Journal of Horticultural Science & Ornamental Plants 11 (3): 192-203, 2019 ISSN 2079-2158 © IDOSI Publications, 2019 DOI: 10.5829/idosi.jhsop.2019.192.203

# The Effect of Ozone Treatments Under Vacuum on Aflatoxin of Chilli Pepper and Caraway Processed for Export

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**Abstract:** Aflatoxins are carcinogenic, mutagenic and immunosuppressive to most animal species and humans. Humans are exposed to aflatoxins via risky foods. Numerous studies proved the contamination levels of spices with poisons aflatoxins B1 (AFB1) and B2 (AFB2). The objectives of this study are to evaluate the effect of ozone gas concentration under vacuum on the detoxification of crushed chilli pepper (*Capsicum annum* L.) and caraway seeds (*Carum carvi* L.). The results indicated that, ozone treatments under vacuum significantly reduced B1, B2 and total aflatoxin contents of crushed dried chilli pepper comparing with other treated samples at atmospheric pressure and control. No aflatoxin contents were exhibited in caraway seeds due to all treatments. Prolonging storage period up to 6 months for untreated samples caused gradually significant increase in B1, B2 and total aflatoxin contents of control sample. Antioxidant activity, total phenols and flavonoids contents gradually decreased with extending the storage period. After six months of storage, ozone treatment at level 40ppm under vacuum had a slightly decreased in capsaicin content of crushed dried chilli pepper. Least significant content was observed by ozone 40ppm under vacuum compared to control sample. Generally, ozone treatments under vacuum were more effective than at atmospheric pressure in decontaminating spices from aflatoxins even after 6 months of storage.

Key words: Aflatoxin B1 • Aflatoxin B2 • Total aflatoxins • Red chilli pepper • Caraway seeds • Antioxidant activity • Total phenols • Total flavonoids • Total alkaloids • Capsicine content • Storage period

# **INTRODUCTION**

Aflatoxins are toxic compounds that are naturally produced by certain types of fungi including *Aspergillus flavus* and *Aspergillus parasiticus* that can produce mycotoxins grow on numerous foodstuffs such as cereals, dried fruits, nuts and spices which cause a health risk to the consumer by producing acute toxic syndromes and carcinogenic [1].

The Egyptian Standard No. ES: 1875 - 1 [2, 3] set the maximum levels for aflatoxin at 5 µg/kg and for sum of B1, B2, G1 and G2 at 10 µg/kg.

El-Kady *et al.* [4] monitored different samples from the Egyptian market and evaluated the mycotoxins in red pepper and caraway samples and found that all samples were positive. Also, Tajkarimi *et al.* [5] monitored some of the rejected samples of pepper in the European Union during 2010 and imported from India and Pakistan and found that the aflatoxin levels ranged from 20 to 40  $\mu$ g/kg. In the same context, Riordan and Wilkinson [6] monitored the presence of some chilli samples in various retail outlets in Ireland that were detected aflatoxin contamination above the regulatory limits of the European Union.

Ozone, the triatomic form of oxygen ( $O_3$ ), is one of the most powerful disinfectants and sanitizing agents, as well as, it can be directly applied as an antimicrobial agent in the food industry. In post-harvest treatment, gaseous and aqueous ozone phases are applied to prevent fungal decay, control storage pests and degrade aflatoxin [7-9]. The mechanisms of ozone to inhibit microbial populations in food occur via the progressive oxidation of vital cellular

**Corresponding Author:** K.M.K. El-Tobgy, Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, A.R.C., Giza, Egypt. components. However, ozone oxidizes polyunsaturated fatty acids or sulfhydryl group and amino acids of enzymes, peptides and proteins to shorter molecular fragments. In addition, ozone degrades the cell wall envelope of unsaturated lipids resulting in cell disruption and subsequent leakage of cellular contents [10]. Several previous studies have shown positive ozone effect in reducing the content of aflatoxin in agricultural crops. Inan et al. [11] found that a reduction in aflatoxin B1 of red pepper when treated with ozone at 66 mg / L for 1 h. Wang et al. [12] have confirmed that ozone as a powerful oxidant, has been successfully used for controlling stored-product pests, fungi and sometime degrading mycotoxins. El-Desouky et al. [13] exposed wheat samples to ozonation at various ozone concentrations (20 and 40 ppm). The reduction percentages of aflatoxin B1 in artificially contaminated wheat. Trombete et al. [14] mentioned that one way to reduce of mycotoxins using ozone in food processing. Due to its high potential as an oxidant, O<sub>3</sub> or the radicals generated in the ozonation process react with mycotoxins that lose their toxicity due to molecular degradation. Kamber et al. [15] showed a decrease in aflatoxin B1 (AFB<sub>1</sub>) content of red pepper samples was dependent on ozone dose and reduction percentage in the AFB<sub>1</sub> content ranged from 6.1 to 74.1%.

Spices occupy a prominent place in the traditional culinary practices and are indispensable part of daily diets of millions of people all over the world. Spices such as pepper, paprika, cumin, ginger, saffron and clove are extensively used to flavoring foods as well as for medication and are highly valuable due to their preservative and antioxidant properties. However, very little information is available on the mycoflora of spices worldwide [16].

Chili pepper (*Capsicum annuum* L.) is one of the important crops of Egypt where the production in 2016 was about 1658 tons. Although the peppers are the second most traded spice in the world, however, Egypt's exports are somewhat limited due to the presence of aflatoxin higher than permitted limits [17].

Caraway (*Carum carvi* L.) a well-known spice belonging to *Apiaceae* family; which is a most important medicinal and aromatic seeds product in Egypt where the production in 2016 was 6990 tons that accounts 47% of the quantity of medicinal and aromatic seeds produced in Egypt in 2016 [18].

The objectives of this study was a trial to evaluate the effect of ozone under vacuum; as an innovation method, at different doses on the detoxification of dried chilli pepper and caraway seeds samples comparing with exposed samples by same doses in normal atmospheric pressure. As well as, study the effect of different doses of ozone on most bioactive compound contents and antioxidant activity to improve quality characteristics and safety to be fit for exportation.

## MATERIALS AND METHODS

**Materials:** Crushed red chilli pepper (*Capsicum annuum* L.) was obtained from a private farm in Assiut city while, caraway seeds (*Carum carvi* L.) were obtained from medicinal and aromatic plants research department, Horticulture Research Institute, A.R.C..

Folin-Ciocalteu reagent was purchased from Sigma Chemical Co. Radical 2, 2diphenylpicrylhydrazyl (DPPH) was purchased from Aldrich Chemical Co. All other solvents and chemicals were purchased from El-Nasr Chemical Co., Cairo, Egypt.

**Ozonation:** An innovative laboratory device was designed to treat the tested samples by ozone under vacuum. The device consists of three parts as shown in Fig. (1).

- The first part was a vacuum pump (DryFast, Model NO. 20187-02, Flow 54 L/min, USA).
- The second part was the ozone generator machine (Healthy life AK-103 ozonizer, 6 gm/hr equipment designed by EXPONENT, Egypt).
- The third part was a 5-liter conical glass flask. The tested samples treated with ozone were placed inside, the conical flask, that fitted with three tubes. The first tube was connected to the vacuum pump to extract the air from the flask and the vacuum occurs. The second tube was connected to the ozone generator while the third one was connected to a pressure gauge to show the pressure level inside the conical flask.

### Methods

**Preparation of Treated Samples:** Fourteen samples of crushed chilli peppers and caraway seeds (during two seasons, 2017 and 2018) were treated with ozone at different doses (10, 20 and 40ppm) at atmospheric pressure and under vacuum as follows:

- Control,
- Exposure to Ozone 10ppm at atmospheric pressure,
- Exposure to Ozone 20ppm at atmospheric pressure,
- Exposure to Ozone 40ppm at atmospheric pressure,
- Exposure to Ozone 10ppm under vacuum,
- Exposure to Ozone 20ppm under vacuum,
- Exposure to Ozone 40ppm under vacuum.



Fig. 1: Ozone device under vacuum

All treatments were ambient stored for six months. Samples were taken at 0, 3 and 6 months during storage period and quality attributes of crushed red chilli pepper and caraway seeds were assessed.

## **Quality Attributes**

**Determination of Aflatoxin Contents:** Aflatoxin B1, B2 and total aflatoxins were determined according to the method described by the A.O.A.C. [19].

Determination of bioactive compounds

Antioxidant activity (%) was evaluated by 1, 1diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method according to the procedure of Chen *et al.* [20].

Total phenols were analyzed spectrophotometrically using the method described by Swain and Hillis [21]. Results were expressed as g. gallic acid /100 g.D.Wt..

Total flavonoids content was determined according to Zhuang *et al.* [22] as mg catechin/100g. D.Wt..

Total alkaloids (mg/100g.) was analyzed spectrophotometrically using the method described by Fazel *et al.* [23].

Capscicine content of the crushed dried chilli pepper was determined by High Performance Liquid Chromatography (HPLC) at the National Organization for Drug & Control Research according to Thapa *et al.* [24]. Extraction and fractionation of caraway seeds volatile oil

Volatile oil of caraway seeds was extracted using Clevenger apparatus (hydro-distillation method) as Guenther [25]. Fractionation of caraway seeds volatile oil was performed using GC/MS Shimadzu model QP5000 at the Central laboratory of Horticulture Research Institute, A.R.C. as the method described in Egyptian pharmacopeia [26].

**Statistical Analysis:** The statistical analysis of the present data was carried out according to Snedecor and Cochran [27]. Averages were compared using the new L.S.D. values at 5% level [28].

### **RESULTS AND DISCUSSION**

Effect of Ozone Treatments on Aflatoxin Contents of Crushed Chilli Pepper During Storage: As shown in Table (1), B1, B2 and total aflatoxin contents of crushed chilli pepper during storage period was markedly affected by all ozone treatments in seasons 2017 and 2018.

Ozone treatments under vacuum significantly reduced B1, B2 and total aflatoxin contents of crushed chilli pepper comparing with those shown at atmospheric pressure and control samples. Least significant aflatoxin contents occurred by ozone (40ppm) under vacuum followed by ozone (20ppm) under vacuum, while control sample had the highest ones in both seasons.

With respect to the effect of storage period, it was mentioned that gradually significant increase in B1, B2 and total aflatoxin contents observed with prolonging the storage period for all treatments in both seasons. Highest significant B1, B2 and total aflatoxin contents of crushed chilli pepper obtained after six months of storage period in both seasons.

Regarding interaction between treatments and storage period, it is worth mentioning that control sample exhibited the highest significant B1, B2 and total aflatoxin contents after six months of storage period that recorded 8.13, 039 and 8.52ppm, respectively while, ozone (40ppm) under vacuum at zero time significantly attained the least values of B1, B2 and total aflatoxin contents (2.79, 0.0 and 2.79ppm and 1.99, 0.0 and 1.79ppm, respectively for first and second seasons) (Table 1). These differences may be due to the increase of ozone penetration as a result of the air vacuum and the voracious of the ozone receiving in all spaces between the seeds of the caraway seeds or red chilli pepper granules. Increasing the concentration of ozone reduced the remaining residue of aflatoxin. Also, high increased in aflatoxin content of control samples occurred due to the fungal growth on samples during storage period in two seasons which produced

					Storage period (S) (Months)							
	Aflato	xin B1			Aflato	oxin B2			Total aflatoxins (B1+B2)			
Treatments (T)	0	3	6	Means (T)	0	3	6	Means (T)	0	3	6	Means (T)
						2017	season					
Control	15.22	22.35	30.87	22.81	0.69	0.74	0.91	0.78	15.91	23.09	31.78	23.59
Ozone 10ppm at atmospheric pressure	5.01	5.13	5.61	5.25	0.35	0.41	0.44	0.40	5.36	5.54	6.05	5.65
Ozone 20ppm at atmospheric pressure	4.93	5.01	5.64	5.19	0.33	0.36	0.39	0.36	5.26	5.37	6.03	5.55
Ozone 40ppm at atmospheric pressure	3.62	3.94	4.27	3.94	0.32	0.33	0.37	0.34	3.94	4.27	4.64	4.28
Ozone 10ppm under vacuum	3.68	3.81	4.01	3.83	0.30	0.32	0.35	0.32	3.98	4.13	4.36	4.15
Ozone 20ppm under vacuum	3.17	3.47	3.54	3.39	0.00	0.14	0.20	0.11	3.17	3.61	3.74	3.50
Ozone 40ppm under vacuum	2.79	2.82	2.98	2.86	0.00	0.00	0.09	0.03	2.79	2.82	3.07	2.89
Means (S)	5.49	6.65	8.13		0.28	0.33	0.39		5.77	6.98	8.52	
New L.S.D. at 0.05 (T) =	1.94				0.06				1.67			
New L.S.D. at 0.05 (S) =	1.27				0.04				1.09			
New L.S.D. at 0.05 (TXS) =	3.37				0.11				2.89			
						2018	season					
Control	18.35	29.68	38.38	28.80	0.55	0.58	0.91	0.68	18.90	30.26	39.29	29.48
Ozone 10ppm at atmospheric pressure	5.61	6.15	6.84	6.20	0.29	0.32	0.51	0.37	5.90	6.47	7.35	6.57
Ozone 20ppm at atmospheric pressure	4.47	4.51	5.13	4.70	0.25	0.28	0.28	0.27	4.72	4.79	5.41	4.97
Ozone 40ppm at atmospheric pressure	4.00	4.09	4.23	4.11	0.23	0.27	0.24	0.25	4.23	4.36	4.47	4.35
Ozone 10ppm under vacuum	3.97	4.03	4.11	4.04	0.21	0.20	0.21	0.21	4.18	4.23	4.32	4.24
Ozone 20ppm under vacuum	3.04	3.12	3.21	3.12	0.00	0.00	0.10	0.03	3.04	3.12	3.31	3.15
Ozone 40ppm under vacuum	1.99	2.10	2.19	2.09	0.00	0.00	0.00	0.00	1.99	2.10	2.19	2.09
Means (S)	5.92	7.67	9.16		0.22	0.24	0.32		6.14	7.90	9.48	
New L.S.D. at 0.05 (T) =	2.03				0.11				1.77			
New L.S.D. at 0.05 (S) =	1.33				0.07				1.16			
New L.S.D. at 0.05 (TXS) =	3.52				0.19				3.07			

Table 1: Effect of ozone treatments on B1, B2 and total aflatoxin contents of crushed chilli pepper during storage period in seasons 2017 and 2018

aflatoxins. These results are matched with the findings of de Alencar *et al.* [29] and Kamber *et al.* [15] who reported that, there was a relationship between ozone treatments and the reduction of aflatoxin contents.

Generally, it could be concluded that, samples treated by ozone during the two seasons were within the limits according to the Egyptian Standards ES: 1875-1 and European Commission Regulation (EC) No 1881 [2, 3] except for the samples treated with 10 ppm of ozone at atmospheric pressure in both seasons, but after storage for 6 months, the accepted samples were numbers 4, 5, 6 and 7, that treated with ozone at 40 ppm in normal atmosphere and with concentrations of 10, 20 and 40 ppm under vacuum, respectively.

Effect of Ozone Treatments on Aflatoxin Contents of Caraway Seeds During Storage Period: The results presented in Table (2) show B1, B2 and total aflatoxin contents of caraway seeds, that significantly affected by ozone treatments during storage period in seasons 2017 and 2018.

No aflatoxin contents were exhibited due to the absence of fungal growth in all conducted ozone treatments of caraway seeds comparing with control sample in both seasons. As for the effect of storage period, gradually significant increases in B1, B2 and total aflatoxin were observed with the extension of the storage period which were definitely attributed to increase in control samples during storage in both seasons.

Regarding interaction between treatments and storage period, it is worth mentioning that, control samples attained the highest significant B1, B2 and total aflatoxin contents (5.02, 0.12 and 5.14ppm, respectively) after six months of storage period while, all ozone treatments significantly attained zero values of B1, B2 and total aflatoxin contents during storage period in both seasons. This may likely to be the powerful act of caraway volatile oil as antifungal which produces aflatoxins [30].

It is worth mentioning that, the control sample, which was not treated by ozone, had aflatoxin B1 content after 6 months of the storage period above the limits allowed in the (5 ppm) [2, 3]. These may be due to the lack and the potential deterioration of aromatic oil of caraway seeds, which led to a weak effect on fungi, resulting the presence of aflatoxin B1 at the end of the storage period. On the other hand, treating with ozone either at atmospheric pressure or under vacuum was very effective in inhibiting fungal activity and caraway seeds had no aflatoxin content during storage period in both seasons.

					Storage period (S) (Months)							
	Aflatoxin B1			Aflatoxin B2			Total aflatoxins (B1+B2)					
Treatments (T)	0	3	6	Means (T)	0	3	6	Means (T)	0	3	6	Means (T)
						2017,	season					
Control	4.01	4.45	5.02	4.49	0.00	0.00	0.12	0.04	4.01	4.45	5.14	4.53
Ozone 10ppm at atmospheric pressure	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ozone 20ppm at atmospheric pressure	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ozone 40ppm at atmospheric pressure	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ozone 10ppm under vacuum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ozone 20ppm under vacuum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ozone 40ppm under vacuum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Means (S)	0.57	0.64	0.72		0.00	0.00	0.02		0.57	0.64	0.73	
New L.S.D. at 0.05 (T) =	0.05				0.03				0.11			
New L.S.D. at 0.05 (S) =	0.03				0.02				0.07			
New L.S.D. at 0.05 (TXS) =	0.08				0.05				0.19			
					2018, season							
Control	3.84	4.56	5.27	4.56	0.34	0.39	0.51	0.41	4.18	4.95	5.78	4.97
Ozone 10ppm at atmospheric pressure	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ozone 20ppm at atmospheric pressure	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ozone 40ppm at atmospheric pressure	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ozone 10ppm under vacuum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ozone 20ppm under vacuum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ozone 40ppm under vacuum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Means (S)	0.55	0.65	0.75		0.05	0.06	0.07		0.60	0.71	0.83	
New L.S.D. at 0.05 (T) =	0.09				0.02				0.14			
New L.S.D. at 0.05 (S) =	0.06				0.01				0.09			
New L.S.D. at 0.05 (TXS) =	0.16				0.03				0.24			

Table 2: Effect of ozone treatments on B1, B2 and total aflatoxin contents of dried caraway seeds during storage period in seasons 2017 and 2018

From the data presented in Tables (1 and 2), it could be concluded that aflatoxins B1 and B2 in stored chilli pepper and caraway seeds directly degraded by ozone gas as reported by Inan *et al.* [11] and El-Desouky *et al.* [13]. Ozone would react across the 8, 9 double bond of the furan ring of aflatoxin through electrophilic attack, causing the formation of primary ozonides followed by rearrangement into monozonide derivatives such as aldehydes, ketones and organic acids [31].

Effect of Ozone Treatments on Antioxidant Activity and Bioactive Compounds of Crushed Chilli Pepper During Storage Period: As shown in Table (3), antioxidant activity, total phenols and total flavonoids of crushed chilli pepper during storage period were significantly affected by ozone treatments in seasons 2017 and 2018.

Concerning to effect of treatments, it is clear that ozone treatments under vacuum significantly declined the aforementioned parameters content in crushed dried chilli pepper comparing with those at atmospheric pressure and control. Least significant contents were observed by 40ppm Ozone under vacuum followed by 20ppm ozone under vacuum, while control had the highest value in both seasons. In concern to effect of storage period, it is obviously noticed that, the antioxidant activity, total phenols and total flavonoids contents gradually decreased and least significant happened of crushed chilli pepper after six months of storage in both seasons.

Samples treated with ozone (40ppm) under vacuum after six months significantly attained the least values of all parameters contents due to high oxidation effect of ozone. Contrarily, control sample at zero time recoded the highest values of these parameters in both seasons. These results are in agreement with those obtained by Iqbal *et al.* [32], who reported that, increasing storage period, gradually and steady decreased phenolic compounds (19.0%) and antioxidants activity which gradually decreased during storage period (150 days). Also, on dried Piper betle, despite the degradation of some phenolic compounds, more than 90% of the antioxidant activity was retained after 180 days [33].

Effect of Ozone Treatments on Antioxidant Activity and Bioactive Compounds of Caraway Seeds During Storage Period: Table (4) show the effect of ozone treatments at atmospheric pressure and under vacuum on antioxidant activity, total phenols and total flavonoids of caraway seeds during storage for six months.

Table 3: Effect of ozone treatments on antioxidant activity (%) and bioactive compounds of crushed chilli pepper during storage period in seasons 2017 and 2018

					Storage period (S) (Months)							
	Antiox	idant act	ivity (%)	)	Total	phenols	s (%)		Total flavonoids (mg/100g)			
Treatments (T)	0	3	6	Means (T)	0	3	6	Means (T)	0	3	6	Means (T)
						2017	season					
Control	29.37	21.79	15.23	22.13	0.76	0.67	0.50	0.64	0.307	0.289	0.249	0.282
Ozone 10ppm at atmospheric pressure	26.39	21.43	15.38	21.07	0.75	0.67	0.47	0.63	0.300	0.282	0.239	0.274
Ozone 20ppm at atmospheric pressure	25.46	21.66	14.94	20.69	0.75	0.66	0.40	0.60	0.285	0.269	0.223	0.259
Ozone 40ppm at atmospheric pressure	24.21	19.83	13.96	19.33	0.73	0.64	0.49	0.62	0.287	0.261	0.217	0.255
Ozone 10ppm under vacuum	24.71	19.77	13.16	19.21	0.74	0.59	0.42	0.58	0.289	0.261	0.218	0.256
Ozone 20ppm under vacuum	22.89	18.43	13.02	18.11	0.73	0.55	0.41	0.56	0.244	0.233	0.215	0.231
Ozone 40ppm under vacuum	22.91	15.70	11.15	16.58	0.70	0.50	0.40	0.54	0.234	0.202	0.198	0.211
Means (S)	25.14	19.80	13.83		0.73	0.61	0.44		0.278	0.257	0.223	
New L.S.D. at 0.05 (T) =	4.48				0.14				0.026			
New L.S.D. at 0.05 (S) =	2.93				0.09				0.017			
New L.S.D. at 0.05 (TXS) =	7.76				0.24				0.045			
						2018	season					
Control	28.25	20.25	15.27	21.26	0.75	0.68	0.49	0.64	0.299	0.287	0.271	0.286
Ozone 10ppm at atmospheric pressure	26.20	20.02	14.98	20.40	0.72	0.67	0.48	0.62	0.287	0.278	0.242	0.269
Ozone 20ppm at atmospheric pressure	25.55	20.68	14.15	20.13	0.70	0.64	0.47	0.60	0.284	0.259	0.238	0.260
Ozone 40ppm at atmospheric pressure	24.01	19.66	13.79	19.15	0.69	0.61	0.47	0.59	0.277	0.258	0.220	0.252
Ozone 10ppm under vacuum	24.20	19.96	12.39	18.85	0.69	0.59	0.43	0.57	0.274	0.255	0.219	0.249
Ozone 20ppm under vacuum	23.54	17.35	12.77	17.89	0.63	0.55	0.47	0.55	0.239	0.227	0.211	0.226
Ozone 40ppm under vacuum	22.99	15.36	10.91	16.42	0.62	0.50	0.42	0.51	0.228	0.199	0.182	0.203
Means (S)	24.96	19.04	13.46		0.68	0.60	0.46		0.270	0.252	0.226	
New L.S.D. at 0.05 (T) =	4.85				0.12				0.024			
New L.S.D. at 0.05 (S) =	3.17				0.08				0.016			
New L.S.D. at 0.05 (TXS) =	8.40				0.21				0.042			

 Table 4: Effect of ozone treatments on antioxidant activity and bioactive compounds of caraway seeds during storage period in seasons 2017 and 2018

 Storage period (S) (Months)

	Antiox	idant act	ivity (%)	)	Total	phenols	s (%)		Total flavonoids (mg/100g)			
Treatments (T)	0	3	6	Means (T)	0	3	6	Means (T)	0	3	6	Means (T
						2017	season					
Control	27.58	24.55	20.58	24.24	0.42	0.35	0.20	0.32	0.039	0.017	0.012	0.023
Ozone 10ppm at atmospheric pressure	26.03	23.87	19.59	23.16	0.32	0.30	0.19	0.27	0.028	0.016	0.012	0.019
Ozone 20ppm at atmospheric pressure	24.13	22.98	19.20	22.10	0.31	0.28	0.17	0.25	0.026	0.015	0.011	0.017
Ozone 40ppm at atmospheric pressure	23.86	22.53	18.11	21.50	0.35	0.30	0.11	0.25	0.021	0.015	0.011	0.016
Ozone 10ppm under vacuum	22.57	19.95	17.20	19.91	0.33	0.30	0.18	0.27	0.020	0.014	0.010	0.015
Ozone 20ppm under vacuum	21.26	19.67	15.17	18.70	0.30	0.29	0.14	0.24	0.019	0.014	0.010	0.014
Ozone 40ppm under vacuum	19.95	17.25	15.15	17.45	0.28	0.27	0.11	0.22	0.019	0.014	0.010	0.014
Means (S)	23.63	21.54	17.86		0.33	0.30	0.16		0.025	0.015	0.011	
New L.S.D. at 0.05 (T) =	1.82				0.03				0.011			
New L.S.D. at 0.05 (S) =	1.19				0.02				0.007			
New L.S.D. at 0.05 (TXS) =	3.15				0.05				0.019			
						2018	season					
Control	27.54	25.99	20.58	24.70	0.48	0.39	0.24	0.37	0.036	0.016	0.012	0.021
Ozone 10ppm at atmospheric pressure	26.49	24.40	19.59	23.49	0.39	0.36	0.21	0.32	0.029	0.016	0.011	0.019
Ozone 20ppm at atmospheric pressure	24.37	22.85	19.20	22.14	0.39	0.32	0.19	0.30	0.027	0.015	0.011	0.018
Ozone 40ppm at atmospheric pressure	23.96	22.98	18.11	21.68	0.33	0.30	0.18	0.27	0.022	0.015	0.010	0.016
Ozone 10ppm under vacuum	22.99	19.73	17.20	19.97	0.29	0.21	0.17	0.22	0.021	0.014	0.011	0.015
Ozone 20ppm under vacuum	21.05	18.35	15.75	18.38	0.28	0.19	0.14	0.20	0.018	0.013	0.010	0.014
Ozone 40ppm under vacuum	20.32	17.58	15.17	17.69	0.26	0.16	0.13	0.18	0.017	0.014	0.010	0.014
Means (S)	23.82	21.70	17.94		0.35	0.28	0.18		0.024	0.015	0.011	
New L.S.D. at 0.05 (T) =	1.71				0.06				0.006			
New L.S.D. at 0.05 (S) =	1.12				0.04				0.004			
New L.S.D. at 0.05 (TXS) =	2.97				0.11				0.011			

Ozone treatments under vacuum had significantly declined the aforementioned parameter contents of caraway seeds comparing with atmospheric pressure and control samples. Least significant contents were observed by ozone (40ppm) under vacuum, while control sample recorded the highest value in both seasons.

In concern to effect of storage period, it is obviously noted that antioxidant activity, total phenols and total flavonoids contents of caraway seeds gradually decreased with extending storage period and the least significant contents obtained after six months of storage in both seasons.

Samples treated with ozone (40ppm) under vacuum after six months significantly attained the least values being 15.15%, 0.11% and 0.010mg/100g. for antioxidant activity, total phenols and total flavonoids, respectively. Contrarily, control sample at zero time recoded the highest values (27.58%, 0.42% and 0.039 mg/100g. for the first season and 27.54%, 0.48% and 0.036 mg/100g. respectively for antioxidant activity, total phenols and total flavonoids). The results are in accordance with those obtained by Tiwari *et al.* [34] and El-Kady *et al.* [35] who explained that these decrements may be due to high oxidation effect of ozone.

As usual, the largest reduction at the end of the storage period was in the control samples where the shortage occurred during the 2017 and 2018 seasons. This loss may be due to the act of light, oxygen, temperature and other environmental factors [36].

Volf *et al.* [37] cited that polyphenols stability under different conditions is a very important aspect that must be taken into account to ensure that phenolic compounds possess the desired properties and maintain their activity under different storage conditions, which can involve high temperatures and light.

Effect of Ozone Treatments on Alkaloids Content of Crushed Chilli Pepper During Storage: Hot and spicy taste of chili pepper is due to the class of compounds called capsaicinoids, arising from the secondary metabolism of the alkaloid groups, capsaicin, high antioxidant capacity and it represents an excellent source of vitamins A, C, E and carotenoids [38]. Therefore, the total alkaloids content of crushed chilli pepper treated with ozone during storage period were determined and the results are presented in Table (5).

Total alkaloids content of crushed chilli pepper during storage period significantly affected by ozone treatments in seasons 2017 and 2018. Ozone treatments under vacuum significantly decreased total alkaloids content of crushed dried chilli pepper comparing with those at atmospheric pressure and control samples. Least significant contents were observed by ozone (40ppm) under vacuum followed by ozone (20ppm) under vacuum, while control samples had the highest values in both seasons.

Furthermore, total alkaloids content gradually decreased with extending storage period and the least significant total alkaloids content of crushed chilli pepper obtained after six months of storage in both seasons.

Regarding the interaction between treatments and storage period, it is worth mentioning that ozone 40ppm under vacuum after six months significantly attained the least values of total alkaloids content being 0.90 and 0.90 mg/100g., respectively in first and second seasons. Contrarily, control sample at zero time recoded the highest values of total alkaloids content in both seasons.

Regarding total alkaloids contents of caraway seeds data in Table (6) show that there were significant decreases due to ozone treatments during storage period in seasons 2017 and 2018.

Ozone treatments under vacuum significantly decreased total alkaloids content of caraway seeds comparing with those at atmospheric pressure and control samples as well as, the highest decrease observed by Ozone (40ppm) under vacuum, while control samples had the highest value in both seasons.

Total alkaloids content gradually decreased with extending the storage period. Least significant total alkaloids content of caraway seeds obtained after six months of storage in both seasons.

Regarding the interaction between treatments and storage period, it is worth mentioning that ozone (40ppm) under vacuum after six months significantly attained the least values of total alkaloids contents (0.63 and 0.61mg/100g, respectively) in the first and second seasons. Contrarily, control sample at zero time recoded the highest values (0.86 and 0.90 mg/100g, respectively) of total alkaloids content in both seasons.

Effect of Ozone Treatments on Capsaicin Content of Crushed Chilli Pepper During Storage Period: Tables (7) show the effect of ozone treatments at atmospheric pressure and under vacuum on capsaicin content of crushed chilli pepper during storage at zero time and after six months in season 2018.

Ozone treated samples at 40 ppm under vacuum had significantly decreased capsaicin content of crushed chilli pepper comparing with atmospheric pressure at 40 ppm and control. Least significant content observed by ozone (40 ppm) under vacuum; however control sample had the highest value.

	Storage period (S) (Months)							
Treatments (T)	0	3	6	Means (T)				
		2017 Season						
Control	1.26	1.13	0.96	1.12				
Ozone 10ppm at atmospheric pressure	1.13	1.10	0.94	1.06				
Ozone 20ppm at atmospheric pressure	1.10	0.99	0.94	1.01				
Ozone 40ppm at atmospheric pressure	1.03	0.96	0.91	0.97				
Ozone 10ppm under vacuum	1.03	0.94	0.91	0.96				
Ozone 20ppm under vacuum	0.99	0.94	0.91	0.95				
Ozone 40ppm under vacuum	0.93	0.91	0.90	0.91				
Means (S)	1.07	1.00	0.92					
		2018 Season						
Control	1.30	1.01	0.99	1.10				
Ozone 10ppm at atmospheric pressure	1.20	1.01	0.93	1.05				
Ozone 20ppm at atmospheric pressure	1.06	0.94	0.93	0.98				
Ozone 40ppm at atmospheric pressure	1.04	0.93	0.93	0.97				
Ozone 10ppm under vacuum	1.04	0.99	0.93	0.99				
Ozone 20ppm under vacuum	1.01	0.94	0.91	0.96				
Ozone 40ppm under vacuum	1.01	0.94	0.90	0.95				
Means (S)	1.09	0.97	0.93					
New L.S.D. at 0.05 for	(T)	(S)	(TXS)					
2017 Season	0.09	0.06	0.16					
2018 Season	0.17	0.11	0.29					

# Table 5: Effect of ozone treatments on total alkaloids (mg/100g) in dried chilli pepper during storage period in seasons 2017 and 2018

Table 6: Effect of ozone treatments on total alkaloids (mg/100g) of caraway seeds during storage period in seasons 2017 and 2018

	Storage period (S) (Months)							
Treatments (T)	0	3	6	Means (T)				
		2017 Season						
Control	0.86	0.79	0.68	0.78				
Ozone 10ppm at atmospheric pressure	0.83	0.76	0.68	0.76				
Ozone 20ppm at atmospheric pressure	0.81	0.75	0.67	0.74				
Ozone 40ppm at atmospheric pressure	0.80	0.72	0.67	0.73				
Ozone 10ppm under vacuum	0.78	0.71	0.66	0.72				
Ozone 20ppm under vacuum	0.72	0.70	0.65	0.69				
Ozone 40ppm under vacuum	0.72	0.70	0.63	0.68				
Means (S)	0.79	0.73	0.66					
		2018 Season						
Control	0.90	0.77	0.69	0.79				
Ozone 10ppm at atmospheric pressure	0.87	0.77	0.67	0.77				
Ozone 20ppm at atmospheric pressure	0.81	0.75	0.66	0.74				
Ozone 40ppm at atmospheric pressure	0.81	0.74	0.65	0.73				
Ozone 10ppm under vacuum	0.80	0.71	0.62	0.71				
Ozone 20ppm under vacuum	0.78	0.71	0.61	0.70				
Ozone 40ppm under vacuum	0.77	0.70	0.61	0.69				
Means (S)	0.82	0.74	0.64					
New L.S.D. at 0.05 for	(T)	(S)	(TXS)					
2017 Season	0.06	0.04	0.11					
2018 Season	0.11	0.07	0.19					

	Storage period (S) (Months)						
Treatments (T)	0		Means (T)				
Control	695.43	633.52		664.48			
Ozone 40ppm at atmospheric pressure	694.21	628.41		661.31			
Ozone 40ppm under vacuum	690.39	627.63		659.01			
Means (S)	693.34	629.85					
New L.S.D. at 0.05 for	(T)	(S)	(TXS)				
	2.27	1.85	3.21				

Table 7: Effect of ozone treatments on capsaicin content ( $\mu g/g$ ) of crushed chilli pepper during storage period in 2018 season	Table 7: Effect of ozone treatments of	on capsaicin content (µg/g) o	f crushed chilli pepper durin	ig storage period in 2018 season
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Capsaicin content gradually decreased with extending storage period and least significant capsaicin content of crushed chilli pepper obtained after six months of storage.

Ozone (40ppm) under vacuum after six months attained the least values of capsaicin content being 627.63  $\mu$ g/g. On the other hand, control sample recoded the highest capsaicin content (695.43  $\mu$ g/g) at zero time.

These results are in agreement with those obtained by Topuz and Özdemir [39] and Wang *et al.* [12] who found that capsaicin and dihydrocapsaicin had very similar stability and showed similar reduction rates during heating and storage, which indicates that the C = C bond in capsaicin is relatively stable and does not account for its decomposition during heating and storage. Furthermore, they also added that the amounts of capsaicin and dihydrocapsaicin decreased continuously during nine months of storage and dihydrocapsaicin showed a somewhat higher reduction rate than capsaicin. Dihydrocapsaicin and capsaicin decreased at room temperature.

As for the effect of ozone treatments, there was no oxidation effect on capsaicin content. This could be attributed to the stability of C = C bond of capsaicin which is relatively stable and does not account for its decomposition during heating and storage.

Increasing storage period, gradually and steady decreased capsaicinoids (12.7%). The trends of capsaicinoids gradually decreased during storage period (150 days) [32].

Effect of Ozone Treatments on Volatile Oil Content of Caraway Seeds During Storage: Results presented in Table (8) show the volatile oil content of caraway seeds during storage period which had significantly affected by ozone treatments in 2018 season.

Ozone treatments had no clear trend on volatile oil content in caraway seeds comparing with control samples. It is obvious that insignificant differences among all ozone treatments and control sample. On the other hand, volatile oil content of caraway seeds gradually decreased with extending storage period and the least significant volatile oil content obtained after six months of storage period.

After six months of storage, all ozone treatments and control samples recorded the least contents of volatile oil (mean of 0.94%) comparing with those at zero time (mean of 1.16%).

Effect of Ozone Treatments on Fractionation of Caraway Seeds Volatile Oil During Storage: Table (9) shows the effect of storage for 6 months and ozone treatments at atmospheric pressure and under vacuum on volatile oil fractions of caraway seeds. Carvone was the predominant component in caraway essential oil recording 69.867%, followed by limonene (23.792%) and trans-anethole (4.260%) at zero time. After 6 months of storage, no clear trend was noticed either control samples or ozone treated ones at atmospheric pressure and under vacuum.

The results are in accordance with those obtained by Bailer *et al.* [40]; Sedláková *et al.* [41] and Rezvanpanah *et al.* [42] who mentioned that the major compounds of caraway were carvone (57.7%) and limonene (35.5%). These results were accordance to previous studies that reported carvone and limonene were the main components of C. *carvi* oil. Also, Carvone and limonene are the main components of caraway essential oil which characterized by high contents of oxygenated monoterpenes and sesquiterpenes [43]. Furthermore, Limonene (77 – 19% of the total oil) and carvone (20 – 79%) were the major ones [44].

By prolonging the storage time especially at room temperature, the concentration of constituents with a lower molecular weight decreased. This phenomenoncan be due to evaporation, oxidation and other unwanted changes in essential oil components during storage period [45].

The volatile compounds of essential oil are also affected by the storage conditions. The relative amount of monoterpenes and sesquiterpenes decreased, while

	Storage period (S) (Months)							
Treatments (T)	0	3	6	Means (T)				
Control	1.13	1.02	0.90	1.02				
Ozone 10ppm at atmospheric pressure	1.17	1.08	0.98	1.08				
Ozone 20ppm at atmospheric pressure	1.19	1.10	0.97	1.09				
Ozone 40ppm at atmospheric pressure	1.15	1.01	0.89	1.02				
Ozone 10ppm under vacuum	1.14	1.06	0.95	1.05				
Ozone 20ppm under vacuum	1.19	1.09	0.93	1.07				
Ozone 40ppm under vacuum	1.18	1.07	0.95	1.07				
Means (S)	1.16	1.06	0.94					
New L.S.D. at 0.05 for	(T)	(S)	(TXS)					
	N.S.	0.09	0.24					

#### Table 8: Effect of ozone treatments on volatile oil percentage in caraway seeds during storage period in 2018 season

At zero time After 6 months of storage

Component	Control	Ozone 40 ppm atm. Pressure	Ozone 40 ppm under vacuum	Control	Ozone 40 ppm atm. Pressure	Ozone 40 ppm under vacuum
t						
α-Pinene	0.078	0.074	0.076	0.070	0.077	0.080
Sabinene	0.049	0.052	0.044	0.060	0.063	0.055
β-Pinene	0.024	0.026	0.026	0.024	0.027	0.027
β-Myrecene	0.098	0.128	0.128	0.100	0.130	0.130
Limonene	23.792	23.718	23.721	24.253	24.177	24.180
Trans-β-ocimene	0.068	0.069	0.069	0.069	0.070	0.070
α-Terpinene	0.081	0.078	0.078	0.083	0.080	0.080
Linalool	0.089	0.084	0.087	0.058	0.055	0.057
Cis-Limonene oxide	0.048	0.044	0.044	0.049	0.045	0.045
Trans-Limonene oxide	0.157	0.147	0.147	0.160	0.150	0.150
Cis-dihydrocarvone	0.289	0.275	0.275	0.189	0.180	0.180
Trans-dihydrocarvone	0.214	0.202	0.202	0.140	0.132	0.132
Trans-Carveol	0.321	0.321	0.321	0.210	0.210	0.210
cis-Carveol	0.145	0.144	0.144	0.095	0.094	0.094
Carvone	69.867	69.886	69.886	71.220	71.240	71.240
Perillaldehyde	0.245	0.255	0.255	0.250	0.260	0.260
Trans-Anethole	4.260	4.321	4.321	2.790	2.830	2.830
Trans-β-Caryophyllene	0.177	0.177	0.177	0.180	0.180	0.180

the oxygenated terpenoids increased. This might have happened due to evaporation of more volatile compounds and the oxidation process [46].

### RECOMMENDATIONS

From the aforementioned data, it could be recommended that using ozone under vacuum decontaminate spices as crushed red chilli pepper and caraway seeds, other than ozone at atmospheric pressure. Using ozone under vacuum had effectively reduced in B1, B2 and total aflatoxin contents than at atmospheric pressure.

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Table 9: Effect of ozone treatments on fractionation of caraway seeds volatile oil

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