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# Influence of Ozone Gas on Cowpea Beetle (*Callosobruchus maculatus*) as Well as Seed Technological Characteristics

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**Abstract:** The present study aimed to investigate the effects of ozone gas exposure (at 2, 4 and 6 hours) treatments on controlling the life stages of *Callosobruchus maculatus* insects (eggs, larvae and adults) for infesting cowpea seeds and their effects on some insect enzymes (phenoloxidase, peroxidase, α-esterases and β-esterases). The ozonated cowpea seed samples (ozone exposure for 6 h) were assessed with respect to some physicochemical and technological characteristics. The insect eggs were highly tolerant to ozone followed by adults and larvae stages which were the lowest tolerant. The maximum reduction percentage in $F_1$ progeny was found in eggs followed by larvae stages at 6 h of the exposure period. The ozone decreased the activity of peroxidase, α-esterases and β-esterases in adult insects except for phenoloxidase which was higher in treated insects compared with control sample. The ozonated cowpea seeds were lower in germination percentages, the 1000-seed weights and starch granules size compared with control. Ozone treatment increased lightness, redness and yellowness values of seeds. Results showed, also, an increase in fiber and ash contents and *in vitro* protein digestibility; while the moisture, protein, fat and minerals (calcium, iron, zinc and phosphorus) were lower in ozonated samples compared with the control. Ozone treatment reduced the cooking time and total soluble solids. Ozonated cowpea seed samples were accepted in all tested sensorial attributes compared with control samples before and after storage periods (12 weeks).

**Key words:** Cowpea seeds · Ozone gas · *Callosobruchus maculatus* · Enzyme profile · Physicochemical analysis · Cooking properties · Sensory attributes

## INTRODUCTION

Legumes have an important nutritional role in the diet as a protein source. They are considered a vital component in achieving food and nutritional security [1]. The cowpea (*Vigna unguiculata*, L.) is one of the highly nutritive legumes worldwide cultivated and is generally used in human food. The total production of cowpea seeds in Egypt was 7215 Tons in 2020 [2]. Cowpea is a nutritious food, *i.e.,* high in protein (~24%) and dietary fiber (~11%), with low lipid contents (<2%) [3]. Cowpea also contains essential amino acids and polyphenols with an antioxidant activity [4].

Insect pests cause a serious damage to economically important legumes during storage all-over the world. Cowpea beetle [*Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae)] is regarded as one of the most common damaging brushed species of various legumes, including cowpea. The larval and pupal stages develop inside the seeds, burrowing into the seed and the larvae consume the cotyledons. These insects attack cowpea seeds causing a physical damage and deteriorate the seeds quality. *C. maculatus* is a cosmopolitan pest of cowpea in world tropics and subtropics countries, which originates from Western Africa [5, 6]. *O*-phenoloxidases are phenol oxidases (PO) that show tyrosinase-like

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activity and they may hydroxylate tyrosine as well as oxidize *o*-diphenols to quinones [7, 8]. Insect PO is produced as pro PO zymogens, which are activated by proteolytic cleavage at a specific location in response to infection or injury [9]. Esterases enzymes are one of the main detoxifying insect enzymes and at least one of them is involved in insecticides detoxification [10].

Ozone ($O_3$) gas is an effective, economic and friendly environment fumigant for monitoring stored product insects with no effect on grain quality. It is attracting much attention as an alternative to commercial insecticides due to its short half-life, rapid decomposing to oxygen without leaving any residues on the stored product and it can penetrate a large mass of seeds [11]. Ozone gas can be effectively used for controlling *C. chinensis* and *C. maculatus* and it could sufficiently protect stored cowpea seeds and it has the ability for degradation of mycotoxin and pesticide residues. Therefore, it is an effective alternative to conventional fumigants against many pests, microorganisms and mycotoxins [12-14]. In addition to that, the US Food and Drug Administration (FDA) approved treating food products and water by ozone gas as a safe treatment [15].

The present study aims to examine the insect enzymes profile after exposure to ozone gas and to evaluate the effect of ozone treatments on the physicochemical, technological and sensory characteristics of cowpea seeds.

## MATERIAL AND METHODS

**Materials:** Cowpea beetles (*Callosobruchus maculatus*) were reared at 28±2°C and 65±5% RH in the laboratory of the Department of Stored Products and Grains Pests, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt. Cowpea seeds were obtained from local markets, Cairo, Egypt. Bovine serum albumin, pepsin and pancreatin enzymes and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma–Aldrich Chemical Co., St. Louis, USA. All used chemicals were of analytical grade.

**Bioassay Test:** Eggs (0-48 h), larvae (10-15 days old) and adults (1 day old) of *C. maculatus* were used in the experiment and each stage of the insect was separately designed. Three replicates of each treatment, each replicate were put in small jute bags, containing 30 g of cowpea seeds which were separately infested with different stages of insects. The bags were well closed and secured with rubber bands. Ozone gas was produced from the air using an ozone generator (Model OZO-6 VTTL OZO Max Ltd, Shefford, Quebec, Canada) from purified extra dry oxygen feed gas at the Laboratory of Food Toxicology and Contaminants, National Research Center. An incubation chamber, with a 50 litter volume, was used for the ozone treatment. The exposure was done as described by Abdelfattah *et al*. [16]. Three different exposure times 2, 4 and 6 h at 200 ppm concentration were left and closed in glass containers for 24 h. Untreated group (control) was conducted as previously mentioned without ozone gas. Eggs and larvae were checked after first generation ($F_1$) progeny emerged while adults mortality was checked after 24 hours after exposure to ozone and the reduction % was calculated using the formula of Henderson and Tilton [17].

$$Reduction\ (\%) = (Control - Treated\ /\ Control)\ x\ 100$$

**Determination of the Tested Enzyme Activity of *C. maculatus* Adult:** The activity of phenoloxidase, peroxidase, alpha esterases (α-esterases) and beta esterases (β-esterases) enzymes in cowpea adult beetles after exposure to 6 h of ozone gas were measured. Phenoloxidase activity was determined according to the modification of Ishaaya [18]. Peroxidase activity was determined according to Vetter *et al*. [19]. Alpha esterases (α-esterases) and beta esterases (β-esterases) were determined according to Van Asperen [20] using α-naphthyl acetate or β-naphthyl acetate as substrates, respectively.

**Physicochemical Analysis and Technological Evaluation:** Regarding the results of mortality percentages in Table 1, cowpea seeds exposed to ozone gas for 6 h were used in physicochemical, technological and sensory evaluation. Cowpea seeds were milled using a laboratory mill (IKA-Laboratechnic, Janke and Kunkel Type: MFC, Germany) to obtain the whole meal powder for chemical analysis and then packed in polyethylene bags and kept in a freezer until further analyses.

**Scanning Electron Microscopy:** The Scanning Electron Micrographs of control and ozonated cowpea (whole meal powder) were captured by using a Scanning Electron Microscope (Model JSM-IT 200, JEOL Ltd., USA). Samples were mounted using carbon paste on an

aluminum stub and coated with gold up to a thickness of 400 A in a sputter-Coating Unit, then observed at an accelerating voltage between 15 and 20 kV. The micrographs were captured at 1500 x magnification.

**Germination Test:** The germination test was conducted in the laboratory using 25 seeds of each sample and was separately placed on a surface of a layer of cotton wool in a petri dish (6×1 cm) which was wetted daily with tap water for 1 week. Germination of cowpea seeds was measured after 1 week of plantation by counting the viable seed samples and the germination percentages were calculated for both seeds (control and after exposure to ozone gas for 6 h). The germination percentages were averages of three replicates [21].

**Physical Properties of Cowpea Seeds:** Control and ozonated cowpea seeds were examined for physical properties (the weight of 1000 seeds and color parameters). The total weight of 1000-seeds of cowpea seeds was measured according to AACC [22]. The external seeds color was measured using a hand-held Chromameter (model CR-400, Konica Minolta, Japan). Color parameters were expressed as the values of lightness ($L^*$), redness ($a^*$) and yellowness ($b^*$). All measurements were averages of three replicates.

**Proximate Analysis of Cowpea Seeds:** Moisture, protein, fat, crude fiber and ash contents of the cowpea seeds, before and after exposure to ozone gas for 6 h were determined according to AOAC [23]. The nitrogen content was estimated by the Kjeldahl method, using a conversion factor of 6.25. The total carbohydrate content was calculated by subtracting the contents of protein, fat, ash and crude fiber from 100 g of samples. The proximate composition values were averages from three replicates. Iron, zinc and calcium contents were determined in samples according to the method outlined in the AOAC [23] using the Agilent Technologies Microwave Plasma Atomic Emission Spectrometers (Model 4210 MPAES, USA). Phosphorus was determined by the colorimetric method of Trough and Mayer [24].

**Determination of Total Phenols:** Total phenols were determined as described by Singleton and Rossi [25]. 1 g sample was mixed with 10 ml methanol (80%) and shaked for 2h. The mixture was filtrated and the color was developed by 0.250 ml Folin-Ciocalteu phenol reagent and 0.50 ml sodium carbonate solution (10%) and the volume was completed to 5 ml with distilled water. The reaction mixture was kept in the dark at room temperature for 30 min. Absorbance of the reaction mixture was measured at 725 nm against blank using a Jenway spectrophotometer (Model 6715 UV/Vis, Cole-Parmer Ltd, Staffordshire, UK). The total phenol content was expressed as mg/100g gallic acid equivalent based on the previously designed standard curve.

**DPPH Free Radical Scavenging Activity:** The antioxidant activity of the previous methanol extract was measured according to the method of Brand-Williams *et al.* [26]. 3.90 ml of DPPH methanol solution (2.40 mg of DPPH were dissolved in 100 ml of methanol) was added to 0.10 ml of sample extract. The reaction mixture was vigorously shaken and allowed to stand in a dark place for 30 min at room temperature and the absorbance was measured at 515 nm using a Jenway spectrophotometer (Model 6715 UV/Vis, Cole-Parmer Ltd, Staffordshire, UK). The DPPH radical scavenging percentage was calculated using the following equation:

$$\textit{Radical scavenging}\ (\%) = [(A0 - A\ 1) / A0]\ x\ 100$$

$A_0$ = Absorbance of the control reaction (containing all reagents except the test compounds).

$A_1$ = Absorbance in the presence of the tested extracts after 30 min.

***In vitro* Protein Digestibility:** *In vitro* protein digestibility was determined according to the method of Akeson and Stahmanna [27]. 1 g sample was added to 15 ml of 1.5 mg pepsin, dissolved in 0.1 M HCl and then incubated at 37°C for 3h. The obtained suspension was neutralized with NaOH (0.2 M), then treated with 7.50 ml of pancreatin (4 mg in 0.2 M phosphate buffer, pH 8.0) and then the mixture was gently shaken and incubated for 24 h at 37°C. The samples were treated with 10% trichloroacetic acid and centrifuged at 5000 xg for 20 min at room temperature. Protein in the supernatant was estimated using the Kjeldahl method [23]. The percentage of protein digestibility was calculated using the following equation:

$$\textit{In vitro protein digestibikity}\ (\%) = (N\ \textit{in supernatant} - N\ \textit{in Blank}) / N\ \textit{in sample}\ x\ 100$$

N = Nitrogen.

**Technological Evaluation**

**Cooking Quality and Total Soluble Solids:** 5 g of cowpea seeds were cooked with 100 ml of distilled water for 40 min at 100°C (where it was higher during storage). After cooking, the total soluble solids (TSS) and water uptake ratio were measured [28]. Total soluble solids (TSS) were detected by drying the cooking water containing soluble materials in an oven at 100°C for 16-18 h. Then the residue was weighed and calculated as a percentage of the initial weight of the seeds before cooking to obtain the total soluble solids percentage as the following equation:

*Total soluble solids* (*TSS*)% = *Weight of residue* (*g*) / *Initial weight of seeds* (*g*) *x* 100

**Water Uptake Ratio:** The previous cooked seeds were drained and weighed. The water uptake ratio was calculated using the following equation:

*Water uptake after cooking* (*g*/*g*) = (*Weight of cooked seeds – Initial weight of seeds*) / *Initial weight of seeds*

**Sensory Evaluation:** The cooked cowpea seeds were coded and submitted to a 10 member panel from Food Technology Research Institute staff, for evaluation, using the method described by Larmond [29]. Panelists were asked to score the products on a 9-point hedonic scale (9 = like extremely and 1 = dislike extremely) for the tested quality attributes (color, taste, texture, odor and overall acceptability).

**Statistical Analysis:** Mortality was statistically analyzed by using the log-probit software program Bakr [30]. The data for cowpea beetles stages were subjected to one-way analysis of variance (ANOVA) at $p<0.05$ followed by Duncan's new multiple range tests to assess differences between the group means and mean values (n=3) and standard deviation are recorded. The collected data of control and ozonated samples (for 6h) were statistically analyzed in triplicate except for sensory evaluation (n=10) for the analytical data, mean values and standard deviation are reported. The obtained data were subjected to an independent t-test and analysis of variance [because it compares the means of two independent groups (control and ozonated samples were exposed to ozone gas for 6h), in order to determine whether there is statistical evidence or the two groups are different from one another], at $p<0.05$, by using SPSS version 21 [31].

## RESULTS AND DISCUSSION

**Bioassay Results:** From the results listed in Table 1, it could be noticed that increasing exposure period increased the death rate in the adult stage, as well as the number of the first generation resulting from exposing the eggs and larvae in their stages decreased. The same Table showed that the egg stage is more affected than the other stages, which could be due to that the larvae live inside the seed and the adult insects have a hard cuticle that protects them more than the egg stage, which is externally placed on the cowpea seeds. The data revealed that time had a clear effect in all beetle stages and distinguished the treatments at 2, 4 and 6 hours compared with the control. On the other hand, the statistical analysis showed that there were no different effects at 2 and 4 h of ozone gas exposure in the eggs and larvae stages. The mortality percentage reached to 100% in the adult stage and the reduction percentage of the first generation ($F_1$) was 99.90% in the egg stage, while it was 67.47% in the larva stages after 6 h of ozone gas exposure.

Table 2 shows the values of 50% and 95% of lethal times for the cowpea beetle adult stage after exposure to different periods of ozone gas. The results are in the same line with Gad *et al*. [32] who reported that the mortality percentage of all life stages of *C. maculatus* was significantly increased with increasing ozone gas exposure time. Besides, the longer exposure time to ozone also caused a higher reduction in adult emergence from eggs, larvae and pupae. While treatment with ozone gas for 5 h caused 72.30% of egg mortality. As well as, treatment with ozone gas for 5 h resulted in 75, 100 and 94.10% reduction in adult emergence from eggs, larvae and pupae, respectively. The larvae and adults were more susceptible to ozone gas than the eggs and pupae [32].

From the results of Tables 1 and 2, data revealed that exposure to ozone gas with a dose of 200 ppm for 6 hours is sufficient to reach the desired limit for controlling the cowpea beetle. Therefore, the further analysis, whether on the insect adults or seeds was continued after ozone exposure for 6 hours.

**Enzyme Activities of Cowpea Adult Beetles:** Table 3 presents the enzymes activities of the adult beetles after exposure to ozone gas for 6 hours compared with the control (untreated cowpea seeds). An independent t-test was conducted to examine the effect of the enzyme activity of cowpea adult beetles after ozone gas exposure (6 h). As for the phenol oxidase enzyme, the enzyme

Table 1: The response of different *C. maculatus* beetles in the different stages after exposure to ozone gas

| | Stages | | | | |
|---|---|---|---|---|---|
| | Adults | Eggs | | Larvae | |
| Exposure time (hour) | Mortality (%) | $F_1$of progeny* | Reduction (%) | $F_1$of progeny* | Reduction (%) |
| Zero h (control) | $0.00^{d}$±0.00 | $423.67^{a}$±22.36 | - | $399.00^{a}$±51.40 | - |
| 2 | $37.33^{c}$±4.16 | $38.00^{b}$±6.16 | $91.00^{b}$ | $348.00^{a}$±46.00 | $12.80^{b}$ |
| 4 | $76.67^{b}$±4.16 | $26.30^{bc}$±4.16 | $93.73^{b}$ | $289.00^{ab}$±76.73 | $27.57^{b}$ |
| 6 | $100.00^{a}$±0.00 | $0.33^{c}$±0.57 | $99.90^{a}$ | $129.67^{b}$±11.60 | $67.47^{a}$ |
| F value | 671.83 | 812.72 | 5055.60 | 10.20 | 20.13 |

*$F_1$= First generation.

Values are mean ±SD. Means in the same column for each parameter with different superscripts are significantly different at $p< 0.05$.

Table 2: Ldp-line analysis readings of adult stage of *C. maculatus* after exposure to ozone gas

| | | Confidence limits of $LT_{50\%}$ | | Confidence limits of $LT_{95\%}$ | | | |
|---|---|---|---|---|---|---|---|
| *$LT_{50\%}$ | *$LT_{95\%}$ | Lower | Upper | Lower | Upper | Slope ± SD | Chi square $X^2$ |
| 2.5 | 8.11 | 2.11 | 2.82 | 6.42 | 12 | 3.20±0.46 | 0.31 |

*LT= lethal times.

Table 3: Enzymes activity of cowpea adult beetles after exposure to ozone gas

| Treatments | Control | 6 h ozone treated | t value | Sig. (2-tailed) |
|---|---|---|---|---|
| Phenoloxidase (unit/min/g.b.wt)* | 94.00±4.58 | 121.00±9.53 | - 4.419 | 0.023 |
| Peroxidase (unit/min/g.b.wt)* | 23.70±1.21 | 23.50±1.27 | 0.223 | 0.835 |
| α-Esterases (µg α-naphthol/min/g.b.wt)* | 545.30±17.47 | 434.70±13.86 | 8.592 | 0.001 |
| β-Esterases (µg β-naphthol/min/g.b.wt)* | 144.70±13.8 | 139.70±8.38 | 0.536 | 0.626 |

*g.b.wt= gram of body weight, Control= untreated cowpea seeds.

Values are means of three replicates ± SD. Data in each row are examined to independent t-test and analysis of variance at $p<0.05$

activity significantly ($p<0.05$) increased in ozone treated cowpea adult insects compared with control cowpea adult insects, which recorded 121.00 and 94.00 units/g for ozone treated and control, respectively. The results showed that there was a difference between control and ozone treatments in phenol oxidase and this may be due to the insect's exposure to oxidizing factors under the influence of the ozone gas stream or may be due to the damage of insect's body, that tried to overcome by secreting higher amount of enzymes to compensate of body damage. Phenolases are responsible for the hardness and darkening of the cuticle and the darkening of all damaged tissues. The results indicated that the peroxidase, α-esterases and β-esterases of control cowpea adult beetles had higher activities than ozone treated beetles and recorded 23.70 and 23.50 unit/min/g.b.wt for peroxidase enzyme, 545.30 and 434.70 µg α-naphthol/min/g.b.wt for α-esterases enzyme and 144.70 and 139.70 µg β-naphthol/min/g.b.wt for β-esterases enzyme. Cadenas [33] and Gacar and Tasksn [10] mentioned that, if the insect is exposed to an external effect, it loses the ability to perform some of its biological functions, which results a change in its enzymes. Besides, the organisms have evolved antioxidant enzymes that catalyze the removal of reactive oxygen species, thus preventing tissue damage. Esterases are one of the main detoxifying insect enzymes and are involved in the detoxification of insecticides. Ozone gas is a strong oxidant, reactive to biomolecules and has a toxic effect on insect pests [34]. Sousa *et al.* [35] found that *Tribolium castaneum* was susceptible to ozone gas. Likewise, insecticide toxicity was associated with biochemical defense mechanisms in insect populations.

**Physicochemical and Cooking Quality of Cowpea Seeds**

**Scanning Electron Microscopy:** The shape and size of granules affect their functional properties which are needed for food and industrial uses. Figure 1 shows the images of cowpea obtained by the Scanning Electron Microscopy (SEM) of control and after 6 h of ozone gas exposure. Data indicated that cowpea starch granules in control cowpea have relatively oval to kidney shape and the size varied between 12.06 and 16.90 µm in width (Figure 1a). Besides, there was a presence of some cracks in the starch surface in both samples. The granules of cowpea starch (Figure 1b) of ozone treatment (for 6 h of exposure), were slightly smaller in size (varied between 9.874 and 14.06 µm in width) compared with control cowpea. Abu *et al*. [36] mentioned that cowpea seed starch granules are made up of several shapes (oval to kidney).

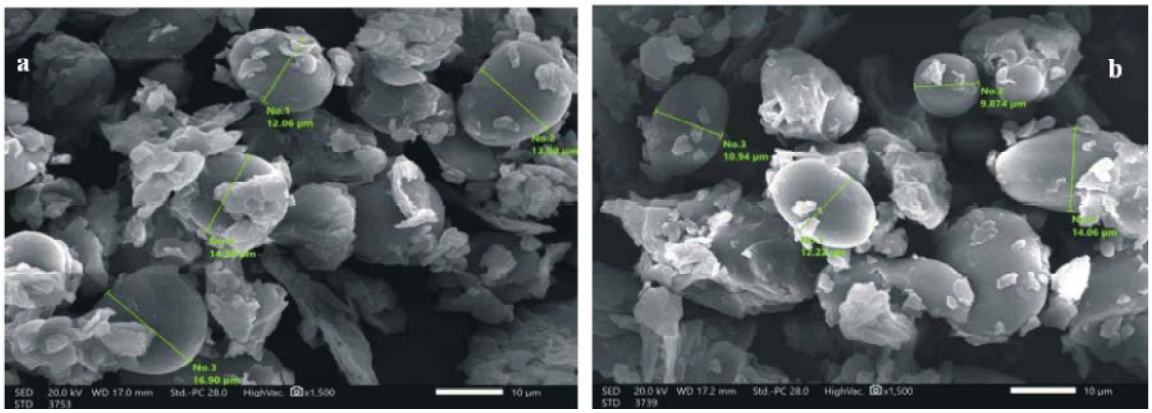


Fig. 1: Scanning electron micrographs (SEM) of control (a) and ozonated cowpea for 6 h (b) (1500 x magnification)

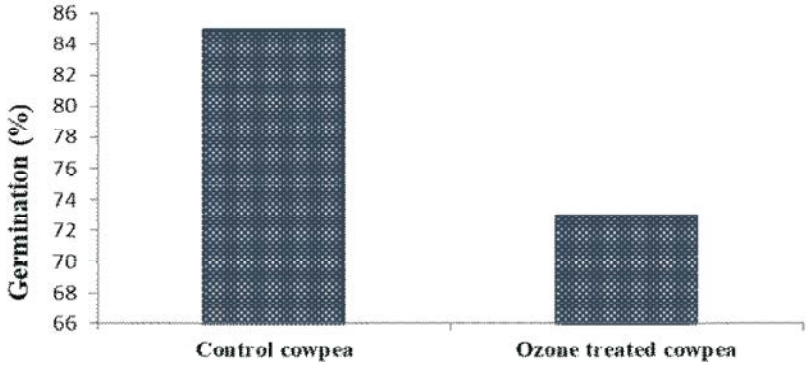


Fig. 2: Germination percentage of ozonated cowpea seeds for 6 h

Table 4: 1000-seeds weight and color parameters of ozonated cowpea seeds during storage

| | Zero time | | 4 weeks | | 8 weeks | | 12 weeks | |
|---|---|---|---|---|---|---|---|---|
| Parameters | Control | Ozonated | Control | Ozonated | Control | Ozonated | Control | Ozonated |
| 1000-seeds weight (g) | 144.90±0.13 | 136.30±0.10 | 143.70±.2.70 | 138.00±1.80 | 145.50±0.70 | 138.20±2.60 | 143.10±0.70 | 138.70±1.50 |
| t value | 9.617 | | 2.981 | | 4.606 | | 4.658 | |
| Sig. (2-tailed) | 0.001 | | 0.041 | | 0.010 | | 0.010 | |
| | | | | | ----------Color parameters---------- | | | |
| *L** | 68.37±1.98 | 69.02±1.32 | 68.50±1.03 | 68.85±0.93 | 68.16±1.89 | 67.98±0.29 | 69.14±1.66 | 67.87±1.88 |
| t value | -0.517 | | -0.429 | | 0.163 | | 0.878 | |
| Sig. (2-tailed) | 0.633 | | 0.690 | | 0.879 | | 0.430 | |
| *a** | 4.35±1.02 | 4.47±0.24 | 4.34±0.10 | 4.68±0.30 | 4.75±0.37 | 4.96±0.51 | 4.87±0.11 | 5.10±1.22 |
| t value | -0.199 | | -1.846 | | -0.580 | | -0.324 | |
| Sig. (2-tailed) | 0.852 | | 0.183 | | 0.593 | | 0.762 | |
| *b** | 16.67±0.42 | 16.91±0.63 | 16.54±0.12 | 16.83±0.06 | 16.47±0.22 | 16.80±0.21 | 16.07±0.29 | 16.25±0.40 |
| t value | -0.558 | | -3.702 | | -1.844 | | -0.623 | |
| Sig. (2-tailed) | 0.607 | | 0.035 | | 0.139 | | 0.576 | |

Control= untreated cowpea seeds. Ozonated= cowpea seeds exposure to ozone gas for 6 h. Values are means of three replicates ± SD.
Data in each row are examined to independent t-test and analysis of variance at $p<0.05$. L*= Lightness, a*= redness and b*= yellowness.

**Germination of Cowpea Seeds:** Figure 2 shows the germination percentage of control and ozonated cowpea seeds after exposure for 6 h. The percentage of germination was higher in the control sample (85.00%) compared with ozonated seeds, which recorded 73.00%. Gad *et al*. [37] indicated that there was non-significant effect of ozone treatments on germination of cowpea seed compared with control seeds (untreated seeds). Mahroof and Amoah [38] mentioned that prolonged exposure to ozone gas decreased germination percentages.

**1000-Seeds Weight and Color Parameters of Cowpea Seeds:** Table 4 represents the 1000-seeds weight and color parameters of control and ozonated cowpea samples. From the results, it could be observed that exposure to ozone significantly decreased ($p$<0.05) the 1000-seeds weight as a response to ozone treatment at all periods of storage (12 weeks). This reduction could be attributed to the lower moisture content. Mishra *et al.* [39] mentioned that the 1000-seeds weight of ozonated wheat grains decreased compared with untreated grains.

The same Table shows the color parameters of control and ozonated seeds. The color of the seed coat of legumes such as cowpea influences consumer acceptability. The exposure of cowpea seeds to ozone non-significantly ($p$>0.05) increased the lightness ($L$*), redness ($a$*) and yellowness ($b$*) at zero time (from 68.37 to 69.02 from 4.35 to 4.47 and from 16.67 to 16.91, respectively). Marston *et al.* [40] found that ozone gas has the ability to decolorize some food components by oxidizing pigments in food. Weiwei and Xueling [41] reported that food samples treated with ozone gas improved color by increasing lightness values.

**Proximate Analysis and *In vitro* Protein Digestibility:** The relationship between ozone treatment and nutrient changes should be known in order to assess the acceptability of treated seeds. The chemical composition of cowpea samples after 6 h of exposure to ozone is presented in Table 5. The data for ozonated samples, showed a decrease in moisture, protein and fat contents, while the contents of fiber, ash and total carbohydrates increased compared with control samples. The results revealed that there was a decrement in moisture and fat contents and this may be due to the oxidation by ozone. Crude fiber content was higher in ozonated cowpea seeds than control. Tiwari *et al.* [12] mentioned that ozone gas has a slight effect on the physicochemical characteristics. Gad *et al.* [37] found a minor decrease in moisture, protein and fat contents and minor increase in ash and fiber contents, compared with control cowpea seeds. The reduction in protein content after ozone gas treatment could be attributed to the degradation of the protein through oxidation by ozone gas [39].

From the results in Table 5, the minerals content decreased in the cowpea seeds treated with ozone gas and recorded 5.54, 1.66, 47.41, 1400.00 and 341.72 mg/100g for iron, zinc, calcium, potassium and phosphorus, respectively compared with control seeds which recorded 6.92, 2.06, 51.81, 1485.71 and 366.17 mg/100g, respectively. The reduction percentage of minerals was 19.94, 19.42, 8.49, 5.77 and 6.68% for iron, zinc, calcium, potassium and phosphorus, respectively.

The same Table shows the contents of total phenols and antioxidant activity of cowpea seeds before and after ozone gas treatment. Total phenol contents and antioxidant activity of ozonated cowpea seeds were non-significantly ($p$>0.05) increased compared with the control. Jackowska *et al.* (2019) [42] found that the phenols content in rapeseed was decreased after ozone gas treatment. The ozone gas could provide a sufficient protection of stored cowpea seeds for controlling *C. maculatus* insects [43].

Concerning the data of protein digestibility (%), the digestibility of protein significantly ($p$<0.05) increased after ozone treatment compared with control samples and this may be due to the effect of ozone gas on macromolecules like protein. Besides, ozonation of seeds changes the morphology of protein, making it more digestible, which can be highly useful in the preparation of healthy food products [44].

**Cooking Quality:** Cooking quality of cowpea seeds during storage (12 weeks) was evaluated by determining the cooking time, water uptake ratio and total soluble solids (Table 6). The data revealed that cowpea treated with ozone showed a significant ($p$<0.05) decrease in cooking time than the control, which will save energy and time for processing. Besides, cooking time increased during the storage of cowpea seeds (ozonated and control samples). Water uptake ratio was lower for ozonated cowpea samples compared with control one. Total soluble solids (TSS) of cooked control seeds were found to be higher than ozonated seeds. The water uptake and TSS significantly decreased ($p$<0.05) after storage. Tiwari *et al.* [12] found that ozone gas treatment provides unique benefits for food seed processing. The proportions of chemical components such as carbohydrate and protein proportions influence seed cooking time [45].

**Sensory Evaluation:** Sensory acceptability scores of cowpea seeds in terms of color, taste, odor, texture and overall acceptability are represented in Table (7). An independent t-test was conducted to compare the effect of ozone treatment (exposure to ozone gas for 6 h) before and after storage periods (12 weeks) of seeds with control cowpea. Data revealed that ozone treatment was non-significantly ($p$>0.05) affect the color, taste, odor, texture and overall acceptability of cowpea seeds.

Table 5: Proximate analysis and *in vitro* protein digestibility of ozonated cowpea seeds

| Characteristics | Control | Ozonated | t value | Sig. (2-tailed) |
|---|---|---|---|---|
| Chemical composition (%)* | | | | |
| Moisture | 9.29±0.18 | 8.53±0.02 | -1.109 | 0.000 |
| Protein | 27.61±0.05 | 27.58±0.05 | 0.737 | 0.502 |
| Fat | 1.74±0.16 | 1.34±0.08 | 46.820 | 0.000 |
| Crude fiber | 3.42±0.02 | 3.43±0.03 | -1.109 | 0.330 |
| Ash | 4.38±0.07 | 4.44±0.06 | -1.050 | 0.353 |
| Total carbohydrates | 62.85±0.02 | 63.21±0.07 | -39.061 | 0.000 |
| Minerals (mg/100g) | | | | |
| Iron | 6.92±0.04 | 5.54±0.03 | 47.805 | 0.000 |
| Zinc | 2.06±0.18 | 1.66±0.15 | 2.945 | 0.042 |
| Calcium | 51.81±4.81 | 47.41±2.77 | 1.176 | 0.305 |
| Potassium | 1485.71±8.90 | 1400.00±7.50 | -12.755 | 0.000 |
| Phosphorus | 366.17±11.38 | 341.72±14.86 | 2.262 | 0.086 |
| Total phenols (mg/100g) | 63.64±1.55 | 64.96±1.39 | -1.112 | 0.952 |
| Antioxidant activity (%)** | 36.78±1.19 | 37.31±0.85 | -0.633 | 0.561 |
| *In vitro* protein digestibility (%) | 65.59±1.27 | 68.69±1.21 | -4.229 | 0.013 |

*Protein, fiber, fat ash and carbohydrate contents were calculated based on the dry weight basis.
**Antioxidant activity (radical scavenging activity) was measured as DPPH.
Values are means of three replicates ±SD. Data in each row are examined to independent t-test and analysis of variance at $p<0.05$

Table 6: Cooking quality of ozonated cowpea seeds during storage

| | Cooking time (min) | | Water uptake (g/g) | | TSS (%) | |
|---|---|---|---|---|---|---|
| Storage (weeks) | Control | Ozonated | Control | Ozonated | Control | Ozonated |
| 0 time | 55±0.50 | 40±0.50 | 1.19±0.04 | 1.07±0.02 | 9.27±0.17 | 9.18±0.23 |
| t value | 36.742 | | 274.780 | | 151.810 | |
| Sig. (2-tailed) | 0.000 | | 0.000 | | 0.000 | |
| 4 weeks | 60±0.50 | 43±0.50 | 1.19±0.01 | 1.07±0.01 | 9.77±0.19 | 8.21±0.27 |
| t value | 41.641 | | 204.057 | | 1465.980 | |
| Sig. (2-tailed) | 0.000 | | 0.000 | | 0.000 | |
| 8 weeks | 65±0.50 | 46±0.50 | 1.18±0.03 | 1.08±0.04 | 10.09±0.24 | 8.26±0.14 |
| t value | 48.990 | | 242.499 | | 1734.095 | |
| Sig. (2-tailed) | 0.000 | | 0.000 | | 0.000 | |
| 12 weeks | 68±0.50 | 49±0.50 | 1.18±0.02 | 1.09±0.06 | 10.05±0.05 | 8.14±0.01 |
| t value | 51.439 | | 7.835 | | 65.644 | |
| Sig. (2-tailed) | 0.000 | | 0.001 | | 0.000 | |

*TSS= Total soluble solids after cooking.
Values are means of three replicates ± SD. Data in each column are examined to independent t-test and analysis of variance at $p<0.05$

Table 7: Sensory evaluation of ozonated cowpea seeds during storage

| | 0 time | | 4 weeks | | 8 weeks | | 12 weeks | |
|---|---|---|---|---|---|---|---|---|
| Parameters | Control | Ozonated | Control | Ozonated | Control | Ozonated | Control | Ozonated |
| | | | | Color | | | | |
| | 8.60±0.50 | 8.65±0.34 | 8.60±0.52 | 8.55±0.44 | 8.60±0.42 | 8.55±0.43 | 8.60±0.52 | 8.60±0.39 |
| t value | -0.256 | | 0.234 | | 0.234 | | 0.000 | |
| Sig. (2-tailed) | 0.801 | | 0.818 | | 0.818 | | 1.000 | |
| | | | | Taste | | | | |
| | 8.60±0.39 | 8.50±0.47 | 8.50±0.47 | 8.40±0.30 | 8.50±0.47 | 8.30±0.42 | 8.40±0.40 | 8.45±0.55 |
| t value | 0.514 | | 0.514 | | 1.000 | | -0.234 | |
| Sig. (2-tailed) | 0.613 | | 0.613 | | 0.331 | | 0.818 | |
| | | | | Odor | | | | |
| | 8.50±0.47 | 8.45±0.70 | 8.50±0.47 | 8.60±0.51 | 8.40±0.39 | 8.30±0.42 | 8.50±0.47 | 8.40±0.52 |
| t value | 0.183 | | -0.452 | | 0.548 | | 0.452 | |
| Sig. (2-tailed) | 0.857 | | 0.656 | | 0.591 | | 0.656 | |
| | | | | Texture | | | | |
| | 8.50±0.33 | 8.80±0.42 | 8.40±0.39 | 8.45±0.55 | 8.40±0.61 | 8.55±0.60 | 8.25±0.26 | 8.50±0.41 |
| t value | -1.765 | | -0.234 | | -0.553 | | -1.627 | |
| Sig. (2-tailed) | 0.095 | | 0.81 | | 0.587 | | 0.121 | |
| | | | | Overall acceptability | | | | |
| | 8.60±0.39 | 8.75±0.35 | 8.50±0.47 | 8.40±0.39 | 8.40±0.51 | 8.30±0.42 | 8.30±0.42 | 8.40±0.39 |
| t value | -0.896 | | 0.514 | | 0.474 | | -0.548 | |
| Sig. (2-tailed) | 0.382 | | 0.613 | | 0.641 | | 0.591 | |

Values are means of ten replicates ± SD. Data in each row are examined to independent t-test and analysis of variance at $p<0.05$

The overall acceptability of ozonated cowpea seeds was slightly higher than the control. During storage (12 weeks), there were non-significant ($p$>0.05) changes in sensory attributes of ozonated and control cowpea seeds. This indicates that even though cowpea seeds were treated with ozone, sensory attributes of cowpea seeds were acceptable.

## CONCLUSION

Ozone could be used as an effective fumigant to control cowpea insects. As ozone does not leave any residues after treatment, it could be considered as a convenient fumigant for food products. The insect eggs were highly tolerant to ozone gas followed by adult and larvae stages. The ozone decreased the activity of insect enzymes (peroxidase, α-esterases and β-esterases) in adult insects, while phenoloxidase increased in ozone treated insects compared with control. Ozone treatment decreases moisture, protein, fat, carbohydrates and minerals contents, while it increases fiber, ash and *in vitro* protein digestibility compared with control cowpea seeds. The sensory characteristics of cowpea seeds were not influenced after ozone treatment. Besides, ozone treatment reduced the cooking time and total soluble solids of the cowpea seeds. Generally, it could be concluded that ozone treatments are beneficial treatment to control legume insects and to avoid using insecticides that may affect human health without any adverse effects on legumes technological characteristics and sensorial attributes.

## REFERENCES


1. Nedumaran, S., P. Abinaya, P. Jyosthanaa, B. Shraavya, P. Rao and C. Bantilan, 2015. Grain legumes production, consumption and trade trends in developing countries. Telangana: International Crops Research Institute for the Semi-Arid Tropics, ICRISAT Research Program Markets, Institutions and Policies, Working Paper Series No. 60, pp: 57.
2. FAO, 2020. Food and Agriculture Organization. FAOSTAT crop production data. Cowpea Production Quantity in Egypt. https://www.fao.org/faostat/en/#data/QCL
3. USDA, 2021. United States Department of Agriculture. Food Data Central. https://fdc.nal.usda.gov/ (accessed June 11, 2021).
4. Affrifah, N.S., R.D. Phillips and F.K. Saalia, 2021. Cowpeas: nutritional profile, processing methods and products - a review. Legume Sci., 4(3): e131.
5. Tuda, M., K. Kagoshima, Y. Toquenaga and G. Arnqvist, 2014. Global genetic differentiation in a cosmopolitan pest of stored beans: effects of geography, host-plant usage and anthropogenic factors. PLoS ONE 9: e106268.
6. Musa, A.K. and A.A. Adeboye, 2017. Susceptibility of some cowpea varieties to the seed beetle *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae). J. Agric. Sci., 62: 351-360.
7. Zibaee, A., A.R. Bandani and D. Malagoli, 2011. Purification and characterization of phenoloxidase from the hemocytes of *Eurygaster integriceps* (Hemiptera: Scutelleridae). Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol., 158: 117-123.
8. Qari, S.H., N.A.H. Abdelfattah and A.A. Shehawy, 2017. Assessment of DNA damage and biochemical responses in *Rhyzopertha dominica* exposed to some plant volatile oils. J. Pharm.Toxico., 12: 87-96.
9. Cerenius, L. and K. Soderhall, 2004. The prophenoloxidase-activating system in invertebrates. Immunol. Rev., 198: 116-126.
10. Gacar, F. and V. Tasksn, 2009. Partial base sequence analysis of MdaE7 gene and ali-esterase enzyme activities in field collected populations of house fly (*Musca domestica* L.) from Mediterranean and Aegean Regions of Turkey. Pestic. Biochem. Physiol., 94: 86-92.
11. McDonough, M.X., L.J. Mason and C.P. Woloshuk, 2011. Susceptibility of stored product insects to high concentrations of ozone at different exposure intervals. J. Stored Prod. Res., 47: 306-310.
12. Tiwari, B.K., C.S. Brennan, T. Curran, E. Gallagher, P.J. Cullen and C.P. O'Donnell, 2010. Application of ozone in grain processing. J. Cereal Sci., 51: 248-255.
13. Kouchesfahani, M.M., M. Alimohammadi, G. Jahed Khaniki, R. Nabizadeh Nodehi, Z. Aghamohseni, M. Moazeni and S. Rezaie, 2015. Antifungal Effects of ozonated water on *Aspergillus parasiticus*: a new approach to prevent wheat contamination. J. Food Saf., 35: 295-302.
14. Savi, G.D., K.C. Piacentini and V.M. Scussel, 2015. Reduction in residues of deltamethrin and fenitrothion on stored wheat grains by ozone gas. J. Stored Prod. Res., 61: 65-69.
15. FDA, U. 2001. United States Food and Drug Administration, Rules and Regulations, Part 173- secondary direct food additives permitted in food for human consumption (21 CFR Part 173 Authority: 21 USC. 321, 342, 348). Fed. Regist., Vol. 66, pp: 123.

16. Abdelfattah, N.A.H., A.R. Al-Qahtani and S.H. Qari, 2021. SCoT-marker analysis of *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae) and stored date kernels of *Phoenix dactylifera* (L.) fumigated with ozone and phosphine gases. J. Asia-Pacific Entom., 24(3): 843-849.
17. Henderson, C.F. and E.W. Tilton, 1955. Tests with acaricides against the brow wheat mite. J. Econ. Entomol., 48: 157-161.
18. Ishaaya, I., 1971. In the armored scale *Aonidiella aurantii* and observation on the phenoloxidase system *Chrysomphalus aonidum*. Comp. Biochem. Physiol., 39 B: 935-943.
19. Vetter, J.L., M.P. Steinberg and A.1. Nelson, 1958. Quantitative determination of peroxidaese in sweet corn. Agric. Food Chem., 6(1): 39-41.
20. Van Asperen, K., I962. A study of house fly esterase by means of sensitive colorimetric method. J. Insect Physiol., 8: 401-416.
21. Abdelfattah, N.A.H. and M.M.A. Gnedy, 2021. Preparation of some safe synthetic botanical materials as fumigant tablets as alternatives for hazard phostoxin for controlling cowpea beetle *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) infesting leguminaceous seeds. Egypt. J. Plant Prot. Res. Inst., 4(4): 545-553.
22. AACC, 2002. Approved Method of American Association of Cereal Chemists. Approved Methods of AACC Published by the American Association of Cereal Chemists. 13th. Ed, St. Paul, Inc. Minnesota.
23. AOAC, 2019. Official Methods of Analysis of Association of Official Analytical Chemists international. Latimer, G. (Ed.), 21th ed., Association of Official Analytical Chemists, Washington, DC, USA.
24. Trough, E. and A.H. Mayer, 1929. Improvement in the deingess calorimetric method for phosphorus and areseni. Indian Eng. Chem. Annual Ed., 1: 136-139.
25. Singleton, V.L. and J.A. Rossi, 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic., 16: 144-158.
26. Brand-Williams, W., M.E. Cuvelier and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Tech., 28: 25-30.
27. Akeson, W.R. and M.A. Stahmann, 1964. Pepsin pancreatin digest index of protein quality evaluation. J. Nutr., 83: 257-261.
28. Wani, I.A., D.S. Sogi and B.S. Gill, 2013. Physical and cooking characteristics of black gram (*Phaseolus mungoo* L.) cultivars grown in India. Inter. J. Food, 48: 2557-2563.
29. Larmond, E., 1977. Laboratory methods for sensory evaluation of food. Research Branch Canadian Department of Agriculture Publication, 1637: 56-59.
30. Bakr, E., 2000. LdP line software. Software to calculate probit analyses used to illustrate the relation between stimulus and response in toxicological and biological studies. http://www.ehabsoft.com/ldpline/ http://www.ehabsoft.com/
31. Elliott, A.C. and W.A. Woodward, 2007. Statistical analysis quick reference guide book. With SPSS examples. 1st Edition, SAGE Publications, Inc., pp: 280.
32. Gad, H.A., T.M. Sileem, R.S. Hassan and S.A.M. Abdelgaleil, 2021. Toxicity of gaseous ozone to the different life stages of cowpea beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae), under laboratory conditions. Hellenic Plant Protect. J., 14: 31-38.
33. Cadenas, E., 1989. Biochemistry of oxygen toxicity. Annual Rev. Biochem., 58: 79-110.
34. Ahmadi, A.S.S., R.A. Ibrahim and S.A. Ouf, 2009. Application of ozone to control insect pests and moulds of date fruits. Biosci. Biotech. Res. Asia, 6(2): 435-446.
35. Sousa, A.H., L.R.D. Faroni, R.N.C. Guedes, M.R. Tótola and W.I. Urruchi, 2008. Ozone as a management alternative against phosphine-resistant insect pests of stored products. J. Stored Products Res., 44: 379-385.
36. Abu, J.O., K.G. Duodu and A. Minnaar, 2006. Effect of γ-irradiation on some physicochemical and thermal properties of cowpea (*Vigna unguiculata* L. Walp) starch. Food Chem., 95(3): 386-393.
37. Gad, H.A., G.F. Abo Laban, K.H. Metwaly, F.S. Al-Anany and S.A.M. Abdelgaleil, 2021. Efficacy of ozone for *Callosobruchus maculatus* and *Callosobruchus chinensis* untreated in cowpea seeds and its impact on seed quality. J. Stored Prod. Res., 92(4): 101786-101796.
38. Mahroof, R.M. and B.A. Amoah, 2018. Toxic effects of ozone on selected stored product insects and germ quality of germinating seeds. In: Proceedings of the 12th International Working Conference on Stored Product Protection, Berlin, Germany 7-11October 2018, pp: 591-595.
39. Mishra, G., A. Palle, S. Srivastavaa and H.N. Mishra, 2019. Disinfestation of stored wheat grain infested with *Rhyzopertha dominica* by ozone treatment: process optimization and impact on grain properties. J. Sci. Food Agric., 99: 5008-5018.

40. Marston, K., H. Khouryieh and F. Aramouni, 2015. Evaluation of sorghum flour functionality and quality characteristics of gluten-free bread and cake as influenced by ozone-treatment. Food Sci. Tech. Inter., 21: 631-640.
41. Weiwei, X. and Z. Xueling, 2008. Effects of ozone treatment on color of wheat flour. Cereal Feed Indus., 5: 7-9.
42. Jackowska, I., M. Bojanowska, M. Staszowska-Karkut and M. Sachadyn-Krol, 2019. Low concentration short time ozonation of rapeseed seeds reduces the stability of the oil and content of some antioxidant components. International J. Food Sci. Tech., 54: 3175-3184.
43. Hansen, J., M. Sato and R. Ruedy, 2012. Perception of climate change. Proceed. Natural Acad. Sci., 109: 14726-14727.
44. Nickhil, C., D. Mohapatra, A. Kar, S.K. Giri, U.S. Verma, S. Muchahary, 2022. Gaseous ozone treatment of chickpea grains: Effect on functional groups, thermal behavior, pasting properties, morphological features and phytochemicals. J. Food Sci., 87: 5191-5207.
45. Henshaw, F.O., K.H. McWatters, O.A. Akingbala and M.S. Chinnan, 2003. Thermal properties of cowpea flour: a study by differential scanning calorimetry. Nahrung/Food, 47(3): 161-165.