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Effects of Modified Atmospheres and Ozone on *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae), Quality of Wheat Flour and Safety of Wheat Grains to Rats

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Abstract: This study aimed to evaluate different modified atmospheres (MAs) and ozone (O_3) on the adult survival and larval development of *Sitophilus oryzae* (L) and *Tribolium castaneum* (Herbst) in addition to quality analyses of wheat flour and safety of wheat grains to rats, at 30±1°C, 65 ±5% RH. Adults and larvae were exposed to two MAs containing CO_2 (40% CO_2, 12% O_2& 48% N_2 and 60% CO_2, 8% O_2& 32% N_2), two MAs containing N_2 (97% N_2& 3% O_2 and 98% N_2& 2% O_2) and O_3 (500 ppm). Flour and grains of wheat were exposed to MAs containing 60% CO_2 and 98% N_2 and 500 ppm O_3 to determine the quality analysis of wheat flour and safety of wheat grains to rats. The MA containing 60% CO_2 is suitable for controlling adults and larvae of *S. oryzae* and *T. castaneum* in short time. Latent effect of O_3 (7 days after exposure) was more preferable for controlling adults and larvae of both *S. oryzae* and *T. castaneum* than only one day after exposure. MAs containing 60% CO_2 and 500 ppm O_3 did not affect the milling and baking qualities of wheat flour. Rats that were fed wheat grains exposed to MAs and O_3 grew normally without any significant alterations in body weights, internal organ weights (brain, kidney and liver) and biochemical analyses (total protein, alkaline phosphatase, and total lipids). It could be concluded that MAs containing 60% CO_2 for 5 days and 500 ppm ozone for 7 days are adequate to eliminate the adults and larvae of *S. oryzae* and *T. castaneum* from stored wheat grains and their products. Also, they did not have adverse effects on the quality of wheat flour and grains.

Key words: Insect Survival · Integrated Pest Management · Larval Development · Reduced Risk Management · Stored Product

INTRODUCTION

Cereal grains are often infested by various stored-product insects, which cause severe economic losses [1]. For example, food product commodities can be affected by insect pests during the storage period, and visible contamination due to insect individuals or their remains may be present in the final product [2]. There are increasing restrictions on chemical compounds registered for pest control and on the maximum residue levels (MRLs) allowed in the final food products. Therefore, it is necessary to implement alternative strategies for the control of insects in stored products. Modified atmospheres (MAs) and ozone (O_3) are safe and environmentally friendly alternatives to control pests in stored products.

Modified atmospheres have an important role in integrated pest management (IPM) systems that emphasize treatment without leaving pesticide residues [3]. Carbon dioxide (CO_2) and nitrogen (N_2) are currently used as eco-friendly alternative gases to fumigants for the control of insects in stored grain. Carbon dioxide is absorbed and desorbed easily without chemical reaction by various grains, and it has almost no effect on grain quality. Many researchers have confirmed the effectiveness of atmosphere control using either CO_2 or

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N_2 for insect control [4]. Modified atmospheres have been used for disinfesting raw or semi-processed food products, such as cereal grains and dried fruits, while still in storage. Treatments based on reduced oxygen (O_2) and high CO_2 or N_2 contents are technically suitable alternatives for arthropod pest control in durable commodities [[5 - 8]. Atmospheres rich in CO_2, with more than 40% in the air, are faster at controlling pests than those with high contents of N_2 [7]. Data on the effects of different types of CO_2 treatments and dosages on key pests are available for many species and stages of stored-product pests under particular sets of conditions [3, 9, 10]. Depending on the temperature, CO_2 treatments may take from a few days to several weeks to be effective in gas-tight chambers or silos [11]. The toxicity of CO_2 to insects is known to vary among species, developmental stages and age groups. Parameters of the physical environment, such as temperature, humidity, and CO_2 levels in storage, also influence toxicity. In the majority of studies involving CO_2, much attention has been focused on determining the time required to kill insect pests [12, 13].

Ozone (O_3) is an unstable molecule with a half-life of 20 – 50 min [14] that can be generated locally, which eliminates the requirements for its handling, storage, and transport. O_3 is highly reactive and a strong oxidizing agent, and is classified as "GRAS" (Generally Recognized as Safe) by the United States Environmental Protection Agency (US-EPA). Throughout the world, O_3 has been used to purify drinking water, kill bacteria, sanitize food, deodorize, and decrease aflatoxin contamination [15 - 18]. One advantage of O_3 is that it breaks down into atmospheric oxygen, eliminating the need to store or dispose of hazardous chemicals. In addition to this information, the enrichment of the atmosphere with O_3 is recognized as an important alternative for the control of stored products pests [14, 19 - 21]; this is because pests of stored products do not show cross-resistance between phosphine and O_3. Additionally, O_3does not leave a residue in the grain because O_2 is the degradation product [20, 22]. Ozone is a gas that is derived from the rearrangement of oxygen atoms that occurs during electrical discharges or from exposure to high-energy electromagnetic radiation (ultraviolet light) in the atmosphere [23, 24].

Several studies have established that ozone treatments can kill stored grain insects, including maize weevil, *Sitophilus zeamais* (Motsch), (Coleoptera: Cuculionidae) rice weevil *Sitophilus oryzae* (L) (Coleoptera: Cuculionidae), red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), confused flour beetle *Tribolium confusum* (Jacqueline duVal) (Coleoptera: Tenebrionidae), Indian meal moth *Plodia interpunctella* (Hübner) (Lepidoptera: Phycitidae) and Mediterranean flour moth *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae:) [14, 19, 22, 25, 26].

The objective of this research was to evaluate efficacy of CO_2, N_2 and O_3 against two stored-product insect species, *S. oryzae* and *T. castaneum*. Additionally, effects of CO_2, N_2 and O_3 on the quality of treated food were assessed. *Sitophilus oryzae* was chosen as a model for primary insect pests on cereal grain, and *T. castaneum* as a model for secondary insect pests which infest the cereal products. In this study, the effects of two concentrations of CO_2 (40% and 60%), two concentrations of N_2 (97% and 98%) and two exposure times of 500 ppm O_3 (1 day and 7 days) were evaluated for their efficacy against adult insects and their ability to reduce adult emergence from exposed larvae of *S. oryzae* and *T. castaneum*.

MATERIALS AND METHODS

Insects: *Sitophilus oryzae* was obtained from a stock culture maintained at the Stored Grain and Product Pests Department, Plant Protection Research Institute. It was reared on whole wheat grains at 30 ± 1°C and 65 ±5 R.H. in continuous darkness. *Tribolium castaneum* was obtained from a stock culture maintained at the Laboratory of Modified Atmospheres, Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University. It was reared on wheat flour under the same conditions used in rearing of *S. oryzae*. Adults (0- 7 days old) and the larvae of *S. oryzae* and *T. castaneum* (20 days old) were used in the experiments.

The Effect of Modified Atmospheres (MAs) on *S. oryzae* and *T. castaneum*: Adults and larvae of *S. oryzae* and *T. castaneum* were exposed to two different concentrations of CO_2 in air and two concentrations of N_2. at 30±1°C, 65 ±5% r.h. and different exposure periods. The MAs containing CO_2 were MA1 containing 40% CO_2, 12% O_2 and 48% N_2 and MA2 comprising 60% CO_2, 8% O_2 and 32% N_2. The MAs containing N_2 were MA3 containing 97% N_2 and 3% O_2 and MA4 with 98% N_2 and 2% O_2. The exposure periods of the tested MAs lasted for 3–5, 5–10, 2–7 days for *S. oryzae* adults, *S. oryzae* larvae and *T. castaneum* larvae and adults, respectively.

Adults and larvae of *T. castaneum* were separated from the culture with a camel hair paint brush and carefully transferred to glass tubes (1×5 cm) contained 2 g of wheat flour. Adults of *S. oryzae* were obtained from culture diets by sieving and were carefully transferred to glass tubes which contained 2 g of wheat grains. Twenty adults for each insect species or 20 larvae of *T. castaneum* were put into a glass tubes. Whereas larvae of *S. oryzae* were treated by exposing wheat grains encountering larvae 20 days after artificial infestation by eggs. Approximately 5 g of wheat grains infested by larvae obtained from the stock culture were exposed to the MAs treatments. Kernels infested by larvae were placed into the tubes. The tubes were closed using cloth and rubber bands to hold cloth material in tubes in place. Tubes were introduced into the Dreshel flask for MA treatment according the method described by Hashem *et al*. [27]. Carbon dioxide or O_2 concentrations were measured at the beginning and the end of exposure periods to check that there was no significant variation in the gas level during exposure. Dreshel flasks were transferred to incubators adjusted to constant temperatures of 30 ± 1°C and 65 ± 5% r.h.

At the end of the tested exposure periods, Dreshel flasks were aerated, and the adults and larvae of *T. castaneum* and adults of *S. oryzae* were counted to determine level of mortality. Treated grains infested by *S. oryzae* larvae were transferred to glass jars (5 cm in diameter and 15 cm high) covered with muslin cloth then incubated under the same optimum conditions and examined daily until the emergence of adult stopped (10 – 15 days). Due to the difficulty of detecting larvae inside the grains, the percentage of reduction in adult emergence was calculated according to the formula of Henderson and Tilton [28]. All the MA treatments were replicated three times; there were three replicates for the untreated control as well.

The Effect of Ozone (O_3) on *S. oryzae* and *T. castaneum*: Ozone gas was produced from air using an ozone generator Model OZO 6 VTTL OZO Max Ltd, Shepherd, Quebec Canada (OZO Max Ltd, Shefford, Quebec, Canada) from purified extra dry oxygen feed gas at the laboratory of Food Toxicology & Contaminants, National Research Centre. The amount of ozone output was controlled by a monitor- controller with a plug-in sensor on board which can be changed for different ranges of ozone concentration and a belt pan in the monitor-controller allows controlling the concentration.

The experiments were conducted using 12 jute bags: (ca: 0.5 kg each) each of 8 jute bags contained 20 g of wheat flour were artificially infested by 30 *T. castaneum* adults and larvae (4 jute bags for adults and 4 jute bags for larvae), other 4 jute bags contained 20 g from wheat grains artificially infested using 30 *S. oryzae* adults, then closed well and secured with rubber bands. All jute bags were exposed to 500 ppm O_3 at 0.5, 1, 2, 4 and 6 h. In the case of *S. oryzae* larvae, 80 g of wheat grains were put in glass jar and artificially infested using 200 *S. oryzae* adults for 48 h; adults were then removed and the glass jar was kept under experimental conditions. After 20 days from infestation, 80 g of wheat were divided to 4 parts inside 4 jute bags as replicates then closed well and secured with rubber bands and exposed to 500 ppm O_3. The control of this experiment was similar except there was no exposure to O_3. All bags were exposed to O_3 inside a glass container of 4-liter capacity as described by Omar [29] and Ismail *et al.* [30]. The glass container consisted of a glass jar with a short neck, closed with rubber stopper with 2 holes; one hole was for the ozone line and the other hole for tubing connected to the ozone destruct unit. There were four replicates for each treatment. Mortality (%) of *T. castaneum* (larvae and adults) and only *S. oryzae* adults were determined after 24 h and 7 days of exposure. In case of *S. oryzae* larvae, percent reduction in progeny emergence was determined according to Henderson and Tilton [28]. In the control treatment, there was no exposure O_3 gas.

The Effect of MAs and O_3 on the Quality Analysis of Wheat Flour

Baking Test: To determine the effect of CO_2, N_2 and O_3 on quality of treated wheat, 1 kg each of wheat flour was exposed to MA containing 60% CO_2, MA containing 98% N_2 for 15 days and 6 h of 500 ppm O_3. After exposure, flour samples were placed in plastic bags and stored at 4°C until used. Untreated wheat flour, control treatment, was not exposed to CO_2, N_2 and O_3. Wheat flour samples were all baked into toast bread at the Egyptian Baking Technology Center

Physical Properties of Toast Bread: The specific volume of bread was calculated according to the A.A.C.C. [31] by dividing volume (cm^3) by weight (g). Loaf volume was measured by rapeseed displacement immediately after removal from the oven and weighting. Loaves were placed in a container of known volume into which rapeseeds were run until the container was full. The volume of seeds displaced by the loaf was considered as the loaf volume.

Loaf Specific Volume (LSV) was calculated according to the following:

L.S.V = Loaf volume (cm^3) / Loaf weight (g) =cm^3 /g.

Sensory Evaluation of Toast Bread: The sensory evaluation of toast bread produced (fresh, MA enriched with 60% CO_2, MA enriched with 98% N_2 and 500 ppm O_3) was done as described by A.A.C.C. (2000) [31] using ten panelists from the Egyptian Baking Technology Center, Giza. The quality score of toast bread included color (20), texture (20), taste (20), flavor (20), general appearance (20) and overall acceptability was calculated (100). Scores: 90–100 very good (V.G), 80–89 Good (G), 70–79 Satisfactory (S), less than 70 questionably.

The Effect of Wheat Grains Exposed to MAs and O_3 on Rats: Eighty adult albino rats weighing 100–110 g were kept under normal laboratory conditions; each rat was in a separate cage. The rats were divided into four groups: the first group fed wheat grains exposed to MA containing 60% CO_2 for a month, the second group fed wheat grains exposed to MA containing 98% N_2 for a month, the third group fed wheat grains exposed to 500 ppm O_3 for a week and the fourth group was fed the untreated wheat grains. Each experimental group included five rats. Every group was repeated four times. The rats were weighed weekly to record the body weight. All groups were maintained for a month and then the rats were slaughtered. Following this, internal organs liver, kidney and brain were taken and weighed. The blood samples were taken and centrifuged to separate the sera those used in measuring alkaline phosphatase; total lipids and total protein [32 - 34].

Statistical Analysis: Mortality counts were corrected using Abbott's formula [35]. The data were then analyzed using the SPSS computing program using ANOVA, as described by Snedecor and Cochran [36]. Data on the effect of exposure periods on the tested insects were subjected to probit analysis, as described by Finney [37]. LT_{50} and LT_{95} values were calculated using the computer program developed by Noack and Reichmuth [38].

RESULTS

The Effect of Modified Atmospheres (MAs) on *S. oryzae*: Modified atmospheres containing 40% CO_2, 60% CO_2, 97% N_2 and 98% N_2 had significant effects on adults and larvae of *S. oryzae* (Table 1). The corrected mortality percentages indicate direct response of adults to MAs, while reduction percentages in adult emergence refer to the response of larvae that were inside the wheat grains. On the first day, no significant mortality was recorded in *S. oryzae* adults excepting MA containing 98% N_2 which recorded 8.33% mortality. On the subsequent days, the adult mortality significantly increased gradually by increase both of the exposure time and CO_2 or N_2 concentration. MAs containing 60% CO_2 and 98% N_2 were more effective on the adults than those containing 40% CO_2 and 97% N_2. Three days were adequate to kill all adults exposed to MA containing 60% CO_2, while 100% mortality was recorded after five days with the rest of the MAs. On the other hand, the larvae responded to MAs earlier than adults. The larvae exposed to MAs containing 40% CO_2, 60% CO_2 and 98% N_2 recorded significant reduction in adult emergence ranging from 36.45 to 57% on the first day excepting 97% N_2 that achieved weak adult reduction (8.79%). It was observed that the reduction in adult emergence significantly increased gradually with the increase of both exposure time and gas concentration like that happened in adult treatments. During the first two-days, MA containing 60% CO_2 was the most effective against the larvae followed by MAs containing 40% CO_2, 98% N_2 and 97% N_2. The larvae took longer time to be killed completely than adults. It took 5-6 and 8–10 days with MAs containing 60-40% CO_2 and 98-97% N_2, respectively to obtain 100% reduction in adult emergence.

The Effect of MAs on *T. castaneum*: Modified atmospheres containing 40% CO_2, 60% CO_2, 97% N_2 and 98% N_2 had significant effects on the adults and larvae of *T. castaneum* (Table 2). The adult mortality significantly increased gradually with increase in both the exposure time and CO_2 or N_2 concentration like that recorded in *S. oryzae* adults. *Tribolium castaneum* adults were more sensitive to MAs at high level. However, they were more tolerant to MAs at low levels. Complete mortality was achieved within two and three days in adults exposed to 98% N_2 and 60% CO_2, respectively. Exposure time increased to 7 days to attain 100% and 90% mortality in adults exposed to 40% CO_2 and 97% N_2, respectively. In larvae, it was observed that the MAs containing N_2 were more effective than those containing CO_2. The MA containing 98% N_2 was the most effective, where; it recorded 100% reduction in adult emergence after two days. To kill all larvae, 3 to 4 days were needed with MAs containing 97% N_2 and 60% CO_2. Some individuals of larvae had ability to adapt themselves with MA containing 40% CO_2. It was found that only 78.57% corrected mortality was recorded in the 7^{th} day.

Table 1: Corrected mortality percentages for adults and percentages reduction in adult emergence from larva of *Sitophilus oryzae* exposed to different modified atmospheres (MAs) combined with several exposure periods.

	Corrected mortality percentage (Mean ± SE)						Reduction percentage in adult emergence (Mean ± SE)					
	Adult stage						Larva stage					
	CO_2 (%)		N_2 (%)				CO_2 (%)		N_2 (%)			
Exposure Period (days)	40	60	97	98	*F*	*P*	40	60	97	98	*F*	*P*
1	00.00±0.00aA	00.00±0.00aA	00.00±0.00aA	8.33±1.66aB	25.00	<0.001	36.97±4.97aAb	57.81±5.63aB	8.79±4.83aA	36.45±16.73aAB	4.49	0.040
2	25.06±8.89bA	48.13±3.43bA	18.33±13.33aA	41.66±8.33bA	2.29	0.155	61.97±0.52bBC	84.37±5.63bC	19.90±10.15aA	55.72±12.11abB	10.13	0.004
3	68.73±14.93cAB	100.0±0.00cB	63.33±11.66bA	74.23±2.98cAB	2.86	0.104	88.02±6.40cA	96.35±2.08cA	70.83±15.27bA	70.31±6.50bcA	2.08	0.181
4	90.00±2.88cA	-	82.43±9.03bcA	98.33±1.66dA	2.04	0.21	97.91±2.08cdB	97.39±1.04cB	96.75±1.22cB	82.81±3.93cA	9.48	0.005
5	100.0±0.00cd	-	100.00±0.00c	100.0±0.00d			99.47±0.52dB	100.0±0.00cB	98.61±0.80cB	82.81±3.93cA	16.80	0.001
6							100.0±0.00dA	-	98.14±0.92cA	91.67±4.06cdA	2.67	0.162
7									98.14±0.92c	91.67±4.06cd		
8									96.29±2.01c	99.47±0.52d		
9									93.98±0.92c			
10									100.00±0.00c			
F	29.57	634.93	22.73	91.98	-	-	56.77	22.19	32.78	6.72		
P	<0.001	<0.001	<0.001	<0.001	-	-	<0.001	<0.001	<0.001	<0.001		

Means followed by the different letters are significantly different from each other at *P<0.05* (Duncan test).

Small letters indicate significant differences among days whereas capital letters indicate significant differences between MAs concentrations.

Table 2: Corrected mortality percentages for adults and larvae of *Tribolium castaneum* exposed to different modified atmospheres (MAs) combined with several exposure periods.

	Corrected mortality percentages (Mean ± SE)											
	Adult stage						Larva stage					
	CO_2(%)		N_2(%)				CO_2(%)		N_2(%)			
Exposure Period (days)	40	60	97	98	*F*	*P*	40	60	97	98	*F*	*P*
1	22.13±1.67aB	30.00±7.63aB	00.00±0.00aA	96.67±1.66aD	108.45	<0.001	00.00±0.00aA	37.96±4.35aB	10.00±5.00aA	10.00±5.00aA	15.49	0.001
2	51.06±9.67bB	98.33±1.66bC	5.00±5.00aA	100.00±8.33aC	67.22	<0.001	30.73±4.27bA	78.33±6.00bB	65.00±5.00bB	100.00±0.00cC	42.48	<0.001
3	70.00±10.40bB	100.0±0.00bC	16.17±8.09abA	-	26.43	0.002	42.83±2.17bA	98.33±1.67cB	100.00±0.00cB		424.97	<0.001
4	87.46±8.03bc	-	30.00±0.00b	-			71.67±7.26c	100.00±0.00c			15.21	
5	93.33±6.67c	-	60.00±2.89c	-			70.53±9.65c	-				
6	93.33±6.67c	-	81.67±6.67d	-			76.67±6.67c	-				
7	100.00±0.00cd	-	90.00±7.63d	-			78.57±9.36c	-				
F	15.75	78.31	47.03	4.00	-	-	20.45	57.57	86.64	324.00		
P	<0.001	<0.001	<0.001	0.116	-	-	<0.001	<0.001	<0.001	<0.001		

Means followed by the different letters are significantly different from each other at *P<0.05* (Duncan test).

Small letters indicate significant differences among days whereas capital letters indicate significant differences between MAs concentrations.

Comparative Lethal Times of *S. oryzae* and *T. castaneum* Exposed to MAs: The calculated LT_{50} and LT_{95} values with their confidence limits for adults and larvae of both *S. oryzae* and *T. castaneum* exposed to MAs containing 40% CO_2, 60% CO_2, 97% N_2 and 98% N_2 (Table 3 and Fig. 1). Both LT_{50} and LT_{95} for adults and larvae indicated that the most effective MAs were those containing 60% CO_2 (LT_{50} up to 2.01 days and LT_{95} up to 2.5 days) and 98% N_2 (LT_{50} up to 1.41 days and LT_{95} up to 2.09 days) on *S. oryzae* and *T. castaneum*, respectively. The MA containing 60% CO_2 had effective on adults and larvae of *T. castaneum* (LT_{50} up to 1.20 days and LT_{95} up to 2.75 days) close to MA containing 98% N_2. Both adults and larvae of *S. oryzae* were more sensitive to MA containing 98% N_2 than that containing 97% N_2 except some larvae may tolerate the atmosphere containing 98% leading to a LT_{95} that was long (10.32 days). The adults of *T. castaneum* were more sensitive to atmosphere with 60% CO_2 than the adults of *S. oryzae* while the larvae of both insect species exhibited similar responses to this MA.

The Efficacy of Ozone (O_3) on *S. oryzae*: Results present in Table (4) showed that corrected mortality % of *S. oryzae* adults increased with increasing exposure times to ozone gas as well as the days post treatment. Significant differences were observed between both exposure times whether after 1 and 7 days of ozone treatment and corrected mortality % of *S. oryzae* adults were 22.14 ± 1.3 and 95.71 % ± 1.84 % at 0.5 and 6 h injection time, respectively, after 1 day ozonation,

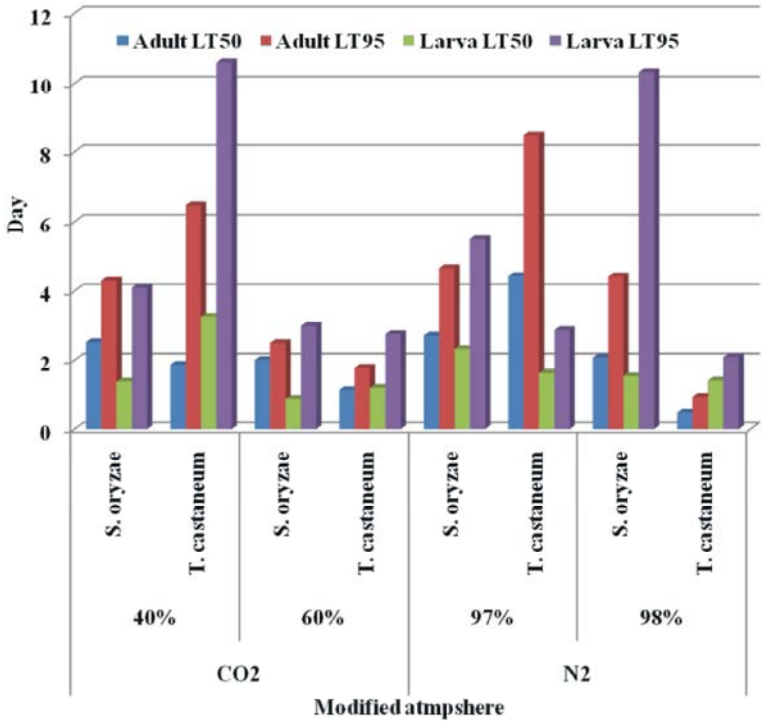

Fig. 1: LT_{50} and LT_{95} values for adults and larvae of *Sitophilus oryzae* and *Tribolium castaneum* exposed to modified atmospheres containing different concentrations of CO_2 or N_2.

Table 3: LT_{50} and LT_{95} values with their confidence limits for adults and larvae of *Sitophilus oryzae* and *Tribolium castaneum* exposed to modified atmospheres containing different concentrations of CO_2 or N_2.

				Confidence limits (d)					
				LT_{50}		LT_{95}			
Gas	Concentration (%)	LT_{50} (d)	LT_{95} (d)	Lower	Upper	Lower	Upper	Slop ± SE	Chi-square χ^2
Sitophilus oryzae adults									
CO_2	40	2.52	4.30	2.38	2.66	3.99	4.74	7.12±0.57	2.57
	60	2.01	2.50	1.93	2.08	2.33	2.94	17.32±3.75	2.13
N_2	97	2.71	4.66	2.56	2.86	4.31	5.15	7.01±0.56	6.26
	98	2.08	4.42	1.65	2.47	3.81	6.49	5.02±0.39	7.88
Sitophilus oryzae larvae									
CO_2	40	1.38	4.10	1.21	1.53	3.61	4.83	3.47±0.29	9.26
	60	0.88	2.99	0.7	1.04	2.62	3.56	3.09±0.32	1.04
N_2	97	2.32	5.48	1.74	2.80	4.71	7.76	4.42±0.25	65.65
	98	1.54	10.32	1.26	1.81	8.32	13.89	1.99±0.18	4.61
Tribolium castaneum adults									
CO_2	40	1.87	6.47	1.38	2.26	5.44	9.87	3.05±0.22	17.11
	60	1.14	1.78	1.07	1.22	1.60	2.09	8.63±1.04	0.46
N_2	97	4.43	8.51	4.22	4.65	7.77	9.57	5.81±0.42	9.13
	98	0.49	0.94	0.44	0.54	0.82	1.16	5.95±0.86	0.45
Tribolium castaneum larvae									
CO_2	40	3.26	10.59	2.63	3.92	9.11	16.71	3.22±0.24	17.59
	60	1.20	2.75	1.08	1.32	2.44	3.23	4.59±0.43	4.60
N_2	97	1.63	2.87	1.55	1.72	2.62	3.23	6.74±0.55	7.61
	98	1.41	2.09	1.05	1.73	2.06	4.04	9.65±0.76	8.39

Table 4: Corrected mortality percentages for adult and reduction percentages in adult emergence from larvae of *Sitophilus oryzae* exposed to ozone gas combined with several exposure periods.

	Corrected mortality percentages (Mean±SE)				Reduction percentages in adult emergence (Mean±SE)			
	Adult stage				Larva stage			
	Exposure time (day)				Exposure time (day)			
Injection Period (h)	1	7	*F*	*P*	1	7	*F*	*P*
0.5	22.14±1.36a	38.57±3.77a	16.71	0.006	23.24±3.38a	49.56±2.71a	36.73	0.001
1	41.42±1.84b	65.00±3.16b	41.34	0.001	45.61±2.37b	57.45±1.49b	17.78	0.006
2	59.28±2.43c	78.60±3.39c	21.25	0.004	52.63±2.77b	72.36±1.31c	41.32	0.001
4	70.71±2.44d	87.85±1.36d	37.54	0.001	73.68±2.26c	88.59±1.13d	34.32	0.001
6	95.71±1.84f	100.0±0.00f	5.40	0.059	87.71±1.60d	99.56±0.43f	50.86	<0.001
F	191.20	73.68			96.47	169.48		
P	<0.001	<0.001			<0.001	<0.001		

Means followed by the different letters are significantly different from each other at $P<0.05$ (Duncan test).

while, after 7 days ozone treatment, corrected mortality % of *S. oryzae* adults was 38.57% ± 3.77 at 0.5 h injection time; complete mortality (100 %) was after the injection time of 6 h. *Sitophilus oryzae* larvae were slightly tolerant to ozone than adults. Reduction % was 23.24 ± 3.38 and 87.71 ± 1.60 % at 0.5 and 6 h injection times, respectively with significant differences between them after only 1 day treatment, but after 7 days ozone treatment, reduction % was 49.56 ± 2.71% at 0.5 h injection time, then significantly increased with increasing injection times to 99.56 % (approximately 100 %) reduction in emerged adults after 6 h injection time.

The Effect of O_3 on *T. castaneum*: The results were obtained in Table (5) reported corrected mortality % of *T. castaneum* adults, it appears that the lowest corrected mortality % was 7.50 ± 2.84% at 0.5 h injection time, while, the highest mortality % was 63.41 ± 3.54 % after 6 h injection time with significant differences between them after 1 day post-ozonation. After 7 days post-ozonation, the lowest mortality % was recorded 20.00 ± 1.35 %, then, significant increased to 73.41± 1.42 for 6 h. In case of *T. castaneum* larvae, data showed previous trend same by adult, but, *T. castaneum* larvae more susceptible to ozone than the adults. It revealed that the lowest mortality % was 23.33 ± 3.04% for 0.5 h, while, the highest mortality % was 78.33 ± 2.15 % for 6 h with significant difference between them after 1 day post ozonation. But after 7 days post-ozonation, the lowest corrected mortality % was 35.83 ± 2.50 % after 0.5 h., whereas, 89.16 ± 2.84 % mortality was only reached after 6 h ozonation with significant differences between them. In general, it appears that mortality or reduction % of *S. oryzae* and *T. castaneum* were affected by different exposure times and days post-ozonation.

Comparative Lethal Times of *S. oryzae* and *T. castaneum* Exposed to Ozone Gas: Table (6) and Fig. 2 show the results LT_{50} and LT_{95} for adults and larvae of *S. oryzae* reported that O_3 gas was more effective when left closed container at 7 days than 1 day time (LT_{95} 10.95 and 15.65 h after 1day for adults and larvae, while, after 7 days were LT_{95} 5.14 and 6.83 h for adults and larvae, respectively). Also, O_3 gas was more effective against adults and larvae of *T. castaneum* when left closed container at 7 days than 1 day (LT_{95} 49.97 and 33.08 h after 1 day for adult and larvae, while after 7 days were LT_{95} 49.89 a nd 18.40 h for adults and larvae, respectively). From the data obtained revealed that adults and larvae of *S. oryzae* was more sensitive to O_3 gas than adults and larvae of *T. castaneum*. It could be concluded from above mentioned results that O_3 gas for 7 days inside air tight containers were more preferable for controlling adults and larvae of *S. oryzae* and *T. castaneum* than 1 day.

Effect of MAs and O_3 on Physical Analysis of Toast Bread: Data in Table (7) show the effect of different treatments on the loaf weight, loaf volume and specific volume of toast bread. The data revealed that specific volume increased in bread made from flour exposed to O_3 but decreased in bread made from flour exposed to N_2 compared to control bread. Also there no difference in specific volume between control bread and bread made from flour exposed to CO_2.

Effect of MAs and O_3 on Sensory Evaluation of Toast Bread: The sensory characteristics of toast bread including color of crust, texture, taste, flavor, appearance and overall acceptability. The results showed significant differences in quality between the samples as compared with control samples (Fig. 3).

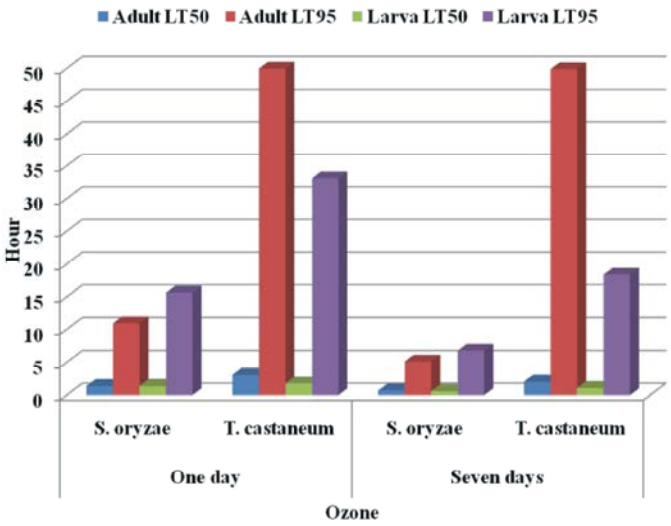

Fig. 2: LT_{50} and LT_{95} values for adult and larva of *Sitophilus oryzae* and *Tribolium castaneum* exposed to 500 ppm ozone.

Table 5: Corrected mortality percentages for adults and larvae of *Tribolium castaneum* exposed to ozone gas combined with several exposure periods.

	Corrected mortality percentages (Mean ± SE)							
	Adult stage				Larva stage			
	Exposure time (day)				Exposure time (day)			
Injection Period(h)	1	7	*F*	*P*	1	7	*F*	*P*
0.5	7.50±2.84a	20.00±1.35a	15.70	0.007	23.33±3.04a	35.83±2.50a	10.07	0.019
1	34.17±2.84b	40.83±1.59b	4.17	0.087	35.00±2.15b	44.16±2.09b	9.30	0.023
2	42.50±2.09b	49.16±0.83c	8.73	0.025	51.67±2.15b	62.50±1.59c	16.35	0.007
4	53.33±2.35c	58.33±2.15d	2.45	0.168	65.00±2.15c	72.50±2.84d	4.41	0.080
6	63.41±3.54d	73.41±1.42f	6.83	0.040	78.33±2.15d	89.16±2.84f	9.22	0.023
F	58.79	168.94			88.39	78.41		
P	<0.001	<0.001			<0.001	<0.001		

Means followed by the different letters are significantly different from each other at $P<0.05$ (Duncan test).

Table 6: LT_{50} and LT_{95} values with their confidence limits for adults and larvae of *Sitophilus oryzae* and *Tribolium castaneum* exposed to ozone.

			Confidence limits (h)					
			LT_{50}		LT_{95}			
Exposure time (day)	LT_{50} (h)	LT_{95} (h)	Lower	Upper	Lower	Upper	Slop ± SE	Chi-square χ^2
Sitophilus oryza adults								
1	1.38	10.95	0.67	2.27	9.99	73.19	1.83±0.16	11.75
7	0.69	5.14	0.55	0.84	3.95	7.44	1.89±0.18	7.72
Sitophilus oryza larvae								
1	1.42	15.65	1.17	1.69	10.56	27.72	1.57±0.16	4.37
7	0.64	6.83	0.21	0.89	5.27	33.58	1.60±0.16	8.37
Tribolium castaneum adults								
1	3.09	49.97	1.90	8.35	50.67	319.43	1.36±0.15	8.57
7	2.07	49.89	1.65	2.62	25.22	152.99	1.19±0.15	3.25
Tribolium castaneum larvae								
1	1.78	33.08	1.44	2.19	18.69	81.31	1.29±015	1.29
7	1.10	18.40	0.85	1.36	11.44	38.38	1.34±0.15	4.68

Table 7: Quality characteristics of toast bread prepared with flour exposed to 60% CO_2, 98% N_2 and 500 ppm O_3 under laboratory conditions.

	Gas treatment					
Toast bread characteristic	Control	60% CO_2	500 ppm O_3	98% N_2	*F*	*P*
Color of crust	19.00 ±0.25c	16.90 ±0.31b	15.40 ±0.30 a	15.10 ± 0.27a	37.90	<0.001
Texture	18.60 ±0.42c	16.70 ±0.36b	17.30 ±0.57b	13.90 ±0.37a	19.78	<0.001
Taste	18.00 ±0.47c	14.80 ±0.61ab	16.40 ±0.69bc	13.00 ±0.94a	9.51	<0.001
Flavor	17.70 ±0.39b	15.70 ±0.74a	17.30 ±0.21b	14.90 ± 0.43a	7.38	<0.001
Appearance	17.00 ±0.36b	16.90 ±0.48b	18.50 ±0.30c	15.50 ±0.42a	9.34	<0.001
Overall acceptability	90.30 ±0.81d	81.00 ±1.11b	84.90 ±1.03c	72.40 ±1.49a	43.55	<0.001

Letters represent significant differences among gas treatments. Means followed by different letters are significantly different from each other at *P<0.05* (Duncan test).

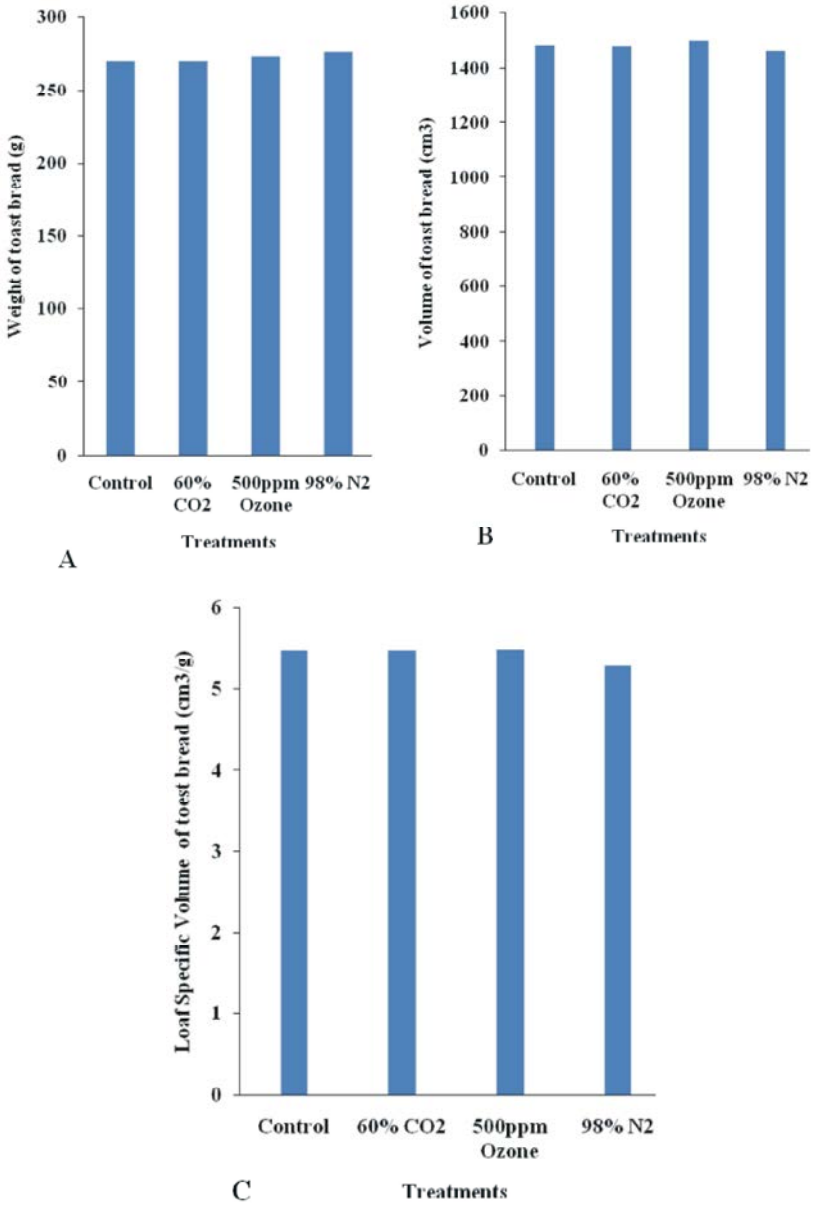

Fig. 3: Physical properties of toast bread prepared with flour exposed to 60% CO_2, 98% N_2 and 500 ppm O_3. A: Weight of toast bread (g), B: Volume of toast bread (cm^3), C: Loaf specific volume of toast bread (cm^3/g).

Table 8: Body weight (g) of albino rats fed wheat grains exposed to different modified atmospheres containing 60% CO_2 or 98% N_2 for one month and 500 ppm O_3 for one week.

	Body weight of rat (Mean ± SE)						
		Week post feeding					
Treatment	Zero time	1st	2nd	3rd	4th	*F*	*P*
Control	100±0aA	114±1.9aB	133±3.4aC	138±2.0C	149±1.8D	85.489	<0.001
CO_2 (60%)	108±1.2cA	125±4.7bB	138±1.2bC	147±2.0D	161±2.5E	54.434	<0.001
N_2 (98%)	100±0aA	112±2.0aB	126±2.9aC	136±2.9D	154±4.0E	59.297	<0.001
O_3 (500 ppm)	104±1.0bA	114±2.4aB	127 ±2.5abC	139±3.6D	152±4.3E	39.859	<0.001
F	23.467	3.880	4.476	3.111	2.213		
P	<0.001	0.029	0.018	056	126		

Means followed by the different letters are significantly different from each other at *P<0.05* (Duncan test).
Small letters indicate significant differences among days whereas capital letters indicate significant differences between MAs concentrations.

Table 9: Weight of different organs (g) and biochemical analyses of albino rats fed wheat grains that exposed to different modified atmospheres containing 60% CO_2 or 98% N_2 for one month and 500 ppm O_3 for one week.

	Weight of Organs (Mean ± SE)			Biochemical Analyses(Mean ± SE)		
Treatment	Brain	Kidney	Liver	Total protein (g/dl)	Alkaline phosphates (U/L)	Total lipids (mg/dL)
Control	1.4±0.09	1.1±0.05	5.8±0.15	6.5±0.4	14.6±3.8	633.6±110
CO_2 (60%)	1.5±0.07	1.1±0.03	6.0±0.16	6.9±0.5	19.9±2.8	791.3±77.4
N_2 (98%)	1.5±0.04	1.0±0.04	5.7±0.08	6.9±0.5	12.7±2.3	585.9±32.3
O_3 (500 ppm)	1.4±0.04	1.1±0.05	5.4±0.3	7.2±0.3	14.4±2.4	625.4±74.6
F	1.516	0.724	2.087	0.528	1.160	1.329
P	0.249	0.552	0.142	0.669	0.356	0.300

According to our results, O_3 and CO_2 treatments had proven to be not effect in the milling and baking qualities of flour treated with it and N_2 reduce the quality of bread.

Effect of Wheat Grains Exposed to MAs and O_3 on Body Weights, Internal Organs and Biochemical Analyses of Albino Rats: Table (8) shows the body weight of albino rats fed wheat grains that exposed to MAs containing 60% CO_2 or 98% N_2 for a month and 500 ppm O_3 for a week. The statistical analysis revealed significant differences between treatments (P< 0.001 – 0.029) among the body weights initially and after the 1st and 2nd weeks. In contrary the body weights of treatments close to each other on the 3rd and 4th weeks without any significant differences between of them (P>0.05). Moreover, rats revealed high significant increases in their weights between weeks (P<0.001) in all treatments recording increase in weights 49, 53, 54, 48 g for control, 60% CO_2, 98% N_2 and 500 ppm, respectively. Table (9) shows the weights of internal organs; brain, kidney and liver, in addition to biochemical analyses of albino rats fed wheat grains exposed to different MAs containing 60% CO_2 or 98% N_2 for a month as well as 500 ppm O_3 for one week. The statistical analysis indicated insignificant differences among the treatments for weights of all internal organs and the measurements of total protein, alkaline phosphatase and total lipids.

DISCUSSION

Wheat grains and their products (i.e wheat flour) are one of the most important sources for human foods and may be stored for a long time. During this long storage period, many insect pests attack them. *Sitophillus oryzae* and *Triboleum castaneum* infest wheat and their products beside other commodities. *Sitophillus oryzae* is a key insect pest where its larvae and adults feed inside the wheat grains, while, *T. castaneum* is the secondary insect pest wherever, its larvae and adults feed flour or broken wheat grains. Therefore, the active stages (adults and larvae) of these two insect pests were chosen to achieve the goals of this study. The main goal was to evaluate the effect of modified atmospheres (MAs) containing 40% CO_2, 60% CO_2, 97% N_2, 98% N_2 and 500 ppm O_3 on the adults and larvae of *S. oryzae* and *T. castaneum*. The larvae and adults of *T. castaneum* and the adults of *S. oryzae* are visible individuals; therefore, their data were recorded as the mortality percentage and corrected later. Whereas, the larvae of *S. oryzae* are concealed individuals, they were inside the wheat grains which were exposed to gases and we wait until larvae developed to the adults and reduction in adult emergence was recorded. The best gas mixtures of MAs (60% CO_2 in air and 98% N_2 with remaining oxygen) those gave the highest effective on *S. oryza* and *T. castaneum* in addition

to 500 ppm O_3 were chosen to achieve the other two goals of this study. The two goals were to evaluate the effect of the selected gas mixtures and O_3 on the quality analyses of wheat flour and safety of wheat grains to rats.

There are a few studies used MAs containing elevated CO_2 or reduced oxygen in controlling *Sitophilus* spp. or *Tribolium* spp. Carvalho et al. [39] evaluated MAs containing 90 -95% CO_2 at 29.6 °C (for 26 day), 34.1 °C (for 10 days) and 22 °C (for 26 days) against *S. oryzae* and *S. zeamais*. They recorded 100% mortality in the adults for all treatments. It is thought that it is a long time in comparing with the results of this study. Also, De Carli et al. [40] achieved complete inhibition in the insects of *Sitophilus* spp exposed to MAs containing 20, 40, 60, 80% CO_2 for 30 days at 26°C. In this research, the time may be reduced in MAs that had high level of CO_2. Conyers and Bell [41] eliminated emergence of *S. granaries* exposed to MAs containing 10 or 20% CO_2 with reducing O_2 (5%) at 20 °C but they did not detect the stages which exposed to MAs. The CO_2 (62%) and N_2 (95%) fumigations were very effective in killing the immature stages of *S. oryzae* at 40, 80 and 120 exposure days [42]. They also attained a long time in comparing with the time required to completely kill *S. oryzae* larvae in this study (5 days). Annis and Morton [10] exposed adults and larvae of *S. oryzae* to 65% CO_2 at 25 °C and they recorded LT_{99} 1.2 days for adults and 1.41 days for larvae. These findings are relatively lower than the results of this study that achieved the LT_{95} 2.5 and 2.99 days for adults and larvae of *S. oryzae* those exposed to 60% CO_2 at 30 °C. This may relate to they tested higher level of CO_2 (65%) comparing with 60% in this study and calculated higher LT_{99} comparing with LT_{95} in this study. Also, Annis and Morton [10] added that adults and larvae of *S. oryzae* were the most susceptible, while eggs and pupae were the most tolerant to the tested MAs. The present study evaluated the susceptibility of adults and larvae of *S. oryzae* and *T. castaneum* to MAs, while the eggs and pupae of these two insect pests might be evaluated in future. The adults of *T. castaneum* recorded 70.5% mortality when they were exposed to MA (3% O_2, 85% N_2 and 12% CO_2) for exposure time 10 days at 26 °C [43]. The gas mixture (3% O_2 and 97% N_2) used in the present study was more effective than that used by Donahaye *et al.* [43], it recorded 90% mortality in *T. castaneum* within 7 days. The modified atmospheres enriched with 45% and 60% CO_2 reduced the time required to achieve 100% mortality of *T. confusum* insects to 7 days at 1 °C [44]. The previous researches were not spot light on the exact time required for achieved 100% mortality in the developmental stages of the tested insects. Therefore, the novel idea in this study is to follow the mortality of active stages (adults and larvae) of both *S. oryzae* and *T. castaneum* daily to reach the lower time that recorded complete killing in the active stages of the two tested insects.

The results of the present study revealed that the adult mortality from exposed adults to MAs and reduction percentage of adult emergence from exposed larvae to MAs significantly increased gradually by increase the exposure time and CO_2 or N_2 concentration in both *S. oryzae* and *T. castaneum*. This finding agrees with the previous studies those used MAs in controlling insect pests of stored products [45, 46]. The current study also added that three days were adequate to kill all *S. oryzae* adults exposed to MA containing 60% CO_2, while 100% mortality was recorded after five days with the rest of MAs. This finding agrees with Zidan [47] who found that *S. oryzae* was killed completely after five days when was exposed to MAs containing 40% and 60% CO_2. This finding disagrees with Riudavets, *et al*. [11] that exposed the adults and larvae of *S. oryzae* to MAs containing 50% and 90% CO_2 in air at 25 °C and recorded 100% mortality after 4 days for adults of the two tested MAs. This may due to the initial date for their investigation was after 4 days and the specimens may dead before this time. The exposure time extended to 12 days to obtain 100% mortality in larvae. There was 7 days more than recorded in this study in *S. oryzae* larvae. This may be due to overlapping between larvae and pupae those inside the grains during the exposure time, wherever, the pupae of *S. oryzae* is more tolerant to modified atmosphere [10]. The complete mortality was recorded within two and three days in *T. castaneum* adults exposed to 98% N_2 and 60% CO_2, respectively. This finding disagrees with Riudavets *et al*. [11] that exposed the adults of *T. confusum* to MAs containing 50% and 90% CO_2 in air and recorded 100% mortality after 4 days in the two tested MAs. This may due to the same reason mentioned above. They recorded 100% mortality after 4 days for MA containing 90% CO_2 and after 12 days in MA containing 50% CO_2. This probably due to the difference between insect species or the conditions of the experiment where, the experiment was conducted at 25 °C comparing with this study that conducted at 30 °C. In the present study, to kill all *T. castaneum* larvae, 2 to 4 days were needed with MAs containing 98% N_2 and 60% CO_2. However, the larvae of *S. oryzae* took longer time to be killed completely than adults. It attained 5-6 and 8-10 days with MAs containing 60-40% CO_2 and 98-97% N_2, respectively to obtain 100% reduction in adult emergence. Surprisingly, the MAs containing N_2 were more effective than those

containing CO_2 on *T. castaneum* larvae, while, MA containing 60% CO_2 was the most effective on the *S. oryzae* larvae. This probably attribute to the feeding of larvae that occurred inside the grains in *S. oryzae* that affected by the CO_2, while, the larvae of *T. castaneum* feed outside of the grain and affected by N_2.

In agreement with Osman [48], the current study revealed that the adults of *S. oryzae* were more susceptible to O_3 than the adults of *T. castaneum*. Hansen, et. al. [49] reported that the freely exposed stage of *S. oryzae* was controlled with 35 ppm O_3 for 6 days. They also showed that full mortality of internal stages of *S. oryzae* within kernels required exposure to 135 ppm O_3 for 8 days. These results of *T. castaneum* treatments are in agreement with those of Osman [48] who noticed that *T. castaneum* adults were lesser susceptible to O_3 than larvae. Hansen *et al.* [49] reported that the freely exposed stage of *T. castaneum* was controlled with 35 ppm O_3 for 6 days. Leesch [26] found that adult of *T. confusum* was more tolerant to O_3 than of *Plodia interpunctella* adults. MeDonough *et al.* [50] determined O_3 concentration and treatment time needed to effectively kill adult stages of *T. castaneum* and *S. zeamais*, they stated that 100 % mortality for both species were reached at 1800 ppm for 120 min.

According to the results obtained in this study, 500 ppm O_3 and MA containing 60% CO_2 had proven to be not effect in the milling and baking qualities of flour treated while, MA containing 98% N_2 reduced the quality of bread. These results are in accordance with data reported by Abdel-Aal [51] who showed that chemical composition of wheat flour not affected under stored with CO_2 condition for 8 months. Also, Zinhoum [52] showed that there was no significant difference in the milling and baking qualities of flour treated by CO_2. Sandhu *et al*. [53] reported that bread made from flour exposed to O_3 for 4.5 min had greater specific loaf volume and whiter crumb compared to bread made with control flour. El-Desouky *et al.* [54] found that a gradual increase in specific volume of ballad bread according to the time of ozonation.

In the present study, rats fed wheat grains exposed to MAs enriched with either 60% CO_2 or 98% N_2 and 500 ppm O_3 grew normally and recorded highly significant increase in body weight ranged between 49 to 54 g for all treatments. Furthermore, insignificant differences among the treatments were recorded for weights of all internal organs (brain, liver and kidney) and the measurements of total protein, alkaline phosphatase and total lipids. These findings confirm that the modified atmospheres and ozone are safe on wheat grains and the stored products under such conditions can be introduced into mammal consumers.

CONCLUSION

The MA containing 60% is suitable for controlling adults and larvae of *S. oryzae* and *T. castaneum*, while MA containing 98% N_2 is suitable for controlling adults and larvae of *T. castaneum* in short time. The adults and larvae of *S. oryzae* were more sensitive to O_3 than those of *T. castaneum*. The exposure to 500 ppm O_3 for 7 days was preferable for controlling adults and larvae of *S. oryzae* and *T. castaneum*. MAs containing 60% CO_2 and 97% N_2 as well as 500 ppm O_3 had proven to be not effect in the milling and baking qualities of flour treated with it and N_2 reduce the quality of bread. The modified atmospheres and ozone are safe on mammals feed wheat grains exposed to these gases.

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