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Effect of Drying and Freezing on the Phytochemical Properties of Okra during Storage

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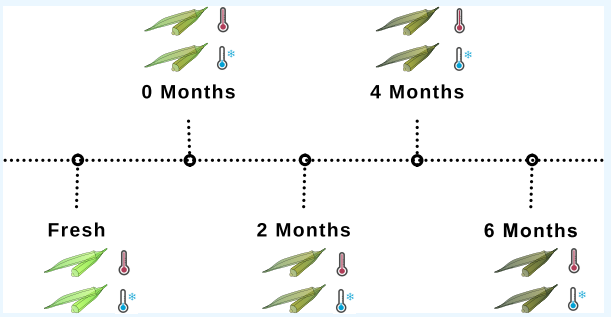
ABSTRACT: Okra (*Abelmoschus esculentus* L.) is known for its high nutritional value, including its content of phytochemicals. This study aims to investigate the effect of drying and freezing conditions on the phytochemical content of okra. Our results indicated that both air-drying and freezing okra during 6 months of storage showed a significant decrease in total phenolic content, flavonoid content, anthocyanin content, and antioxidant activity. Furthermore, higher levels of phytochemicals were found for okra samples treated with Na_2SO_4 solution when compared to untreated okra. The freezing process appeared to better preserve the content of the investigated phytochemicals when compared to the decrease after drying. Our research has determined that both immersing and freezing okra samples consistently yielded better results in the preservation of phytochemical properties over time, compared to other methods. This study is important for the food industry, as it highlights the importance of proper storage methods to retain the nutritional value of okra.



1. INTRODUCTION

Okra belongs to the Malvaceae family and is found in many tropical and warm climates around the world.[1] Plants in the Malvaceae family, such as cotton, mallow, and rosella, are primarily used as sources of fiber, food, beverage, medicine, and timber, and in horticulture.[2] In the Middle East, Africa, and Asia, okra is considered a significant food source.[3] It contains a wealth of minerals and vitamins, such as carbohydrates, crude fiber, ash, iron, calcium, and thiamin.[4] Okra has been proven to be rich in natural phytochemicals, including antioxidants, phenolic compounds, flavonoids, phenolic acids, and anthocyanins, as well as compounds such as vitamins C and E and carotenoids.[5,6] Antioxidants, such as polyphenols and vitamins C and E, are substances that significantly prevent the oxidation of a substrate and are used as protectives against oxidation.[7] Phenolic compounds are secondary metabolites in plants, including simple phenols, hydroxybenzoic acid, flavonoids, and tannins.[8] Anthocyanins, a major antioxidant in fruits, are a group of polyphenolics that give many fruits, vegetables, and flowers their color.[9,10] Flavonoids, a major group of phenolics, have several health-related biological effects.[11] Many studies have concentrated on the phenolic composition and antioxidant effect of many plants, aiming to enhance the product market value by illustrating their preventive and remedial impact on various health disorders, such as malignancy and cardiac, neurodegenerative, and metabolic diseases.[12] Frozen produce is extensively used in the food production industry due to its nutritional value and its content of active compounds. Additionally, these compounds serve to extend the shelf life of fresh produce.[13] Nonetheless, the health advantages associated with these nutritional compounds rely on their bioaccessibility, which means that these compounds must be easily digested and absorbed by the gastrointestinal tract.[13,14]

The estimated global production of okra exceeds 10 million tons annually.[15] Because many crops such as okra have a limited shelf life and are considered perishable due to their seasonality, it is necessary to maintain their quality at the highest levels.[16,17] Pretreatment is a common process used before drying agricultural products to deactivate enzymes, facilitate the drying procedure, and enhance the overall quality of the resulting dried goods.[18] These treatments include chemical solutions (such as alkali, sulfite, and acid), gas treatment, thermal blanching techniques (such as hot water, steam, and microwave heating), and non-thermal processes (such as ultrasound, pulsed electric field, and high hydrostatic pressure).[18] Pretreatment such as high-humidity warm air impingement branching (HHAIB) shows a decrease in the aerobic bacteria, mold, and yeast

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populations and reduces the products' hardness. Moreover, it enhances the drying rate and reduces drying time compared to the untreated samples of red pepper;[19,20] meanwhile, cold plasma (an ionized gas that contains different electrons, ions, and reactive neutral species) enhances the drying rate and increases the antioxidant activity with no significant change in the color of chili peppers.[21]

The most common storage methods for okra include drying, freezing, and preserving in jam.[22] Drying is one of the most prevalent techniques for prolonging the lifespan of fruits and vegetables by reducing the moisture content to a degree that inhibits the proliferation of microorganisms and reduces the rate of numerous degradation processes facilitated by moisture[23] and also decreases the mass and bulk of vegetables, yielding advantages such as the reduction of expenses associated with packaging, storage, and transportation.[24] A study comparing swell drying with shade drying of okra indicated significant differences in the levels of total phenolic and flavonoid compounds, carotenoids, antioxidant activity, and chlorophyll pigments and observed a significantly more porous open solid matrix of swell-dried okra.[25] Drying at room temperature is the most widely used method of food preservation, as it is both simple and cost-effective, requiring minimal energy expenditure.[26,27] However, it can have adverse effects, such as altering the appearance and aroma due to the loss of volatile compounds or the formation of new volatile compounds due to oxidation reactions.[27]

The freezing process has recently become a preferred method of food preservation, overtaking drying, due to its effectiveness in retaining food quality, safety, flavor, and texture.[28] The reduction in temperature inhibits metabolic activity and slows microbial growth, thereby maintaining the quality of the product.[29] Despite its advantages, however, frozen food may experience significant quality degradation compared to fresh products, including water loss, lipid oxidation, altered texture and flavor, and a reduction in antioxidant activity.[30,31]

Total phenolics, antioxidant activity, flavonoids, and anthocyanin compounds in these products are all affected by drying or freezing preservation methods and storage.[32,33] However, various pretreatments can be used to reduce drying time and maintain the quality of fruits and vegetables, such as using sulfur dioxide and sodium acid pyrophosphate before drying, which can inhibit microbiological spoilage and oxidation. These pretreatments can also maintain total phenolic compounds and prevent color deterioration during dehydration, as they are considered effective color preservatives for fruits and vegetables and can inhibit both enzymatic and non-enzymatic browning reactions.[34] To the best of the authors' knowledge, no previous studies have examined the effect of extended storage processes on the phytochemical content of okra. The current study aims to fill the gap in the literature by investigating the impact of extended storage and drying and freezing processes on the phytochemical content of okra. To the authors' knowledge, no prior research has thoroughly explored this topic. Thus, this study seeks to provide valuable insights into the preservation of phytochemical compounds in okra during storage, which could have important implications for the food industry and nutrition.

2. MATERIALS AND METHODS

2.1. Sample Preparation. Okra (*Abelmoschus esculentus*) was purchased from a local market (Irbid, Jordan). six kg of okra was obtained and then washed under tap water to remove dust. The quantity was divided into two equal portions to be preserved by drying and freezing. The 3 kg okra portions were also divided into two samples, one of which was treated by being immersed in a solution of Na_2SO_4 and the other was not treated. Both samples were subjected to drying and freezing processes. Four treatments were studied: immersion drying, immersion freezing, drying, and freezing.

2.2. Okra Preservation. *2.2.1. Drying Processes.* Okra samples were dried by using an air-drying method at room temperature (23 °C ± 2 °C). The air-drying was conducted as described by Hossain et al.,[27] with some modifications, using 3 kg of okra (1.5 kg for each sub-treatment). The drying process was conducted using a tray dryer made of wood strips (60 cm × 40 cm × 20 cm) with small pores to prevent leaf loss. The trays were placed in a dry place in the laboratory until they were completely dried (9 days for the untreated samples and 10 days for the treated samples). The final dried samples were evaluated for appropriate color and scrubbed by hand. The moisture content of the dried okra was evaluated according to the methods described by McClements and was considered completely dry when the moisture content was below 12%.[35] All the samples were collectively weighed on a dry basis from this point forward, and the dried samples were placed in 12 vacuumed plastic bags, approximately 16.3 g in each, and stored at room temperature in the food research laboratory for analysis after 0, 2, 4, and 6 months.

2.2.2. Freezing Processes. Okra samples were frozen at −18 °C in a frost-free commercial freezer. The freezing methods were conducted as described by Poiana et al.,[36] with some modifications. The 3 kg portion of okra was taken and divided into two sub-treatments (1.5 kg for each sub-treatment). The frozen samples were placed equally into 12 vacuumed plastic bags and stored at −18 °C in the food research laboratory for evaluation after 0, 2, 4, and 6 months of freezing.

2.3. Chemical Analysis. *2.3.1. Extract Preparation.* The total phenolic content, flavonoid content, anthocyanin content, and radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of fresh (treated and untreated) okra were determined through sample analysis, following the method described by Rababah et al.[37] To obtain the extracts, 2 g of fresh okra was extracted with 50 mL of methanol in a shaking water bath at 60 °C for 60 min. The extracts were filtered through Whatman filter paper No. 3 made up to a volume of 50 mL and then stored in the dark until analysis.

2.3.2. Determination of the Total Phenolic Contents. The total phenolic contents of extracts were determined according to the Folin−Ciocalteu procedure as follows: 100 of the extracts (triplicate) were transferred into a test tube and then mixed with 0.4 mL of 10% Folin−Ciocalteu reagent;[38] after 3 min, 0.8 mL of 10% Na_2CO_3 was added. The test tubes were allowed to stand for 1 h at room temperature, and the absorbance was measured at 725 nm using a spectrophotometer (CELL, model CE 1020; Cecil Instruments Ltd., Cambridge, England) against a blank containing 100 μL of methanol. The results were calculated as milligrams of gallic acid equivalent per 100 g of dry weight (mg GAE/100 g DW), with gallic acid serving as the calibration standard.

2.3.3. Determination of Radical DPPH-Scavenging Activity. The DPPH-radical scavenging effect was determined according to the method of Matthaus (2002).[39] First, DPPH was dissolved in methanol. A fresh DPPH stock solution (5 mL) was prepared daily. The solution was prepared by weighing 2.4 mg of DPPH (6×10^{-5} M) and dissolving it in 100 mL of methanol, resulting in a purple solution. A volume of 500 μL of

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Table 1. Total Phenolic Contents (mg GAE/100 g) in Okra after Drying and Freezing during 6 months of Storage

	process			
	drying		freezing	
storage period (months)	untreated	treated	untreated	treated
fresh	523.3 ± 59.1[a]	543.7 ± 112.1[a]*	523.3 ± 59.1[a,b]	543.7 ± 112.1[a]*
0	293.8 ± 2.9[b]	328.4 ± 45.1[b]	447.6 ± 12.0[b,c,d]	502.5 ± 63.9[a,b,c]
2	206.3 ± 26.2[c,d]	274.8 ± 2.6[b,c]	440.2 ± 44.1[b,c,d]	472.2 ± 36.7[a,b,c,d]
4	168 ± 53.4[d,e]	253.5 ± 39.6[b,c]	416.2 ± 35.7[c,d]	433.8 ± 14.6[c,d]
6	125.7 ± 4.3[e]	210.6 ± 10.4[c,d]	215.4 ± 11.7[e]	395.9 ± 39.7[d]

All values are calculated as dry basis and means of three replicates. *Means ± SD in the same process column with the same lowercase letters are not significantly different ($P \leq 0.05$). Untreated okra is without any pre-drying treatment, and treated okra is treated with Na_2SO_4.

methanol extracts was then mixed with 0.2 mL of methanolic DPPH solution. The mixture was brought to a total volume of 4.0 mL with the extracting solvent and mixed thoroughly. It was then allowed to stand in the dark for 30 min. Absorbance (A) was then determined at 515 nm, against the blank. The radical scavenging activity was expressed as a percentage of inhibition according to the following formula:[11]

$$\text{inhibition (\%)} = \frac{\text{abs. of the control} - \text{abs. of the sample}}{\text{abs. of the control}} \times 100 \quad (1)$$

2.3.4. Determination of Flavonoid Contents. The flavonoid content was determined using an aluminum chloride colorimetric assay according to Marinova et al.[40] An aliquot of 0.1 mL of the extracts was added to a 5 mL volumetric flask along with 0.3 mL of $AlCl_3$ (10%) and 2 mL of NaOH (4%). The total volume was brought to 5 mL with distilled water. The tubes were allowed to stand in the dark for 60 min at room temperature. The absorption was measured at 510 nm against the blank. The total flavonoid content was expressed as milligrams of (+)-catechin equivalent (CE/100 g dw). The mean value was calculated using three replicates.

2.3.5. Determination of Total Anthocyanin Contents. Preparation of the okra extracts was performed according to a procedure described by Rabino and Mancinelli[41] and Kang et al.;[42] samples of 2 g (in triplicate) of okra were weighed and extracted with 50 mL of acidified methanol (1%). The extraction was conducted in a shaking water bath for 60 min at 60 °C. The extracts were filtered using Whatman filter paper No. 3, placed in a 50 mL volumetric flask, and stored in the dark. The extracts were extracted with acidified methanol, and their absorbance was measured using a CELL spectrophotometer (model CE 1020) at 530 and 657 nm. The formula A = A530 − (0.25 × A657) was used to account for the contribution of chlorophyll and its degradation products to the absorption at 530 nm. The anthocyanin content was reported as milligrams of cya-3-glucoside equivalent per kilogram of dry sample weight (mg of Cya-3-glu/100 g).

2.4. Statistical Analysis. Data were analyzed using the general linear model (GLM) procedure with the JMP statistical package (JMP Institute Inc., Cary, North Carolina, USA). The model included the effect of replicates (three) and storage times (0, 2, 4, and 6 months) for okra. The measured variables were total phenolics, antioxidant activity, anthocyanin, and flavonoid contents, and all analyses were calculated using a dry weight basis. Mean values were separated by LSD analysis using a least significant difference of $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Total Phenolic Contents of Okra during Storage. The results of the analysis for the total phenolic content, antioxidant activity, flavonoid content, and anthocyanin content of okra, both before and after 6 months of storage, are presented in Tables 1, 2, 3, and 4, respectively. The results show that there is no significant difference in the total phenolic content of fresh untreated okra (523.3 mg GAE/100 g) and treated okra (treated with Na_2SO_4). These results are consistent with those reported by Olivera et al.[43] who reported a range of total phenolic content in okra cultivars from 313 to 563 mg GAE/100 g. However, the results differ from those of Adetuyi and Dada,[44] who reported a total phenolic content of 481 mg GAE/100 g for okra. These variations in total phenolic content could be attributed to differences in soil, climate, and methodology.[45]

Table 2. Antioxidant Activity (% of Inhibition) in Okra after Drying and Freezing during 6 months of Storage

	process			
	drying		freezing	
storage period (months)	untreated	treated	untreated	treated
fresh	69.5 ± 0.3[b]	70.4 ± 0.2[b]*	69.5 ± 0.3[b]	70.4 ± 0.2[b]
0	52.7 ± 2.1[d]	63.5 ± 4.9[c]	56.7 ± 3.4[d,e]	64.6 ± 3.8[c]
2	44.7 ± 4.1[e]	53.3 ± 5.3[d]	52.4 ± 5.2[e,f]	58.9 ± 4.5[d]
4	38.9 ± 1.5[f,g]	45.8 ± 4.1[e]	47.8 ± 2.6[f,g]	55.5 ± 1.0[d,e]
6	37.8 ± 3.1[g]	43.4 ± 2.7[e,f]	44 ± 3.0[g]	52.7 ± 3.9[e,f]

All values are calculated as dry basis and means of three replicates. *Means ± SD in the same process column with the same lowercase letters are not significantly different ($P \leq 0.05$). Untreated okra is without any pre-drying treatment, and treated okra is treated with Na_2SO_4.

Regarding the drying process (Table 1), the total phenolic content of okra significantly decreases upon drying ($P \leq 0.05$) but no significant decrease ($P > 0.05$) is observed upon freezing. The reduction in total phenolic content after freezing is 14.5% in untreated okra and 7.5% in treated okra.

Our results agree well with Ellong et al.,[46] who reported a slight decrease in the total phenolic content of untreated okra after freezing. de Ancos et al.[47] found that freezing at −20 °C reduced the total phenolic content of raspberry fruits by 5.0 to 13.0%. The reduction in total phenolic content during freezing can be attributed to the slow enzymatic reactions in frozen products, as enzymes remain active in the presence of non-frozen water.[48]

The average total phenolic content after the drying process was found to be 293.8 mg GAE/100 g for untreated okra and

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Table 3. Total Flavonoid Contents (mg CE/100 g) in Okra after Drying and Freezing during 6 months of Storage

	process			
	drying		freezing	
storage period (months)	untreated	treated	untreated	treated
fresh	137 ± 11.6^{a,b}	140.9 ± 0.95^{a}*	137 ± 11.6^{a,b}	140.9 ± 0.95^{a}
0	125.8 ± 10.0^{a,b,c}	134 ± 29.5^{a,b}	132.2 ± 5.1^{a,b,c}	132.4 ± 4.1^{a,b,c}
2	110.5 ± 10.0^{c,d}	123.5 ± 6.2^{a,b,c}	122.5 ± 4.4^{d,e}	131.2 ± 5.6^{b,c,d}
4	93.7 ± 4.7^{d}	118.7 ± 7.5^{b,c}	118.1 ± 2.5^{e}	128.1 ± 3.3^{c,d}
6	72.3 ± 2.2^{e}	96 ± 6.1^{d}	99.3 ± 0.4^{f}	126.8 ± 4.8^{c,d,e}

All values are calculated as dry basis and means of three replicates. *Means ± SD in the same process column with the same lowercase letters are not significantly different ($P \leq 0.05$). Untreated okra is without any pre-drying treatment, and treated okra is treated with Na_2SO_4.

Table 4. Anthocyanin Content (mg of Cya-3-glu/100 g) in Okra after Drying and Freezing during 6 months of Storage

	process			
	drying		freezing	
storage period (months)	untreated	treated	untreated	treated
fresh	0.041 ± 0.01^{a}	0.043 ± 0.008^{a}*	0.041 ± 0.01^{a}	0.043 ± 0.008^{a}
0	0.006 ± 0.0009^{b}	0.009 ± 0.0005^{b}	0.002 ± 0.0006^{b}	0.002 ± 0.0002^{b}
2	0.005 ± 0.0003^{b}	0.006 ± 0.005^{b}	0.003 ± 0.001^{b}	0.003 ± 0.0003^{b}
4	0.002 ± 0.00002^{b}	0.002 ± 0.0001^{b}	0.004 ± 0.0009^{b}	0.004 ± 0.001^{b}
6	0.002 ± 0.002^{b}	0.002 ± 0.00004^{b}	0.005 ± 0.001^{b}	0.006 ± 0.001^{b}

All values are calculated as dry basis and means of three replicates. *Means ± SD in the same process column with the same lowercase letters are not significantly different ($P \leq 0.05$). Untreated okra is without any pre-drying treatment and treated okra is treated with Na_2SO_4.

328.4 mg GAE/100 g for treated okra. The reduction in total phenolic content after drying was 43.8% for untreated okra and 39.5% for treated okra. To the best of our knowledge, these findings have not been previously reported in the literature. However, similar investigations on different products have been reported. Rababah et al.[49] found that the total phenolic content of sage and mint decreased by 31.7 and 46.0%, respectively, after air-drying. Zhang and Hamauzu[50] found that the total phenolic content of broccoli decreased by between 56 and 72% after the drying process. Enzymatic processes that occur during drying may have caused the loss of total phenolic content observed after drying.[51] Our results indicated that air drying deactivates degradation enzymes such as polyphenol oxidases, which can degrade phenolic compounds before the plant materials are completely dried.

When observing the samples after the freezing process, at 0 month, the decreases in total phenolic contents only were statistically significant ($P \leq 0.05$) after 4 months of storage for both treated and untreated samples.

The storage results in Table 1 show that the highest total phenolic content for both untreated and treated okra was directly after drying, at 293.8 and 328.4 mg GAE/100 g, respectively. The phenolic content decreased significantly with increasing storage periods and reached 125.7 and 210.6 mg GAE/100 g after 6 months of storage for untreated and treated dried samples, respectively. The reduction in total phenolic content from fresh to 2 months of storage was 60.5 and 49.5% for untreated and treated dried okra, respectively.

To the best of the authors' knowledge, there are no studies of long-term storage of untreated and treated okra available in the literature. However, Adetuyi et al.[52] found that the total phenolic content of various types of okra decreased after 10 days of storage, with a reduction ranging from 34.9 to 58.5%. These values are likely lower than those obtained in this study because the storage period in their study was shorter. The reduction in total phenolic content of untreated and treated okra may be due to the oxidation of phenolic compounds by active polyphenol oxidases (PPO) enzymes during storage, as reported in previous studies.[52,53]

Table 1 shows that the phenolic content of treated samples was slightly higher than that of untreated samples for both dried and frozen samples. There was a significant difference in phenolic content between treated and untreated samples as the storage time varied from 4 to 6 months, with the exception of untreated frozen samples.

To the best of the authors' knowledge, there are no studies in the literature that have investigated the long-term storage of untreated and treated dried or frozen okra. Taain et al.[54] found that treating dried okra with ascorbic and citric acids before and after 12 days of storage increased the total phenolic content. Ahmed et al.[55] reported that immersing sweet potatoes in 0.5% sodium hydrogen sulfite before drying improved their quality and increased their total phenolic content. The retention of treated okra samples may be due to the inhibitory effect of these chemicals on the activity of PPO.[56]

Overall, no significant difference was found between frozen okra samples stored for 0, 2, and 4 months. Chaovanalikit and Wrolstad[57] found a 50% loss of total phenolic content in sweet cherries after 6 months of frozen storage. However, de Ancos et al.[47] found no significant effect on the total phenolic content of raspberries after 12 months of freezing. The reduction in total phenolic content during frozen storage could be due to ice crystals damaging the cell wall or cellular disruption caused by the release of oxidoreductive ionic forms of PPO. Freezing okra for 6 months reduces phenolic content in both treated and untreated samples. Brannan and Wang[58] found that ascorbic acid treatment before freezing increases the total phenolic content in pawpaw pulp. The retention of treated okra samples after freezing could be due to reduction in the activity of PPO.[56]

3.2. DPPH-Radical Scavenging Activity of Okra during Storage. The antioxidant activities of okra after drying and during the first 6 months of storage are presented in Table 2.

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ACS Omega 2023, 8, 34448−34457

Results of antioxidant activity showed that there was no significant difference observed between fresh, untreated okra with a percent inhibition (PI) of 69.5% and treated okra with a PI of 70.0%. Our results agree well with the results reported by Adetuyi et al.,[52] who found that the antioxidant activity in different okra cultivars varied from 46 to 73% PI, and Huang et al.,[49,59] who found that the antioxidant activity of okra was 70.7% PI.

The processing and drying results show that the antioxidant activity of okra decreased significantly ($P \leq 0.05$) after drying and freezing for both treated and untreated samples. The PI decreased from 69.5 to 52.7 after drying for fresh, untreated dried samples and from 70.4 to 63.5 for treated samples. The reduction of the antioxidant activity after drying untreated okra was 24.2%, while the reduction of the antioxidant activity of treated okra was 9.8%, which indicates that treating okra with Na_2SO_4 decreases the antioxidant degradation. For frozen untreated and treated okra, they were found to be 56.7 and 64.6% (PI), respectively. The reduction of the antioxidant activity after freezing in untreated okra was 18.4%, and 8.2% for treated okra.

Other investigations of different products reported that the reduction of the antioxidant activity after air-drying different kinds of herbs ranged from 1.3 to 2.4%.[49] In addition, Katsube et al.[60] reported that the antioxidant activity of mulberry leaves decreased significantly after air-drying. In another study, the decrease in antioxidant activity of horseradish and lavage after the drying process was found to be 61.4 and 20.2%, respectively.[61] The loss in antioxidant activity could be caused by the depletion of natural antioxidants in raw materials from plants[62] or that air-drying causes a significant decrease in the total phenolic contents, which consequently resulted in a significant decrease in the antioxidant activity after the drying process.[51] This loss could be due to enzymatic processes that occurred during air drying.

Previous studies have been conducted on different products, and it has been reported that the loss of the antioxidant activity of raspberry fruits ranged from 4.0 to 26.0% after the freezing process at −20 °C.[47] The decrease in antioxidant activity could be attributed to enzymatic reactions in frozen products because these reactions are slow but not completely blocked in frozen products and enzyme activity is linked to the presence of non-frozen water.[48]

Results relating to the untreated and treated dried okra indicate that antioxidant activity reduced significantly ($P \leq 0.05$) after 0, 2, and 4 months but all subsequent storage times did not display a statistically significant reduction. For frozen samples, however, the results indicate that there is no significant difference between samples stored for 0 and 2 months. However, a significant difference between 0 and 4 months of storage is found.

When comparing the treated and untreated dried and frozen okra at the same period of storage, the treated samples displayed a statistically significant higher antioxidant activity. To the best of the authors' knowledge, there are no studies on the changes in antioxidant activity for a long period of storage on untreated and treated okra currently available in the literature. However, Adetuyi et al.[52] found that the antioxidant activity of various varieties of dried okra decreased during 10 days of storage and the reduction of the antioxidant activity ranged from 46.0 to 73.3%.

The reduction in the antioxidant activity of untreated and treated okra could be a consequence of the reduction in the total phenolic contents because the antioxidant activity in a vegetable depends on its total phenolic content.[52] A study on different products conducted by Sen et al.[63] demonstrated that the stored dried apricots treated with sulfur dioxide retained more total phenolic contents and antioxidants than untreated samples. The retention of treated okra samples after drying could be due to the role of these chemicals in reducing the activity of PPO and thus stopping the work of the enzyme reactions.[56]

When comparing the untreated with the treated okra, the reduction of antioxidant activity was significantly higher in the untreated samples at all the time points (0, 2, 4, and 6 months). The overall reduction in the antioxidant activity of untreated okra after 6 months of storage was 36.6%, and for the treated okra, it was 25.0%. Poiana et al.[36] found that the antioxidant activity of various garden fruits decreased during frozen storage at −18 °C and reported similar findings on other fruits. The reduction in antioxidant activity during frozen storage could be attributed to the formation of tiny ice crystals, which may impale the cell wall and ultimately result in the loss of total phenolic compounds from the cell.[58] Additionally, Brannan and Wang[58] found that pawpaw pulp retained more antioxidant activity when treated before freezing with ascorbic acid than untreated samples. The retention of treated okra samples after freezing could be attributed to the decrease in phenol contents of frozen okra.[56]

3.3. Total Flavonoid Contents of Okra during Storage.

The flavonoid contents of okra after drying and during 6 months of storage are presented in Table 3. The drying process itself led to a decrease in the total flavonoid content of okra. However, the total flavonoid contents of untreated and treated okra after drying and freezing were not significantly different ($P > 0.05$). The reduction of the total flavonoid contents after drying was 8.2 and 4.8% in untreated and treated okra, respectively, but these differences were not significant. The literature lacks studies on the flavonoid content of okra during storage. However, similar studies have been conducted on other products. Gupta et al.[64] reported a 51% decrease in the total flavonoid content of seaweed after drying. Rababah et al.[49] found that air drying resulted in a 61.9% decrease in the flavonoid content of thyme. The decline in total flavonoid content can be attributed to harsh drying conditions, which can affect the phytochemical breakdown and result in the migration of some flavonoids. The loss of flavonoids may also be caused by chemical reactions involving oxygen, enzymes, and light.[65]

The processing and freezing results indicate that the freezing decreased the flavonoid content slightly in both treated and untreated samples, but these differences were not significant. The reduction of the total flavonoid contents after freezing in untreated okra was 3.5%, while the percentage reduction of the total flavonoid contents in treated okra was 6.0%. On the contrary, Mullen et al.[66] found that the total flavonoid contents of raspberries were unaffected by the freezing process.

The results of untreated and treated okra indicate that the values of the total flavonoid contents were slightly reduced but not significantly ($P > 0.05$) during a short term of storage, while they were significantly reduced when the storage time was over 4 months. Studies investigating green tea beverages have been conducted and found that the reduction in the total flavonoid contents after 12 days of dried storage was 72%.[67]

The reduction in the total flavonoid contents of untreated okra during 6 months of storage was 47.2%, and 31.9% for the treated okra. The reduction in the total flavonoid contents of untreated okra during 6 months of storage was 27.5%, and for

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Table 5. The Comparison of the Effect of Freezing and Drying Methods on the Total Phenolic, Antioxidant Activity, Total Flavonoid, and Anthocyanin Contents of Okra after Processing at Zero Time of Storage

treatments	total phenolic contents (mg GAE/100 g)	antioxidant activity (% of inhibition)	flavonoids (mg CE/100 g)	anthocyanins (mg of Cya-3-glu/100 g)
fresh	533.5 ± 8.1[a]**	69.9 ± 5.8[a]	138.95 ± 7.7[a]	0.04 ± 0.9[a]
freezing	475.1 ± 5.1[a]	60.6 ± 5.4[b]	132.33 ± 4.1[a]	0.002 ± 4.7[b]
drying	311.2 ± 3.4[b]	58.1 ± 6.8[b]	129.9 ± 2.0[a]	0.008 ± 3.4[b]
Sub-treatment				
treated	458.19 ± 1.2[a]	66.17 ± 4.5[a]	135.79 ± 1.5[a]	0.018 ± 2.0[a]
not treated	421.58 ± 1.1[a]	59.64 ± 7.8[b]	131.69 ± 9.4[a]	0.016 ± 2.0[a]
Interactions				
fresh, treated	543.65 ± 1.1[a]	70.43 ± 2.1[a]	140.9 ± 9.5[a]	0.043 ± 8.7[a]
fresh not treated	523.32 ± 5.9[a]	69.46 ± 3.1[a,b]	137.0 ± 1.2[a]	0.041 ± 1.1[a]
freeze, treated	502.53 ± 6.4[a]	64.6 ± 3.8[b,c]	132.5 ± 4.1[a]	0.0015 ± 1.9[b]
freeze, not treated	447.58 ± 1.2[a]	56.7 ± 3.4[d]	132.2 ± 5.1[a]	0.0019 ± 6.1[b]
drying, treated	328.38 ± 4.5[b]	63.46 ± 5.0[c]	134.0 ± 2.9[a]	0.009 ± 4.7[b]
drying, not treated	293.84 ± 3.0[b]	52.76 ± 2.1[d]	125.8 ± 1.0[a]	0.006 ± 4.9[b]

All values are calculated as dry basis and means of three replicates. *Means ± SD in the same column with the same lowercase letters are not significantly different ($P \leq 0.05$) and untreated okra (without any pre-drying treatment) and treated okra (treated with Na_2So_4).

the treated okra, it was 10.0%. The total flavonoid content of okra can decrease during storage due to glycosylation reactions from glucosidase activity, which affects flavonoid solubility, reactivity, and stability.[68] Salabak[69] found that the total flavonoid content of stored pawpaw pulp remained unchanged during the first 6 months of frozen storage.

Table 3 shows that treated samples had slightly higher total flavonoid content compared to untreated samples. Other studies have found that treating samples with potassium metabisulfite and calcium chloride solutions before drying improved the total flavonoid retention in black carrots.[56,70] The retention of total flavonoid content in treated okra samples may be due to the presence of antioxidants and phenols.[56]

3.4. Anthocyanin Content of Okra during Storage. The anthocyanin contents of okra after drying and freezing and during 6 months of storage are shown in Table 4. There is no significant difference found between fresh, untreated okra (0.41 mg of Cya-3-glu per 100 g) and treated okra (0.43 mg of Cya-3-glu per 100 g). The total anthocyanin contents of okra decreased significantly ($P \leq 0.05$) after drying and freezing in both treated and untreated okra, and the anthocyanin contents were found to be in trace amounts after drying. The reduction in the anthocyanin contents after the drying process (0 months) was 85.4 and 79% for untreated and treated samples, respectively, and that for frozen samples was 95.1 and 95.3% for untreated and treated samples, respectively.

The results indicate that the values of the anthocyanin contents in okra decreased but not significantly ($P > 0.05$) during the 6 months of storage. The reduction in the anthocyanin contents of dried okra after 6 months of storage was 95.1 and 95.3% for untreated and treated samples, respectively, and that for frozen samples was 87.8 and 86.0% for untreated and treated samples, respectively.

To the best of the authors' knowledge, no previous studies have focused on the effect of untreated and treated okra on anthocyanin content over 6 months of storage. Previous studies have found that the anthocyanin content of blueberries decreased after the drying process.[71] Similarly, the anthocyanin contents have been found to decrease during processing, demonstrating that this reduction could be due to its degradation by the breakage of glycoside linkages, which are considered unstable and easily degraded via oxidation reactions.[72]

The reduction in the anthocyanin contents during the different times of storage could be attributed to the function of peroxidase and PPO enzymes.[73] Previous studies on dried blueberries found that they retained more anthocyanin content when samples were treated with the NaCl solution before drying.[71]

For frozen samples, the reduction in the anthocyanin content could be due to cellular disruption caused by the freezing process, producing a release of oxidoreductase enzymatic (PPO).[47] The main reduction in the anthocyanin content appears to happen during the freezing process, and after that, the reduction was very little. Similarly, Lohachoompol et al.[71] found that the anthocyanin contents of frozen blueberries were the same over 3 months of storage.

3.5. The Comparison of the Effect of Freezing and Drying Methods on the Phytochemical Compounds of Okra after Processing at Zero Time of Storage. The total phenolic, antioxidant activity, total flavonoid, and anthocyanin contents of fresh okra that were subjected to drying and freezing at zero time of storage are shown in Table 5. It shows that the total phenolic contents in fresh and frozen samples are 533.5 and 475.1 mg GAE/100 g, respectively, but the differences were not significant. Upon drying, the phenolic contents dropped significantly to 311.2 mg GAE/100 g. Tomsone and Kruma[61] found that the freezing process of horseradish and lovage at −20 °C retained more total phenolic contents than the drying process at 24 °C. This could be because the drying process results in severe damage and deterioration of the integrity of leaf tissue, causing the release of active enzymes that could cause enzymatic degradation. In particular, during periods of drying, the enzymes are inactivated due to decreased water activity.[74] On the other hand, freezing causes a decrease in available water for enzymatic reactions, so that the PPO is no longer active and thus the loss of phenolic contents can be minimized.[75]

Table 5 shows that the highest value of antioxidant activity is attained in fresh samples, at 69.9% PI. Upon drying and freezing performed consecutively, the antioxidant activity dropped significantly to 60.6 and 58.1% for drying and freezing samples, respectively. There was no significant difference ($P > 0.05$) in total flavonoid content between the two processes at zero

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storage time. Furthermore, results indicate that there is no significant difference ($P > 0.05$) between treated and not-treated okra when observing the effect on the total phenolic contents and total flavonoid contents. When comparing the antioxidant activity of treated and non-treated okra, a significant difference ($P \leq 0.05$) was found.

The results of the interactions between treatments and sub-treatments of investigated phytochemicals are shown in Table 5. Frozen products contain more phytochemicals than dried products. In addition, the highest total phenolic contents and antioxidant activity are attained in the treated and not-treated fresh samples. Treated okra exhibited better behavior than non-treated okra. It seems that the best result has been obtained when the okra samples have been subjected to freezing and immersion. The okra interactions do not affect the total flavonoid content ($P > 0.05$). These interactions are expected because, as previously stated, freezing retains more water than drying.

3.6. Effect of Freezing and Drying on Okra Phytochemicals after Processing and Storage. The total phenolic content, antioxidant activity, total flavonoid content, and anthocyanin contents of okra subjected to both drying and freezing processes during 2, 4, and 6 months of storage are shown in Supplementary Tables S1, S2, and S3, respectively. The results indicate that freezing processes retain more total phenolic content, antioxidant activity, and total flavonoid content than drying processes do. This could be attributed to the fact that freezing preserves more investigated phytochemicals than drying. In addition, treated okra retained more total phenolic content, antioxidant activity, and total flavonoid content than non-treated okra did for samples stored for 2 to 6 months.

The interactions between treatments and sub-treatments indicate that the interaction between freezing processes treated and freezing not treated yields good results. This is to be expected because freezing retains more moisture than drying.

4. CONCLUSIONS

This study investigated the impact of processing methods, such as drying and freezing, as well as storage duration on the phytochemical composition of okra. The findings revealed important insights into the changes that occur in the total phenolic, flavonoid, and anthocyanin contents of okra during these processes over 6 months of storage. The results indicated that drying most significantly decreased the phytochemical contents of okra while freezing had a milder effect over time. Treating okra with Na_2SO_4 before processing showed some potential in retaining phytochemical compounds in storage. Moreover, storage duration played a crucial role in the degradation of phytochemicals, with a progressive reduction observed over time.

It is important to note that this study identified gaps in the current literature, particularly in terms of the long-term storage of okra and the impact of treatment methods. Thus, it highlights the need for further research in these areas. Future studies could focus on exploring alternative processing techniques that minimize the loss of phytochemical compounds in okra over time. Additionally, investigations into the mechanisms underlying the degradation of phytochemicals during drying, freezing, and storage specifically in okra could provide valuable insights for preservation strategies. Furthermore, the role of different factors, such as climate and okra variety, on the phytochemical composition of okra warrants further exploration. Understanding how these factors influence the presence and stability of phytochemical compounds in okra could contribute to optimizing cultivation practices and post-harvest handling methods. In conclusion, this study enhances our understanding of the effects of processing and storage on the phytochemical composition of okra. The findings underscore the importance of considering these factors to preserve the nutritional and health benefits associated with okra consumption. By further investigating the areas identified in this study, researchers can develop strategies to maximize the retention of phytochemical compounds in crops such as okra and promote its nutritional value.

■ ASSOCIATED CONTENT

Data Availability Statement

The Data is available upon request.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c02560.

Comparison of the effect of freezing and drying methods on the total phenolic, antioxidant activity, total flavonoid, and anthocyanin contents of okra after processing at after 2, 4, and 6 months of storage (PDF)

Anthocyanins (okra) mg/100 g of dry sample weight; antioxidant (okra) inhibition %; flavonoid (okra) mg of CE/100 g FW; phenolic (okra) mg gallic acid/100 g of dry sample weight (XLS)

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Notes

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Okra, a member of the Malvaceae family, holds significant culinary importance for numerous households across the Middle East, Africa, and Asia. To ensure its long-term preservation, drying and freezing are commonly employed methods. However, these approaches can impact the levels of phytochemicals, including total phenolic compounds, found in okra. To mitigate this effect, pretreatments involving sulfur dioxide and sodium acid pyrophosphate can be utilized before the drying process. These pretreatments serve to inhibit microbiological spoilage, oxidation, and preserve the overall content of total phenolic compounds. Furthermore, they aid in preventing color deterioration during dehydration and impede enzymatic and non-enzymatic browning reactions. The objective of this study is to examine the influence of drying and freezing on the phytochemical composition of okra during storage, ultimately providing practical recommendations for the food industry to maintain the nutritional value of okra throughout its preservation.

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