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 F1 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...
activity and they may hydroxylate tyrosine as well as oxidize α-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 (O₃) 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 (F₁) 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 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:

\[
\text{Radical scavenging (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100
\]

\(A_0\) = 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.

**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].

**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).**
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:

\[
\text{Total soluble solids (TSS)\%} = \frac{\text{Weight of residue (g)}}{\text{Initial weight of seeds (g)}} \times 100
\]

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

\[
\text{Water uptake after cooking (g/g)} = \frac{(\text{Weight of cooked seeds} - \text{Initial weight of seeds})}{\text{Initial weight of seeds}}
\]

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

<table>
<thead>
<tr>
<th>Stages</th>
<th>Adults</th>
<th>Eggs</th>
<th>Larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure time (hour)</td>
<td>Mortality (%)</td>
<td>F of progeny*</td>
<td>Reduction (%)</td>
</tr>
<tr>
<td>Zero h (control)</td>
<td>0.00 ±0.00</td>
<td>423.67±22.36</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>37.33 ±4.16</td>
<td>38.00±6.16</td>
<td>91.00a</td>
</tr>
<tr>
<td>4</td>
<td>76.67 ±4.16</td>
<td>26.30±4.16</td>
<td>93.73b</td>
</tr>
<tr>
<td>6</td>
<td>100.00±0.00</td>
<td>0.33±0.57</td>
<td>99.90c</td>
</tr>
<tr>
<td>F value</td>
<td>671.83</td>
<td>812.72</td>
<td>5055.60</td>
</tr>
</tbody>
</table>

*F = 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

<table>
<thead>
<tr>
<th>Confidence limits of LT 95%</th>
<th>LT 95%</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5</td>
<td>8.11</td>
<td>2.11</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>10.01</td>
<td>2.82</td>
</tr>
</tbody>
</table>

Slope ± SD | Chi square X²
3.20±0.46 | 0.31

*LT= lethal times.

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

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

*µg.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

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

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control</th>
<th>Ozonated</th>
<th>t value</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>9.29±0.18</td>
<td>8.53±0.02</td>
<td>-1.109</td>
<td>0.000</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>27.61±0.05</td>
<td>27.58±0.05</td>
<td>0.737</td>
<td>0.502</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>1.74±0.16</td>
<td>1.34±0.08</td>
<td>46.820</td>
<td>0.000</td>
</tr>
<tr>
<td>Crude fiber (%)</td>
<td>3.42±0.02</td>
<td>3.43±0.03</td>
<td>-1.109</td>
<td>0.330</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>4.38±0.07</td>
<td>4.44±0.06</td>
<td>-1.050</td>
<td>0.353</td>
</tr>
<tr>
<td>Total carbohydrates (%)</td>
<td>62.85±0.02</td>
<td>63.21±0.07</td>
<td>-39.061</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Storage (weeks)</th>
<th>Cooking time (min)</th>
<th>Water uptake (g/g)</th>
<th>TSS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Ozonated</td>
<td>Control</td>
</tr>
<tr>
<td>0 time</td>
<td>55±0.50</td>
<td>40±0.50</td>
<td>1.19±0.04</td>
</tr>
<tr>
<td>t value</td>
<td>36.742</td>
<td>0.000</td>
<td>274.780</td>
</tr>
<tr>
<td>4 weeks</td>
<td>60±0.50</td>
<td>43±0.50</td>
<td>1.19±0.01</td>
</tr>
<tr>
<td>t value</td>
<td>41.641</td>
<td>0.000</td>
<td>204.057</td>
</tr>
<tr>
<td>8 weeks</td>
<td>65±0.50</td>
<td>46±0.50</td>
<td>1.18±0.03</td>
</tr>
<tr>
<td>t value</td>
<td>48.990</td>
<td>0.000</td>
<td>242.499</td>
</tr>
<tr>
<td>12 weeks</td>
<td>68±0.50</td>
<td>49±0.50</td>
<td>1.18±0.02</td>
</tr>
<tr>
<td>t value</td>
<td>51.439</td>
<td>0.000</td>
<td>7.835</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>0 time</th>
<th>4 weeks</th>
<th>8 weeks</th>
<th>12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t value</td>
<td>8.60±0.50</td>
<td>8.65±0.34</td>
<td>8.60±0.52</td>
<td>8.65±0.44</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.801</td>
<td>0.818</td>
<td>0.234</td>
<td>0.234</td>
</tr>
<tr>
<td>Taste</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t value</td>
<td>8.60±0.39</td>
<td>8.50±0.47</td>
<td>8.50±0.47</td>
<td>8.40±0.30</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.514</td>
<td>0.613</td>
<td>0.514</td>
<td>1.000</td>
</tr>
<tr>
<td>Odor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t value</td>
<td>8.50±0.47</td>
<td>8.45±0.70</td>
<td>8.50±0.47</td>
<td>8.60±0.51</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.183</td>
<td>0.857</td>
<td>0.452</td>
<td>0.548</td>
</tr>
<tr>
<td>Texture</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t value</td>
<td>8.50±0.33</td>
<td>8.40±0.42</td>
<td>8.40±0.39</td>
<td>8.45±0.55</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.095</td>
<td>0.81</td>
<td>-0.234</td>
<td>-0.553</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>8.60±0.39</td>
<td>8.75±0.35</td>
<td>8.50±0.47</td>
<td>8.40±0.39</td>
</tr>
<tr>
<td>t value</td>
<td>0.382</td>
<td>0.613</td>
<td>0.514</td>
<td>0.474</td>
</tr>
</tbody>
</table>

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 larval stages. The ozone decreased the activity of insect enzymes (peroxidase, $\alpha$-esteras and $\beta$-esteras) 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 $\textit{in vitro}$ 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**


