storage in the whole frozen chain. Such as explained by Serpen et al., 2007), vitamin C degradation is caused by two factors, L-ascorbic acid oxidation and by destructive effects of specific enzymes which enhanced under temperature fluctuations during storage. In similar study, Favell, (1998) reported changes in vitamin C due to freezing for several vegetables. He found negligible losses in carrots but 20 and 30% losses in broccoli and green peas respectively. Retention of vitamin C can vary tremendously in all products, depending on cultivar and processing conditions among other variables. In general, losses due to the entire freezing process can range from 10 to 80% (Fennema, 1982). In another study, Rickman et al., (2007) vitamin C also continues to degrade during prolonged storage of frozen products. Losses after 1 year for fruits and vegetables stored at −18 to −20 ^{0}C averaged 20–50% WW for products such as broccoli and spinach. Folate, vitamin B, are reduced derivatives of folic acid, which naturally occur in foods, and are necessary for proper functioning of the body. The rich sources of folate in the human diet are green vegetables, (Rampersaud et al., 2003). Unfortunately, these vitamins are very unstable compounds and quickly lose their biologically activity. They undergo degradation at high temperature, sunlight, during storage time and food product preparation (Johansson et al., 2008, Xue et al. 2011). In the same context, Hakes and Villota (1989) found that folate reduction can vary from 22% in asparagus up to 84% in cauliflower due to the use of different technological treatments including freezing. In related to niacin, few studies on changes in it during frozen storage suggest that there is some degradation of these vitamins during storage for most products (Marta et al., 2017). Vitamins A, and E, because these vitamins are fat-soluble, they aren't affected during the pre-treatment process done before freezing. These vitamins will stay in frozen foods until they are thawed and exposed to light and heat. The study of Romeu-Nadal et al., (2008) reported that vitamin E levels did not change at either refrigeration temperature (under 24 h) or at freezing or ultrafreezing temperatures.

Table 6. Effect of freezing process and storage on vitamins content of eggplant (per 100 g of fresh and raw fruit)*

Component	Fresh samples	Freezing storage (Month)			
		0	2	4	6
Vitamin A (µg)	1.22 ± 0.11 [a]	1.21 ± 0.09 [a]	1.20 ± 0.10 [a]	1.20 ± 0.07 [a]	1.19 ± 0.16 [a]
Vitamin C (mg)	2.20 ± 0.19 [a]	1.96 ± 0.10 [b]	1.95 ± 0.21 [b]	1.83 ± 0.31 [b]	1.71 ± 0.21 [b]
Vitamin E (mg)	0.43 ± 0.07 [a]	0.43 ± 0.09 [a]	0.42 ± 0.08 [a]	0.42 ± 0.11 [a]	0.42± 0.09 [a]
Folate (µg)	26.43 ± 2.17 [a]	24.01 ± 1.80 [b]	23.87 ± 2.07 [b]	23.51 ± 1.55 [a]	22.98 ± 3.03 [a]
Niacin (mg)	0.81 ± 0.07 [a]	0.79 ± 0.11 [a]	0.79 ± 0.12 [a]	0.76 ± 0.08 [a]	0.75 ± 0.12 [a]
Vitamin K (µg)	4.11 ± 1.23 [a]	4.04 ± 0.98 [a]	4.01 ± 0.69 [a]	4.00 ± 1.05 [a]	3.98 ± 0.37 [a]

* Each value represents the mean of three replicates ±SD.

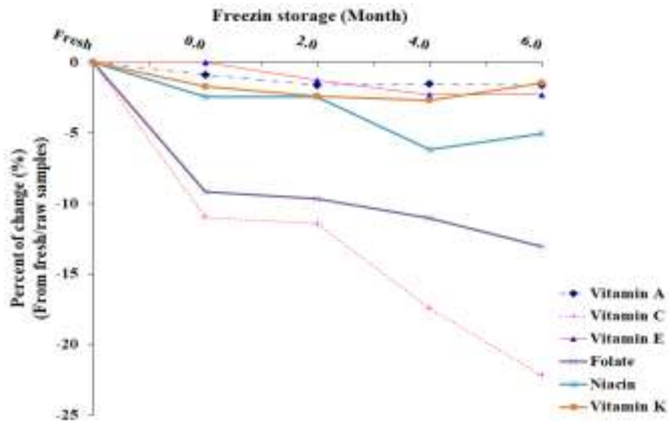

Figure 2. Effect of freezing process and storage on vitamins content (% of change fresh/raw samples) of eggplant fruit. Each value represents the mean of three replicates.

3.7. Effect of freezing process on bioactive compounds content of eggplant fruits

Effect of freezing process on bioactive compounds of eggplant was studied as shown in Table (7) and Figure (3). Freezing has been shown to cause significant($p \leq 0.05$) losses of phenolics, β-carotene, flavonoids and anthocyanins eggplant fruits by the ratio of -6.57, -6.43, -2.52 and -10.23% as compared with the fresh samples. All the bioactive compounds also continue to degrade during prolonged storage of frozen products by ratio of -20.50, -19.52, -13.24 and -18.92% as compared with the fresh samples after 6 months of storage**.** In general, freezing causes increase/decrease of phenolic compounds in vegetables, with retention levels dependent on cultivar. For example, Puupponen-Pimi¨a *et al*., (2003) studied the effects of blanching and freezing on phenolic compounds of peas, carrots, cauliflower, cabbage and potatoes. The authors reported an average loss of 20–30% of total phenolics in most vegetables, although no change was observed in most carrot samples and a 26% increase was observed in cabbage. Changes in total phenolics during frozen storage seem to depend heavily on commodity**.** No statistically significant change was observed in total phenolics of frozen peaches during 3 months of storage on a wet weight basis (Asami et al., 2002). Also, Puupponen-Pimi¨a *et al*., (2003) found some losses of total phenolics during 12 months of frozen storage of vegetables including broccoli, carrots, cauliflower, peas and potatoes. Furthermore, significant decreases in total phenolics and total anthocyanins were found during frozen storage of Bing cherries. Losses of 50 and 87% of total phenolics and total anthocyanins respectively were recorded after 6 months of storage at $-20\ ^{0}$C. In another studies, Gonz´alez *et al*., (2003) studied four raspberry cultivars and found different results for each, ranging from no change to an increase of 12% and decreases of 21 and 28%, during 12months of frozen storage. Such data confirmed that hanges in total phenolics and anthocyanins were dependent on cultivar. For β-carotene, numerous epidemiological studies have demonstrated that populations that consume large amount of fruits and vegetables rich in those compounds have dramatically lower risk of contracting various diseases including cancer, Alzheimer's and Immuno Deficiency virus (Wald et al., 1988 ; Garewal et al., 1992; Zaman et al., 1992).The decrease in β-carotene was found to be insignificant compared to the initial value even after 80 days of storage time at -18^{0}C and for 3 min blanch time (Debjani et al., 2005).

Table 7. Effect of freezing process on bioactive compounds content of eggplant (per 100 g of fresh and raw fruit)*

Component	Fresh samples	Freezing storage (month)			
		0	2	4	6
Phenolics (mg GAE)	1875.02 ± 67.75 ^{a}	1751.75 ± 45.12 ^{b}	1518.06 ± 46.82 ^{c}	1501.68 ± 29.56 ^{c}	1490.62 ± 32.56 ^{c}
β-carotene (µg)	33.14 ± 3.68 ^{a}	31.01 ± 2.13 ^{ab}	28.34 ± 2.09 ^{b}	27.01 ± 4.01 ^{b}	26.67 ± 3.45 ^{b}
Flavonoids (mg QE)	311.21 ± 19.43 ^{a}	303.36 ± 11.56 ^{a}	281.45 ± 20.97 ^{b}	270.67 ± 316.34 ^{b}	269.07 ± 19.53 ^{b}
Anthocyanins (mg DOGE)	1383.07 ± 74.39 ^{a}	1241.54 ± 56.32 ^{b}	1165.67 ± 42.87 ^{c}	1138.89 ± 31.65 ^{c}	1121.45 ± 29.79 ^{c}

* Each value represents the mean of three replicates ±SD. GAE, gallic acid equivalent, QE, quercetin equivalent, DOGE, delphinidin 3-*O*-glucoside equivalent.

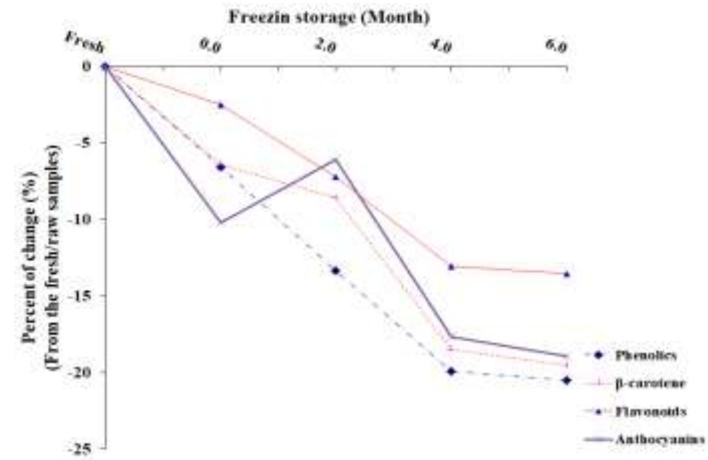

Figure 3. Effect of freezing process and storage on bioactive compounds content (% of change from the fresh/raw samples) of eggplant fruit. Each value represents the mean of three replicates.

3.8. Effect of freezing process on antioxidant activities of eggplant fruit extract

3.8.1. Antioxidant activities

Effect of freezing process on antioxidant activity of eggplant was studied as shown in Table (8) and Figure (4). Freezing has been shown to cause significant ($p \leq 0.05$) increase of antioxidant activities of eggplant fruits. By the methods of estimation used, antioxidant activity (AA, %) and trolox equivalent (TE), the ratio of increasing were 4.57 and 11.74%, respectively as compared with the fresh samples. All the antioxidant activities also continue to slightly increases during prolonged storage of frozen products by ratio of 5.42 and 14.63 % (For AA and TE, respectively), as compared with the fresh samples after 6 months of storage.

Table 8. Effect of freezing process on antioxidant activities of eggplant fruit extract*

Component	Fresh samples	Freezing storage (Month)			
		0	2	4	6
Antioxidant activity, AA (% , α-tocopherol standard, , 50 mg/L)	87.56 ± 1.97 b	91.56 ± 2.21 a	92.15 ± 1.86 a	92.54 ± 3.44 a	92.31 ± 2.58 a
Trolox equivalent (mM TE/g dw)	209.56 ± 14.56 b	234.17 ± 20.51 a	239.84 ± 16.75 a	241.16 ± 10.56 a	240.21± 8.64 a

* Each value represents the mean of three replicates ±SD.

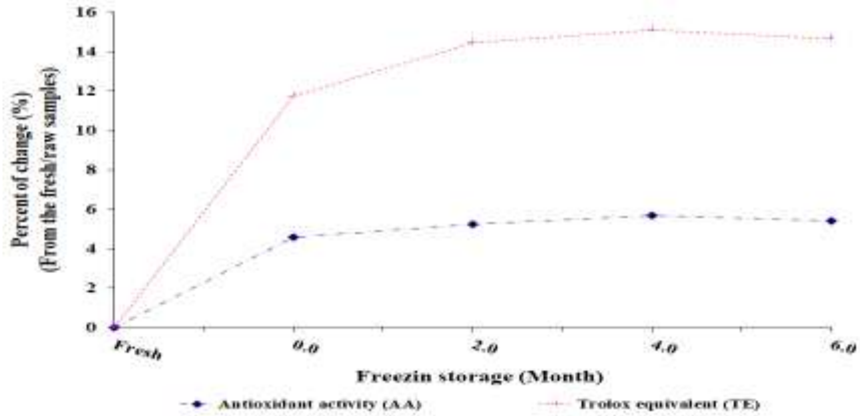

Figure 4. Effect of freezing process on antioxidant activities (% of change from the fresh/raw samples) of eggplant fruit extract. Each value represents the mean of three replicates.

3.8.2. *β* -Carotene Bleaching

The antioxidant activity of fresh and frozen eggplant extracts was assayed by β-carotene bleaching (BCB). This assay is based on the coupled oxidation of β-carotene and linoleic acid (LA) which estimates the relative ability of antioxidants to scavenge the radical of LA peroxide (LOO•) that oxidizes β-carotene (lost the double bonds) in the emulsion phase according to Marco (1968). The decrease in Abs of β-carotene in the presence of different methanolic of fresh and frozen eggplant fruit extracts as well as antioxidants used as standards, BHT and α-tocopherol with the oxidation of β-carotene and linoleic acid is shown in Figure (5). Such data indicated that storage 6 months samples of eggplant fruit recorded the lowest decreasing followed by 4 months samples, 2 months samples and fresh samples. The values of the eggplant fruits samples Abs through 120 min are coming well i.e. closing the line of 50 mg α-tocopherol and 50 mg /L of butyhydroxytoluene (BHT). Such data proved the high stability of the eggplant fruit samples when comparing with that well knowing standards, α-tocopherol and BHT.

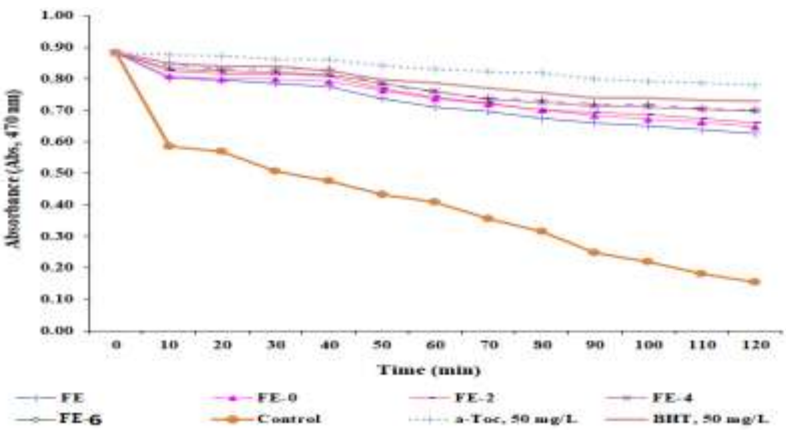

Figure 5. Antioxidant activity of fresh and frozen eggplant fruit extracts as well as references/standard antioxidants assayed by the β-carotene bleaching (BCB) method. FE, fresh eggplant, FE-0, Frozen

eggplant at zero time, FE-2, Frozen eggplant storage 2 months, FE-4, Frozen eggplant storage 4 months days, FE-6, Frozen eggplant storage 6 months

3.8.3. DPPH radical scavenging activity

DPPH radicals scavenging activity test is based on measurement of the a absorption of diene conjugation () in the presence of 2,2-diphenyl-1-picrylhydrazyl (DDPH) (Antolovich et al., 2002). The free radical scavenging activity of eggplant samples and BHT as a standard are showed in Figure (6) and Table (10). Such data indicated that frozen samples storage 6 months extract possessed the highest activity followed by storage 4 months, storage 2 months, frozen 0 storage and fresh samples. At a concentration of 100 µg/mL, the radicals scavenging activity of those samples extracts were 82.83, 81.83, 78.77, 80.90 and 77.54% respectively, whereas, BHT standard was 89.83%. For the IC50, fresh eggplant samples, frozen samples at 0 time, frozen samples at 2 months of storage , frozen samples at 4 months of storage and frozen samples at 6 months of storage extracts were recorded 12.37, 11.89, 11.36, 11.18 and 9.87µg/mL, respectively while BHT standard was 7.90 µg/mL. Therefore, the free radical scavenging activity of eggplant fruit samples extracts and standard was in the following order: standard (BHT)> frozen samples at 6 months of storage> frozen samples at 4 months of storage> frozen samples at 2 months of storage > frozen samples at 0 time> fresh samples.

In general, our previous studies with the others proved that the all previous antioxidant and scavenging activities methods have been also used successively to evaluate the antioxidant activity in various plant parts including eggplant *in vitro* (Aly et al., 2017; Elhassaneen and Abd Elhady, 2014; Elmaadawy et al., 2016; Kashaf, 2018, Mahran et al., 2018; Abd Elalal et al., 2021; Alqallaf, 2021, Elhassaneen et al., 2016, 2020 and 2022). All of these studies reported that the bioactive compounds (polyphenols, flavonoids, carotenoids and anthocyanins) content, such as found in a highly content in fresh and frozen eggplant fruit extracts, are highly correlated with its antioxidant and scavenging activities. Also, all of those studies proved that the antioxidant and free radical scavenging activities are very important to prevent the adverse role of free radicals in different diseases including obesity, diabetes, cancer, neurological, pulmonary, nephropathy and CVD diseases. Therefore, the difference was seen in the degrees of antioxidant and scavenging activities of fresh and frozen eggplant fruit extracts probably due to the type and level of those bioactive compounds present. Also, frozen eggplant fruit samples showed higher antioxidant and scavenging activities than raw/fresh fruits although there is not much difference in the content of bioactive compounds. Such observation could be probably attributed to the pretreatment (grilling) used in our freezing technology. With this context, Das et al. (2011) showed that grilled eggplant presented higher cardio-protective capacity that uncooked fruit. Also, white fruit showed higher antioxidant capacity than purple eggplants after microwaving and grilling (Zaro et al., 2015). This was suggested to result from the novo synthesis of Maillard intermediates with antioxidant properties (Lo Scalzo et al., 2010). In similar study, Nesrin et al., (2022) found that the crude phenolic extracts of the eggplants fruit parts (peel and pulp) exhibited high antioxidant and scavenging activities which correlated with the cultivars. The black and purple eggplants are the cultivars with greater potential benefits in terms of their phenolics and antioxidant values than the white eggplant. Other study indicated that anthocyanins (found by high levels in black and violet eggplant fruit cultivars) contribute to fruit antioxidant capacity, but their participation in total fruit radical scavenging properties is much lower than that of phenolic acids (Zaro et al., 2015). Data of the present study with the others proved that consumption of purple eggplant containing a high concentration of the different bioactive compounds prevents peroxidation of lipids and accumulation of reactive oxygen species (ROS) (Casati et al., 2016). Also, Seeram et al., (2001) reported that anthocyanin found in eggplant peel appears to be vital in preventing obesity by reducing the levels of serum triglyceride and cholesterol, and increasing high-density lipoprotein (HDL). Furthermore, Ghosh et al., (2007) and Matsubara et al., (2005) found that anthocyanins present in eggplant are highly beneficial due to their anti-allergic, antioxidant, anti-inflammatory, anti-mutagenic, anti-microbial, and anti-viral activities, and vision improvement effect.

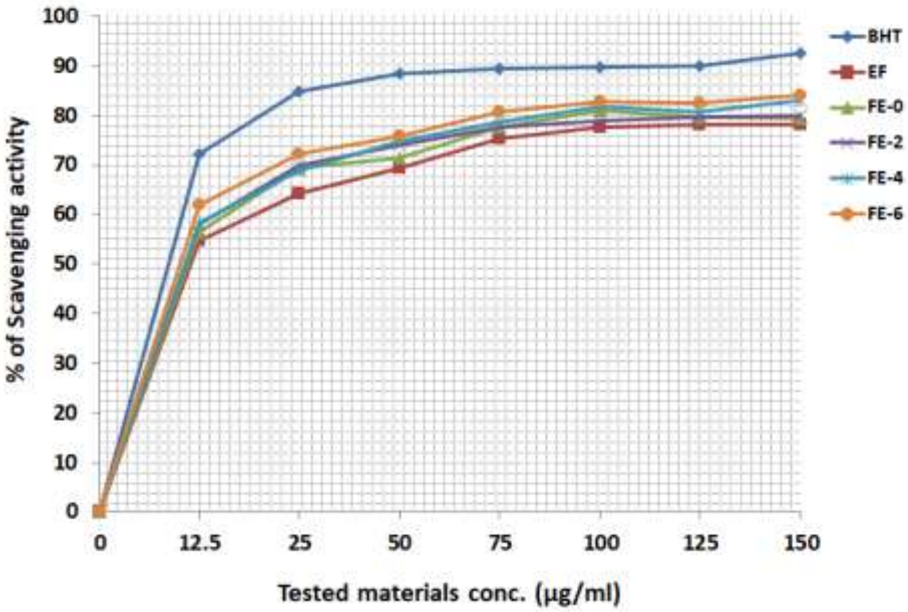

Figure 6. DPPH radical scavenging activity (%) of fresh and frozen eggplant fruit extracts as well as standard (BHT)*

* Each value represents the mean value of three replicates. BHT, Butylated hydroxytoluene, FE, fresh eggplant, FE-0, Frozen eggplant at zero time, FE-2, Frozen eggplant storage 2 months, FE-4, Frozen eggplant storage 4 months days, FE-6, Frozen eggplant storage 6 months

Table 10. IC_{50} (DPPH) of fresh and frozen eggplant fruit extracts as well as standard (BHT)

Sample	BHT	FE	FE-0	FE-2	FE-4	FE-6
IC_{50} (µg/mL)	7.90 ± 0.78 [d]	12.37± 1.04[a]	11.89± 0.55[a]	11.36± 0.83 [ab]	11.18± 0.62 [b]	9.87± 0.39 [c]

* Each value represents the mean value of three replicates ±SD. Values with different superscript letters in the same raw are significantly did different at p≤0.05. BHT, Butylated hydroxytoluene, FE, fresh eggplant, FE-0, Frozen eggplant at zero time, FE-2, Frozen eggplant storage 2 months, FE-4, Frozen eggplant storage 4 months days, FE-6, Frozen eggplant storage 6 months

3.9. Total bacterial count

The effect of freezing process on the total bacterial count of eggplant fruit was shown in Table (11). From such data it could be noticed that freezing induced non-significant increase in total bacterial count which was not affected much by the elongation of the storage period. Also, the values recorded for the total bacterial count in fresh and frozen samples were less than the permissible limits which not exceeded $100x10^3$ cfu/g (Egyptian Standard, ES, 2005). This might indicate that all fresh and frozen samples were sound and safe for human consumption.

Table 11. Effect of freezing process on the total bacterial count of eggplant fruit *

TPC	Fresh samples	Freezing storage (Month)			
		0	2	4	6
Value (Cfu/g)	$12.68x10^3$ [a]	$13.14 x10^3$ [a]	$13.23 x10^3$ [a]	$13.49 x10^3$ [a]	$13.51 x10^3$ [a]
% of change	0.00	3.63	4.34	6.39	6.55

* Each value represents the mean of three replicates.

4. Conclusion

The data of the current study prove that eggplant should receive more attention in many nutritional and medicinal aspects, due to its richness in nutrients such as minerals and vitamins, as well as its high levels of bioactive compounds and antioxidant capabilities. Also, the use of the freezing method that was applied in this study led to the preservation of all these previous important characteristics in addition to the microbial aspect, which were not significantly affected during the storage period, which extended for six months. Therefore, the results of this study, with the new and effective method presented in preserving the eggplant, contribute to increasing the economic value of this important product by making it available in the markets regularly throughout the year, the ease of exporting it to various countries of the world and to be an important source of income for the countries producing it.

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Sample availability

Samples of the frozen eggplant are available upon request from the corresponding author.

Conflicts of Interest

The authors declare no conflict of interest.

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