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Study on laser perforated films as gas permeable packaging for confused flour beetle (*Tribolium confusum* Jacquelin du Val.) control inside food packaging

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ABSTRACT

Storage insect pests are a major threat for packaged foodstuffs and most packages have low permeability to the fumigant gases used for control. In this study, the effects of O_3 gas concentrations (50, 100, 150 ppm) in the atmosphere of 70% CO_2 on adults of Confused flour beetle placed inside three foodstuffs including; wheat (alive), wheat flour and rolled oats (inanimate) packed with laser perforated BOPP film with 80 µ width, were evaluated. The experiments performed at 25 ± 2 °C and 35 ± 5% r.h. with exposure period seven days. The results showed that the regression models between permeability index (PI) of Bopp film and insect mortality in three different foodstuffs and also empty packages were different. Regression model for packaged wheat was quadratic, for wheat flour and rolled oats was power and in empty packages, linear model was fitted with 95% confidence. The additive effect between O_3 and CO_2 gases on pest mortality placed in empty non-perforated film was found. Other results proved that the mean mortality in three products in the same condition is different and highest mortality was observed in wheat. Also, the interactions observed between factors at 0.05 level. Present study suggests laser perforated BOPP films as a proper packaging for inanimate products and non-perforated BOPP films for live products.

1. Introduction

The confused flour beetle, *Tribolium confusum* Du Val is one of the most serious pests of stored products worldwide. It causes damage to an extremely large variety of foodstuffs, and it is a very important pest on wheat flour and broken grain kernels. Moreover, this species is resistant to many residual pesticides and fumigants used for stored-product control (Abd El-Aziz and El-Saved, 2009; Rossi et al. 2010).

Food packaging as one of the most important parts of food industry is related with food security. Food packaging provides not only a method for transporting food safely, but extends product's self-life via preventing from harmful bacteria, contamination and degradation (Chin, 2010). Furthermore packaging can be security for food product, insect can enter goods during transportation, storage in the warehouse, or in retail stores, and also it is possible that the initial contaminants develop and destroy foodstuffs (Allahvaisi et al. 2010). Accordingly, the way, type of packaging can eliminate probable contamination of the food and prevent re-contamination, is one of the underlying subjects in packaging industry. When an infected packaging with an insect's life stage, enters into the warehouse, it can spread the contamination to other packages and in addition reducing food quantity, they annihilate quality.

In Europe, nearly 40% of all plastics are used in the packaging sector, and packaging is the largest sector of plastic's usage (Association of Plastics Manufacturers in Europe, APME). About 50% of Europe's food is packed in plastic packaging (British Plastics Federation, BPF) (Coles et al. 2003). Today, there are several popular types of polymers for foodstuff's packaging. Some of them have virtually no resistance to insects while others may be extremely resistant (Highland, 1981). Among these polymers, polypropylene has very low permeability against the pest insect (Allahvaisi et al. 2010). Orientation increases the versatility of PP film. Oriented PP film (OPP or BOPP) was the first plastic film to successfully replace regenerated cellulose film (RCF) in major packaging applications such as biscuit packing (Coles et al. 2003). In recent years, especially these films (BOPP) have become one of the most popular high-growth films in the world market (Lazic et al. 2010).

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In general, modified atmosphere packaging (MAP) is where a modified atmosphere is applied as the package is sealed and to retain the applied atmosphere, the very low permeability is required. On the other hand, today for fresh produce, films with relatively high oxygen transmission and suitable water vapor transmission rates are required (Winotapun et al. 2010). Laser perforation is the newer method to provide micro holes (Chow, 2003) that can increase gas permeability.

It is worth noting that of the 16 fumigants listed in common use, some 22 years ago by Bond (1984), only very few remain today (Navarro, 2006). Exposure of insects to toxic concentrations of atmospheric gases has been practiced for centuries and has been promoted in recent years as a bio-rational substitute for chemical fumigations (Sadeghi et al. 2011). Ozone, a powerful oxidant, has numerous beneficial applications and is very familiar to the food processing industry. This gas has regulatory acceptance by the Food and Drug Administration (USA), and the Environmental Protection Agency's (USA) MSDS defines it as “pure air” (Mason et al. 2006). CO_2 gas, is relatively safe to use, leaves no known residues, and readily penetrates packages of tightly compressed commodities (Keever, 1989).

When a product is packaged, it may be contamination and because percentage of insect's penetration can depend on the type of packaging material, finding the best wrapper for packaging is inevitable and purpose of this study. So, modified atmosphere system and BOPP film were applied due to least dangerous to storing agricultural products and maximum resistance to penetration by insect respectively.

2. Material and methods

This study was carried out at Department of Plant Pest and Disease, Razavi Khorasan Research Center for Agriculture and Natural Resources during the years 2012–2013. Different concentrations of O_3 and CO_2 gases was tested on packages made of laser perforated BOPP films with 80 μ width containing wheat, wheat flour and rolled oats, and the best gas mixture was selected. This gas mixture was treated on the eight treatments, including seven laser perforated films (LPFs), a non-perforated film and control treatment.

2.1. Insect

Confused flour beetle was collected from a flour silo in Mashhad (36°20′N 59°35′E), a city in Iran. Cultures were established and maintained on healthy uncontaminated food at 25 ± 2 °C and 65 ± 5% r.h. in plastic bottles and were closed with pieces of muslin cloth fixed by rubber bands. Rearing medium used was composed of wheat flour with 5% yeast (Childs and Overby, 1983). All insects were cultured under moderately crowded conditions to ensure proper development and equal size of the resultant adults.

2.2. Supply of gases

Ozone gas was generated by ozone Generator, Ozonica series, Oz 100 model (WWW.ozoneab.com/), that generate 100 g/hour ozone from purified oxygen with four reactors. Purified oxygen produce by oxygen generator, LFY-I-5F–W model, provided by Longfei Group Co. Ltd., and produce purified oxygen 93% ± 3% with flow rate 0–5 L/min. Specified O_3 concentration Was measured based on the volume of the chamber and the default generator. A local factory supplied CO_2 gas needed inside cylinders of 40 kg with 99.9% purity.

2.3. BOPP film

We laminated two BOPP film rolls with 40μ width together and produced film 80μ. Then cut rolls to size 60 × 100 cm. Afterward, perforated them with Golden Laser Machine, JG-13090DT model produced by China with distances 0.75, 0.8, 1, 2, 3, 4 and 5 cm and 200 ± 25μ hole's diameter, and made the packages to size 20 × 30 cm by plastic press machine.

2.4. Bioassay

At the first, the packages 20 × 30 cm were filled with 1 kg of wheat, wheat flour and rolled oats separately. Then a cage (Petri with a diameter of 10 cm) containing 40 adults *T. confusum* 12 ± 4 day old and 3 g food was entered into each package and sealed with a plastic press machine. Three packages from each of the treatment of LPFs and foodstuffs was performed, then transferred into chamber 70 × 120 × 180 cm and placed horizontally at the bottom it and the chamber closed tightly. Afterward, CO_2 gas was injected into the upper left, and air exited from the bottom right until concentration of CO_2 was 40% or 70% and in the final step, we injected O_3 gas daily at a specified time and every day on reaching the specified concentration, ozone injection was stopped. A total, in each experiment seven injections with equal doses during seven days performed. During CO_2 injection and until 1 h after O_3 injection, the system was circulated. During experiments, upper surface of packages exposed chamber atmosphere. Exposure period was considered 7 day at 25 ± 2 °C, 35 ± 5% r.h. After exposure period, the specimens were transferred to a clean jar containing 3 g of food with the same condition. Mortality rates of the insects were recorded 6 h after termination of the treatment.

Preliminary dose-mortality tests to determine a range of doses that produce 25–75% mortality at the lowest and the highest doses were done, respectively (Robertson et al. 2007). Afterward, the interaction of two gases on mortality obtained. Final experiment performed in two parts. At the first, we consider relationship between permeability index (PI) of BOPP film with the formula (Area of all of micro-holes on package/Area of package) × 1000 and mortality rate (MR) of adult insect and another part; treatments were compared for each factor separately and interactions between factors was evaluated.

2.5. Complementary experiments

We performed two complementary experiments for elected treatments, including test of confused flour beetle penetration inside and outside packaging and test of adult emergence from immature stages in a gas treatment. The first experiment for adults was conducted in two parts. At first, the starved insects were placed around packages containing 3 g of foodstuffs and then, the starved insects were entered into packages while on the outside there were foodstuffs. For the test of adult emergence, a sample containing 100 g of rearing medium containing eggs, larvae and pupae of confused flour beetle randomly selected and placed into the packages containing wheat, wheat flour and rolled. After fumigation (70% CO_2 + 150 ppm O_3), the samples were transferred to a clean jar and held at 25 ± 2 °C and 60 ± 10% r.h. for eleven weeks. During this period of incubation, every week, the number of adults emerged, recorded and was removed from the container. The experiment was replicated three times and results were pooled.

2.6. Data analysis

Experiments were performed by a completely randomized design using factorial experiment. All of data were analyzed with

Table 1
Summary of probit analysis and chi-square test for interaction of O_3 and CO_2 gases in empty non-perforated package in the defined conditions on confused flour beetle.

Treatments	LD50	LD95	LD25	Slope	ρ	Type of action
Ozone (ppm)	130.9	262.6	98.4	2.36	–	–
Carbon dioxide (%)	63.6	86.9	55.97	12.4	–	–
Ozone + Carbon dioxide	98.4 + 55.97	–	–	–	0.549	Additive

ρ is significant at 0.05 level.

the Statistical Package for the Social Science (SPSS) software (SPSS Inc. 2007). First, mortality of treatments was modified with Abbott's formula and so, transformed the Arcsin $\sqrt{x}$ for data normalizing. GLM univariate analysis performed to evaluate the main effects and interactions of factors. For means comparison separately, was used the Tukey's test. The regression models were fitted for each foodstuff and its mortality rate separately by curve estimation models and the highest value of R-squares selected as the best models. Furthermore, Probit analysis (Finney, 1971) was used to estimate LD50 and LD95 values and the slopes of the regression lines.

3. Result

For finding the interaction of two gases on mortality, LD50 values of two gases calculated for empty non-perforated packages separately. Afterward LD25 values of both gases were treated in one experiment simultaneously (Kent, 1998) and then, the data analyzed. According to chi-square test ($\chi^2 = 0.549$), There was no significant difference between mortality rate and the rate of alive. As a result, the mortality rate in the mixture treatment (LD25 + LD25) is equal 50 percent and interaction of these gases on mortality in packages without foodstuffs with 95% confidence was additive (Table 1).

The results showed that relationship between mortality rate and permeability index of films in wheat product (alive-product) was quadratic model and in wheat flour and rolled oats products (non-alive products) was power model, but this relation was fitted with linear model in empty packages (Table 2 and Figs. 5 and 6).

In factorial experiment with three factors including foodstuffs, permeability index (PI) and ozone concentration, all factors and interactions were significant at 0.05 level (Table 3). Different behavior wheat (alive product) compared to flour wheat and rolled oats and high degree of freedom experiment seems to be some of reasons for interactions.

For comparison all Arcsin $\sqrt{x}$ mean mortality, Tukey's test was used. Mean mortality in all three foodstuffs showed significant difference and the highest mortality was observed in wheat

Table 3
Factorial experiment for three factors of foodstuffs, permeability index (PI) and ozone concentration on confused flour beetle.

Source	Sum of squares	df	Mean square	F	ρ
Foodstuffs	12.381	2	9.775	9145.834	0.000
PI	0.283	7	0.259	242.112	0.000
Concentration	0.082	3	0.100	93.143	0.000
Foodstuffs * PI	1.389	14	0.168	156.793	0.000
Foodstuffs * Concentration	0.005	6	0.019	18.192	0.000
PI * Concentration	0.014	21	0.003	3.076	0.000
Foodstuffs * PI* Concentration	0.030	42	0.003	2.654	0.000
Error	0.086	188	0.001		
Total	14.269	283			

ρ is significant at 0.05 level.

(Wheat > Rolled oats > Flour wheat, at 0.05 level) (Fig. 1). Despite higher mortality in PI0 permeability index only in wheat (Fig. 3), but the highest mean mortality in permeability index treatment was observed in PI7 (PI0 = PI1 > PI2 = PI3 = PI4 > PI5 > PI6 > PI7, at 0.05 level) (Fig. 2). Ozone treatment in three concentrations was significant and highest mortality was observed in 150 ppm concentration (150 ppm > 100 ppm > 50 ppm > 0 ppm, at 0.05 level) (Fig. 4).

3.1. Complementary experiments

The results of the first experiment indicated that any insect during the test period (two weeks) could not penetrate to the outside or inside of packages.

The test of adult emergence was carried out to record the potential contaminations of life stages in foodstuffs mass after exposure to 70% CO_2 + 150 ppm O_3 atmosphere for 7 days. Results showed that life stages of confused flour beetle represented about 85% mortality inside wheat placed LPF 1 cm packages and 90% mortality inside wheat placed Non-LPF packages compared control treatment. About wheat flour foodstuff observed about 74% mortality in LPF 1 cm packages and 65% mortality in Non-LPF packages. Also rolled oats foodstuff showed about 80% mortality in LPF 1 cm packages and 77% mortality in Non-LPF packages (Table 4). Other results proved that in the chamber and only with 70% CO_2 atmosphere in defined conditions, pest mortality within non-perforated packages filled with wheat (0.862 ± 0.095) was more than empty packages (0.425 ± 0.076) with 95% confidence ($\rho = 0.000$), and at the same time, no mortality was recorded within same packages filled with wheat placed in ambient air. As a result, can be said that wheat foodstuff can influences pest mortality in the experiment conditions and this can justified the results in Table 4.

Table 2
Relationship between permeability index (PI) of packages and mortality rate (MR) in foodstuffs in the defined conditions on confused flour beetle.

Gas treatment	Foodstuffs	R Square	S. E.	Model fitted	ρ	β0	β1	β2
40% CO_2	empty	0.962	0.007	Linear[a]	0.000	0.025	0.147	–
70% CO_2	empty	0.967	0.044	Linear	0.000	0.432	1.266	–
70% CO_2	Wheat	0.770	0.042	Quadratic[b]	0.025	0.865	−1.437	2.720
70% CO_2+50 ppm O_3	Wheat	0.816	0.039	Quadratic	0.015	0.813	−1.527	2.924
70% CO_2+100 ppm O_3	Wheat	0.764	0.046	Quadratic	0.027	0.848	−1.547	3.119
70% CO_2+150 ppm O_3	Wheat	0.823	0.041	Quadratic	0.013	0.855	−1.572	3.307
70% CO_2+150 ppm O_3	Wheat flour	0.958	0.120	Power[c]	0.000	0.390	0.227	–
70% CO_2+150 ppm O_3	Rolled oats	0.896	0.075	Power	0.000	0.759	0.088	–

Permeability Index = (Area of all of micro-holes on package/Area of package) × 1000.
ρ is significant at 0.05 level.
[a] $MR = \beta0 + \beta1 \cdot PI$.
[b] $MR = \beta0 + \beta1 \cdot PI + \beta2 \cdot PI^2$.
[c] $MR = \beta0 \cdot PI^{\beta1}$.

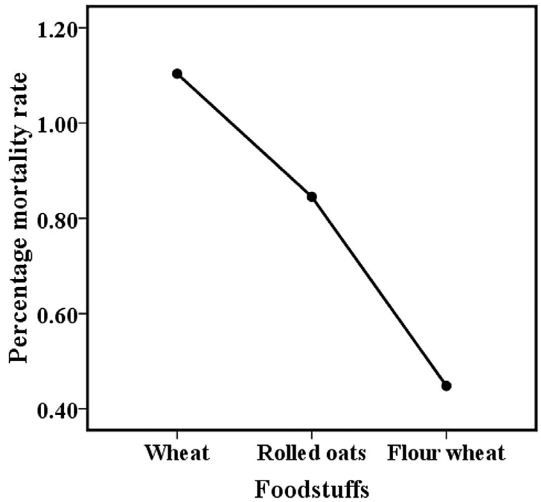

Fig. 1. Comparison of mortality rate in three foodstuffs.

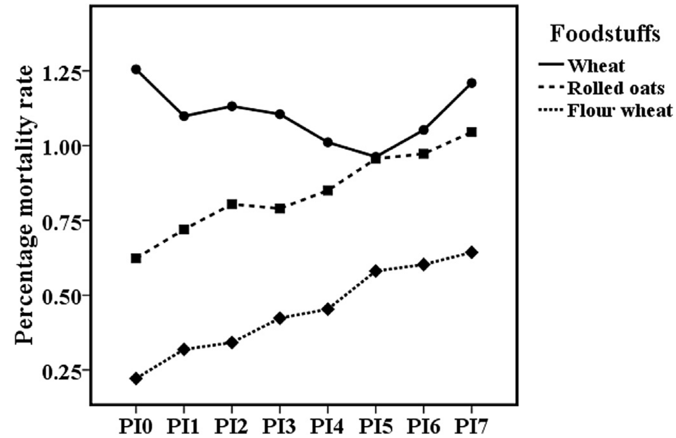

Fig. 3. Comparison of mortality rate in three foodstuffs for different permeability indexes.

4. Discussion

4.1. Gas mixture factor

About ozone gas, the research indicates that decomposition it on the grain surface occurs in two phases. The first phase, due to the high interaction with the grains surface, the penetration rate is low and in the second phase, movement through the grain is rapid with very little impedance (Kells et al. 2001). For this reason, we used low doses of O_3 gas intermittently to achieve minimum damage to the foodstuffs and packages, and maximum performance on the pest control.

The studies show when CO_2 is added to low O_2 environments, there can be a synergistic effect or an antagonistic effect. Some Investigators have observed additive effects of elevated CO_2 and reduced O_2 atmospheres, while others have not. It seems that these different results are probably, in most part, attributable to the different ranges of gases used or metabolic responses (Mitcham et al. 2006). Also, each species and life stages may have different critical concentrations of CO_2 and O_2 to make synergism and antagonism effects. Relative humidity (RH) plays no role in mortality at high humidity levels, but at lower levels it plays an important role in affecting treatment efficacy (Jay et al., 1971). In other words at low humidity, reduced O_2 is lethal due to rapid loss of water through opened spiracles (Navarro, 1978). Therefore we can conclude that reducing the amount of oxygen plus 35 ± 5% RH can have a significant effect on the increase pest mortality in this study.

The insects placed in wheat that were treated with 70% CO_2 + 50 ppm O_3 showed mean lower mortality compared with only 70% CO_2 (Fig. 4), although comparison of mean mortality in these two treatments was not significant in wheat individually ($\rho = 0.205$). So it can be concluded that adding 50 ppm O_3 to the 70% CO_2 cannot increase mortality in wheat. But about hypothesis of an antagonistic effect of low concentrations of O_3 gas on CO_2 gas on mortality rate of insects placed in wheat and packed, it is needed more study.

4.2. Foodstuff factor

Three types of foodstuffs used, are from the main stored products that can be infected by confused flour beetle. Wheat grains are alive and breathing and other two foodstuffs aren't alive. The research shows that the seeds respiration led to an increase in the CO_2 concentration within sealed packages, and this is an important factor influencing mortality (Moreno et al., 1991). And so, wheat respiration increased CO_2 concentration in the packages and this condition led to an increase in pest mortality. Our result showed

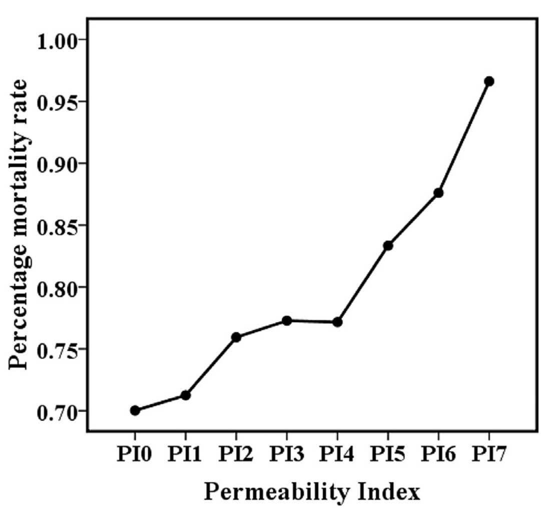

Fig. 2. Comparison of mortality rate in different permeability indexes.

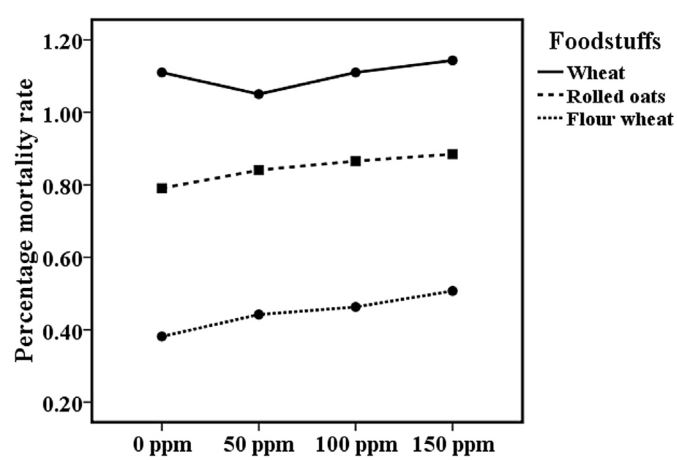

Fig. 4. Comparison of mortality rate in three foodstuffs for different gas mixture.

Table 4
The adult emergence from immature stages pest exposed to 70% CO_2 + 150 ppm O_3 atmosphere for 7 days (hidden infestation tests).

Treatment	Foodstuffs	Emergence at week											
		1	2	3	4	5	6	7	8	9	10	11	Total
Control	Wheat	30	23	4	7	10	16	20	11	9	2	–	132
	Wheat flour	60	6	11	3	8	7	4	9	3	–	–	111
	Rolled oats	41	7	10	9	5	5	3	3	1	–	–	84
LPF 1 cm	Wheat	5	13	–	–	–	–	–	–	–	–	–	18
	Wheat flour	10	2	3	1	4	4	2	3	–	–	–	29
	Rolled oats	–	–	3	4	2	4	3	1	–	–	–	17
Non-LPF	Wheat	2	10	1	–	–	–	–	–	–	–	–	13
	Wheat flour	10	1	–	8	9	5	3	2	1	–	–	39
	Rolled oats	3	–	5	5	2	3	1	–	–	–	–	19

that minimum time to create significant mortality wheat treatment placed inside non-perforated packaging compared control treatment, only by bio-increasing of CO_2 concentration in the defined conditions, was determined 14 days with 95% confidence ($\rho = 0.015$). So can be said that for control of confused flour beetle within foodstuffs packaging in the chamber, about wheat flour and rolled oats was applied only O_3 and CO_2 gases injected into chamber and about wheat, apart from the injected gases, an additional CO_2 gas produced by the respiration of wheat grains within the packages influenced on the pest control. Accordingly it can be concluded that in alive products, packaging films with low permeability can be proper and the results also showed this subject. So, for these products, BOPP film with thickness of 80 microns was appropriate (Fig. 5). On the other hand in inanimate products including wheat flour and rolled oats, because there is no respiration and no bio-increasing in the mount of gases within the packages, pest mortality was almost exclusively influenced by chamber atmosphere and with elevating CO_2 and O_3 concentrations, increased mortality was followed. Therefore, the use of packaging LPFs was suitable for inanimate products and increasing the number of micro-holes or decreasing distances between micro-holes, increased pest mortality inside package. As a result, LPFs with the minimum of holes distance or the maximum value PI was diagnosed most appropriate (Fig. 6).

As expected (Ren et al. 1994), the relationship between mortality mean and permeability index of wheat flour and rolled oats foodstuffs were direct and also, power model fitted both of them (Table 2). It seems to be due to the higher absorption and lower

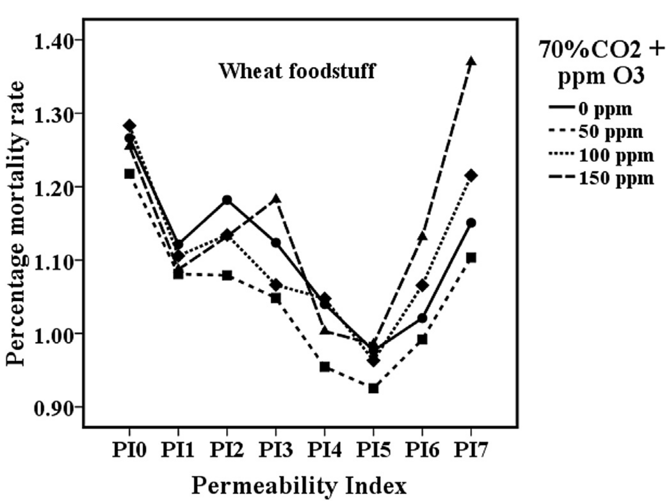

Fig. 5. Comparison of mortality rate in four gas mixture for different permeability indexes in wheat foodstuff treatment.

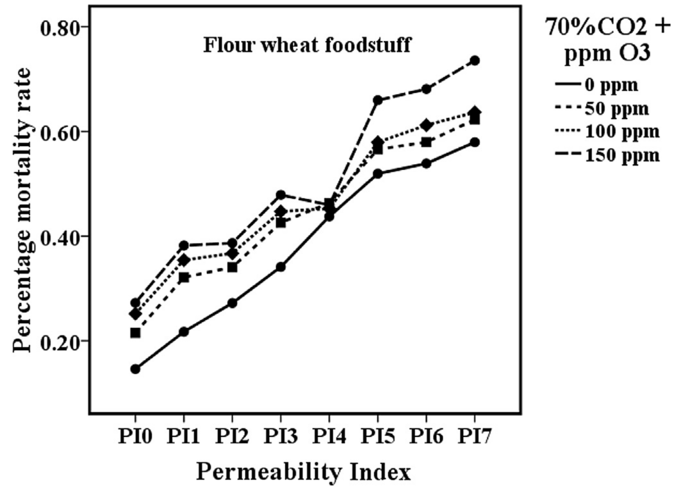

Fig. 6. Comparison of mortality rate in four gas mixture for different permeability indexes in flour wheat foodstuff treatment.

porosity of wheat flour, mortality mean was observed significantly lower than rolled oats foodstuff at 0.05 level (Fig. 1).

4.3. Laser perforated film (LPF) factor

Penetration rate of CO_2 gas into the non-perforated BOPP films is about three times O_2 gas (Lazic et al. 2010) and this difference, is caused O_2 leaving rate to the outside packaging be much lower than CO_2 entering rate into packaging. Therefore, the CO_2 concentration in the packaging increased due to CO_2 entering of chamber (70% CO_2) and wheat seeds and insects respirations and the other hand, can be assumed O_2 concentration in this packaging decreased slowly. On the other hand, the perforated films have very high penetration rate and so, package's atmosphere was approached to the chamber's atmosphere quickly. In other words, perforated packaging been more influenced by the chamber atmosphere and in the greater permeability index, this influence and uniformity of 2 atm to be more, but in non-perforated packaging, minimal uniformity of 2 atm occurred and was formed a new atmosphere into packages. Also, air flow in packages from chamber to packages and vice versa increased with the high permeability index and the minimum air flow in non-perforated packages was assumed.

In this study, contrary to expectation, the relationship between permeability index and mortality mean for wheat was not linear and direct and generally the highest mortality was observed in the lowest and highest penetration rate (Fig. 5) and quadratic model fitted (Table 2). Several hypotheses can be assumed for this event. The first hypothesis, an interaction effect between low concentrations of CO_2 produced by the wheat grains packed with CO_2 gas penetrated from chamber atmosphere into the package. The second hypothesis can be difference of permeability rate of non-perforated BOPP film to O_2 and CO_2 gases that produced new atmosphere in packages. The third hypothesis seems to be difference in the rate of air flow inside the packages. Another hypothesis may be combination of the factors mentioned in the other hypotheses.

Overall can be conclude that mixture CO_2 and O_3 gases is appropriate treatment for control of confused flour beetle and use of O_3 gas with safe concentrations intermittently and with specified interval can reduce the CO_2 concentrations used in modified atmospheres. Also obtained mixture due to the use of two controlling factors can reduce development of pest resistance compared to use of them separately. On the other hand, this study offers a new model according to MAs called Gas-permeable Packaging (GPP) that can prevent insect's penetration into or out of the packaging a lot, and also has a good permeability to gases for eliminating probable initial and secondary contaminations during the supply process. According to the findings of this study, this model is much appropriate for inanimate products.

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