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Evaluating the effect of ozone gas on the qualitative and microbial characteristics of corn seeds

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ABSTRACT

Corn production rank is second among cereals after wheat crop, and usually, a large part of it is not used immediately after harvesting, but is kept in storage for gradual use in other seasons or exported to other regions. The spread of different types of fungi and the production of fungal toxins, especially in areas with high humidity, in addition to quantitative and qualitative damage to stored products, cause an increase in waste and endanger the health of society. To replace the low-risk methods of increasing the storage life, in this research, the effect of ozone gas with two variables of ozone concentration; (25, 50 and 75 ppm) in the duration of ozonation (1, 3, 5 and 7 days) on the dominant corn grain (Single cross number 407) in Golestan region, was evaluated. The microbial characteristics were compared with the control sample in terms of controlling the spread of fungi and changes in the seed qualitative aspects. The results showed that increasing the ozone concentration up to 50 ppm and the ozonation time of 3 days had a significant effect on reducing the growth of fungi and the production of aflatoxin ($P<0.05$). Also, the results indicated that the use of 75 ppm concentration in 1, 3, 5, and 7 days had caused significant oxidative changes in the characteristics of fat (increased acidity indices) and starch (increased carboxyl index) of corn kernels compared to the control sample. Different conditions of ozonation up to the concentration of 50 ppm at varying times did not have a significant effect on the amount of corn seed protein ($P<0.05$).

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1. Introduction

After wheat, corn occupies the most agricultural land in the world and is used in the diet of humans, livestock, birds and aquatic animals due to its relatively high nutritional value compounds [1]. One of the low-risk methods to increase the shelf life of corn kernels is the use of ozone gas (instead of using compounds such as methyl bromide) in corn storage tanks and silos, especially in the livestock and poultry feed industries [2 and 3]. Corn seeds are usually infected with various fungal spores in the field. A delay in harvesting or inappropriate conditions for drying seeds, especially in areas with high humidity, creates suitable environmental conditions for the growth of fungi and the spread of fungal toxins, and can cause quantitative and qualitative damage to the seeds and their health for consumption.. High temperature and humidity during corn storage are the main factors for the growth of fungi such as Aspergillus, Fusarium and Penicillium, which lead to the production of fungal toxins such as aflatoxin.[1]Fomensin[2], dioxynivalenol[3] and subaltern[4] be. Therefore, the health of corn seeds during storage is of particular importance [3]. Ozone gas is a very strong oxidizing agent that attacks the cell walls and mucous membranes of microorganisms and causes them to die, so that it can destroy even the carcasses of microorganisms and is effective on all microorganisms such as fungal spores and pathogens, bacteria, mold and yeasts, viruses and parasites. It is impressive.In recent years, ozone has been used as a suitable substitute for common disinfectants in the fields of preserving and storing agricultural products, washing agricultural products, eliminating pests and field diseases, disinfecting food products and water used in food industry processes.[4]. Ozone or active oxygen destroys dangerous substances such as fungal toxins, viruses, yeasts and algae at the molecular level (by attacking the ring structure of the poison and opening it). Despite the reported effect of ozone on the inactivation of fungi in some cases, especially in inappropriate concentrations, ozone can cause the progress and acceleration of oxidation or chemical decomposition of compounds in corn seeds. Changes such as oxidation of starch, fat, change in the amount of protein, discoloration, and loss of germination power can occur in the seed due to excessive use of ozone [2 and 5]. In the study of the effect of ozone on preventing the production of dioxynivalenol, it was reported that ozone gas was significantly effective in inactivating Fusarium fungus and caused a decrease in dioxynivalenol contamination at a concentration of 600 mmol/mol of ozone gas in 180 minutes. The use of this concentration can reduce the germination power of wheat by 12.5%, but it did not have a significant effect on the nutritional value and qualitative properties of the examined grain (wheat)[6]. In another study, the effect of ozone gas to prevent Fusarium growth and fumanzin production in corn seeds was investigated. The results showed the effect of ozone gas on contaminated samples in concentrations ppm 100 and 200 in the period of 60 and 30 minutes have well inhibited the growth of spores [7]. Today, the use of appropriate concentrations of ozone is important because some physicochemical and qualitative properties of corn may change with increasing gas concentration and exposure time. Therefore, in this research, the effect of ozone gas on the qualitative and microbial characteristics of corn has been investigated with the appropriate concentration and exposure time approach.

2- Materials and methods

2-1-preparation of corn sample

The corn sample under investigation is one of the dominant cultivars of Golestan region (No. Single $Cross_{407}$) was grown with proper agricultural management at Gorgan research station. The samples were harvested at the end of October and after separating the impurities, they were dried to a moisture content of 8%.

1. Aflatoxin
2. Fumonisin
3. Deoxynivalenol
4. Zearalenon

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2-2- Azandhi method

Ozone was produced electrostatically by an ozone generator. Corn drying was carried out in polyethylene cylindrical containers with a capacity of 10 kg and equipped with full rubber caps and gas inlet and outlet pipes. When the gas concentration of the chamber reached the desired level, it was stopped by sealing the chamber, and the gas injection continued until the output ozone concentration was stable. Exposure was evaluated with two main variables of ozone concentration (25, 50, and 75 ppm) and duration of exposure (1, 3, 5, and 7 days) by examining changes in the quality and microbial characteristics of corn. Control sample tests were also performed under similar conditions without ozone gas injection.

2-3-exams

2-3-1-Determining the amount of fungal contamination

50 grams of sample was mixed with 450 milliliters of sterile phosphate buffer for 2 minutes in a shaker and then by transferring 1 milliliter of the sample to 9 milliliters of sterile phosphate buffer solution with dilutions of 0.1, 0.01, 0.001. of each dilution using surface culture[5] to PDA culture medium[6] (Merck, Germany) was transferred. The samples were kept in an incubator with a temperature of 25 degrees Celsius for 5 days, and at the end of this period, the degree of contamination of the seeds was reported as a single colony per gram (cfu/g) [8].

2-3-2-Determining the amount of fungal poison

High-performance liquid chromatography and immunoaffinity column purification were used to detect and determine the amount of aflatoxin in the samples (in micrograms/kg) [9].

2-3-3- Determination of qualitative changes

2-3-4-Acid index

Fat was extracted from 10 grams of corn flour sample by Soxhlet machine and hexane solvent. The extracted fat was extracted in the presence of phenolphthalein until a pink color was obtained with potassium solution of 0.1 normal titer and the acid number was expressed as a percentage according to the amount of potassium consumed [10].

2-3-5- amount of starch carboxyl

Starch isolation was done according to the method of Weiner et al. (2012). The obtained starch was dried in an oven at a temperature of 40 degrees Celsius for 12 hours and was used to measure the amount of carboxyl**..** The amount of isolated starch carboxyl was measured according to the method of Chattopadhyay et al. (1997) and titration against 0.1 normal soda against the control [11, 12].

2-3-6-the amount of protein

The amount of seed nitrogen was measured using a fully automatic microcaldal device. Then, the amount of nitrogen was calculated and the conversion factor of the protein content of the samples was calculated [10].

2-4- Statistical analysis

The data obtained from the experiments were analyzed using a factorial test, with two treatments of concentration and ozonation time in the form of a completely randomized design and in three replications. Average main and interaction effects were analyzed through Duncan's multiple range test at the 5% level.

3. Results and Discussion

3-1- The effect of ozone on the amount of fungi in corn seeds

Table 1 shows the mutual effect of different soil conditions on the amount of fungal contamination of corn seeds. Statistical analysis of the results*Expressing the significant effect of the factors*Concentration, life time and their interaction were significant (P<0.05).

5. Spread-plate

6. Potato Dextrose Agar

Table 1 Effect of ozonation time and concentration on the fungi content of corn seed (cfu/g×10^3)

Ozone concentration(ppm)	Ozonation time (day) 1	3	5	7
25	2.3±0.46[It is]	1.6±5.5[d]	0.9±0.26[c]	0.6±0.21[c]
50	0.25±0.07[b]	0.19±0.06[ab]	0.13±0.08[a]	0.12±0.05[a]
75	0.12±0.03[a]	0.11±0.05[a]	0.10±0.04[a]	0.10±0.03[a]
(Control)	3.4±0. 4[f]			

*Numbers with different letters indicate significant differences in the 5% level of probability.

The results of the effect of the life time factor, regardless of the ozone concentration, showed that there was a significant decrease in the spread of fungi by increasing the ozone concentration from 25 to 50 ppm in 3, 5, and 7 days, while in all the mentioned times, increasing the concentration above 50 ppm had no effect (Figure 1).

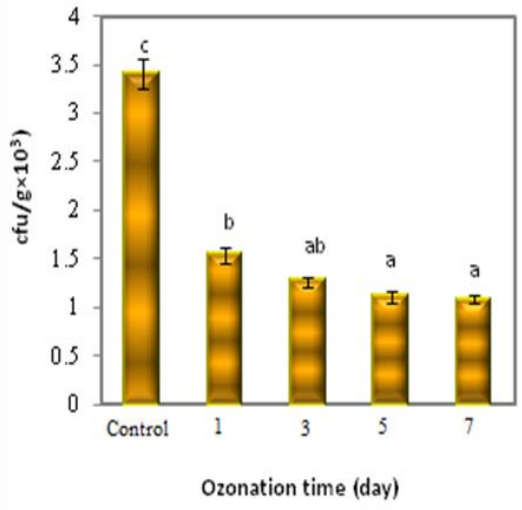

Fig 1 Ozonation time effect on the growth of fungi (P<0.05).

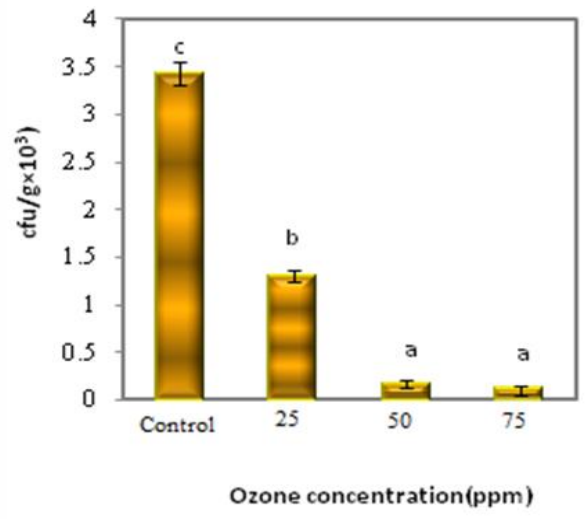

Fig 2 Ozonation concentration effect on the growth of fungi (P<0.05).

The results of the effect of the main factor of ozone concentration, regardless of the exposure time, showed that the growth of fungi was significantly reduced with the increase of ozone concentration, but the comparison test of means indicated that there is no significant difference between the concentrations of 50 and 75 ppm (P<0.05). . The results of the statistical analysis at the concentration of 25 ppm at different times of life showed a decrease in the growth of fungi, so that their rate was 2.3 (unit colony per gram x 10^3) in 1 day of life to 0.6 (unit colony per gram x 10^3) in 7 days of life has decreased. At the concentration of 50 ppm, the growth of fungi decreased with the increase of the living time (P < 0.05). At the concentration of 75 ppm, the decrease in the growth of fungi was not significant between 1 and 7 days (Figure 2). Therefore, it can be concluded that concentration is usedppm 50 for 3 days, the conditions were suitable to reduce the growth of fungi in corn seeds, so that in these conditions, the amount of fungi from 3.4 (one colony per gram x 10^3) in the control sample to 0.19 (one colony per gram x 10^3) is limited.Mason et al. (2005) in the study of the effect of ozone on the control of pests and fungal toxins reported that low concentrations of ozone were significantly effective in inactivating the Fusarium fungus and they stated that a concentration of 25 ppm prevented surface growth, sporulation and the production of fungal toxins by the Fusarium fungus. Is[13]. Mailona et al. (2016) in the study of the effect of ozone gas to inhibit Fusarium growth and fumanzin production in corn seeds showed that the proximity of ozone gas to contaminated samples in concentrations of 100 to 200 mg/kg effectively inhibited the growth of spores and caused a decrease in fungal contamination up to The level is 94%, which is in line with the results of this research [7].

2-3- The effect of ozone on the amount of aflatoxin in corn seeds

Table 2 shows the mutual effect of different conditions on the amount of aflatoxins in corn seeds. Statistical analysis of the results*Expressing the significant effect of the factors*The concentration, residence time and their interaction effect on the amount of aflatoxins in corn seeds were (P<0.05).

Table 2 Effect of ozonation time and concentration on the aflatoxin content of corn seed (ug/Kg)

Ozone concentration(ppm)	Ozonation time (day) 1	3	5	7
25	9.24±0.56[d]	5.15±0.41[c]	3.74±0. 6[b]	2.63±0.52[a]
50	2.14±0.33[a]	ND	ND	ND
75	ND	ND	ND	ND
(Control)	19.15±0.53[It is]			

*Numbers with different letters indicate significant differences in the 5% level of probability.
ND: Non-detectable

Comparing the averages showed that the decreasing trend of aflatoxin production between 3, 5 and 7 days is not statistically significant (Figure 3).

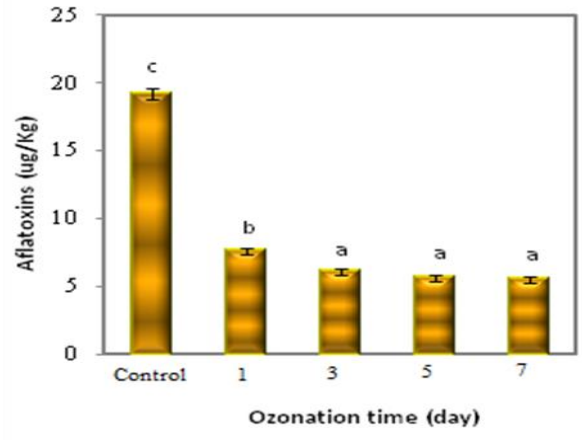

Fig 3 Ozonation time effect on the Aflatoxins (P<0.05).

The results indicate that the injection of ozone gas has significantly reduced the production of aflatoxins compared to the control sample. Increasing the concentration from 25 to 75 ppm has also caused a significant decrease in aflatoxins, so that its amount has reached from 5.11 micrograms per kilogram at a concentration of 25 ppm of ozone gas to unmeasurable at a concentration of 75 ppm (Figure 4).

The comparison test of the means showed that there was no significant change in the amount of aflatoxins at the concentration of 50 ppm with the increase of the exposure time. (P<0.05). In other words, the effect of the ozone concentration factor is more important than the yield time. Therefore, it can be concluded that using a concentration of 50 ppm for 3 days was the right condition in terms of controlling the spread of aflatoxins in corn seeds, and in this condition, the amount of aflatoxins was unmeasurable.

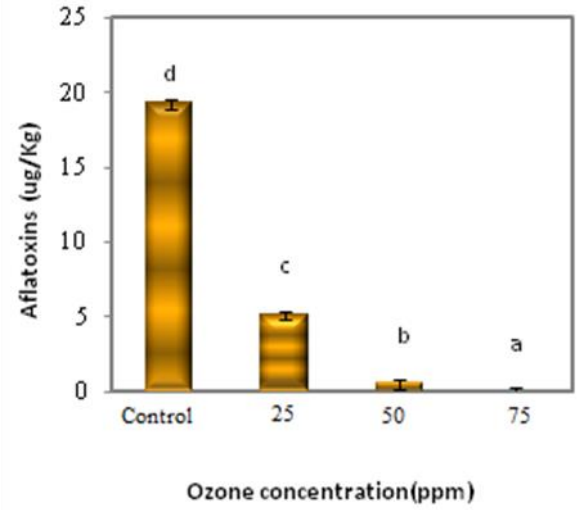

Fig 4 Ozonation concentration effect on the Aflatoxins (P<0.05).

The change in ozonation concentration and time depends on the type of grain and its structure. Corn has a more porous pericarp and endosperm structure than other grains, and ozone gas can penetrate more easily. Ozone is a strong oxidizing agent that connects transversely with the double bands 8-9 of the furan ring of aflatoxins and is electrophilically absorbed by the furan ring and is able to completely decompose aflatoxins in a few minutes [14]. The results of the research of Proctor and colleagues (2004) also showed the destruction of aflatoxins in peanut kernels exposed to ozone gas (destruction of 87% of aflatoxin B1 and 90% of aflatoxin G1), which confirms the results of this research [15].

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3-3- The effect of ozone on the spiciness of corn seed oil

resultsExpressing the significant impact of the effects of the main factorsConcentration, the interaction effect of concentration and timing and with a lesser intensity the effect of the timing factorOnThe spiciness of corn seed oil was high (P<0.05).

Table 3 Effect of ozonation time and concentration on the acidity Value of corn seed (%)

Ozone concentration(ppm)	Ozonation time (day) 1	3	5	7
25	0.46±0.04[a]	0.46±0.03[a]	0.47±0.03[a]	0.48±0.04[a]
50	0.49±0.05[a]	0.49±0.04[a]	0.53±0.05[ab]	0.56±0.02[b]
75	0.54±0.04[b]	0.63±0.02[c]	0.7±0.03[d]	0.83±0.03[It is]
(Control)	0.46±0.05[a]			

*Numbers with different letters indicate significant differences in the 5% level of probability.

Effect of ozone concentration factor On acidity Corn seed oil indicated that with the increase of ozone concentration from zero to 75 ppm, the acidity level increased. The results of the mean comparison test showed that this increase was not significant up to the concentration of 50ppm and above that concentration, the changes with the increase in the residence time of the acidity in corn significantly increased compared to the control sample. The average comparison test showed that between the incubation times from 1 to 7 days, this increase was not significant until 3 days, but this increase was significant at 7 days (Figure 5). At the concentration of 25 ozone gas, the effect of ozone on increasing acidity was not significant at all exposure times. At the concentration of 50 ppm, the acidity level did not change significantly with the increase of the aging time until 3 days (P<0.05) and there was a significant increase only in the 5th and 7th days of aging (Figure 6). Therefore, it can be said that the use of ozone concentration up to 50 ppm and exposure time up to 3 days is recommended in terms of controlling the rancidity of oil in corn seeds. Oil oxidation may occur due to the oxidation of unsaturated fatty acids of seed oil by ozone gas. Ozone gas can react with the chemical compounds in the grain and depending on the concentration and duration of exposure, it can cause oxidative changes in the grain [6]. Similarly, Prudent and King (2002) also did not observe a significant change in the fatty acid profile of corn and its oxidative stability [15]. Wang et al. (2008) reported that low concentrations of ozone gas have no effect on the oxidation of corn oil, but higher concentrations caused oxidation damage in the seeds, which confirms the results of this research [16].

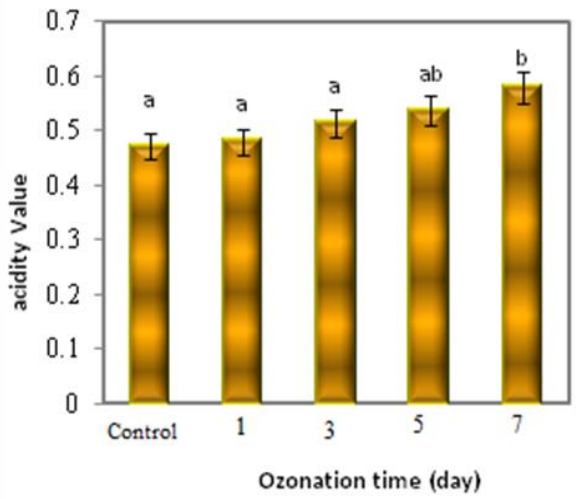

Fig 5 ozonation time effect on the acidity value (P<0.05).

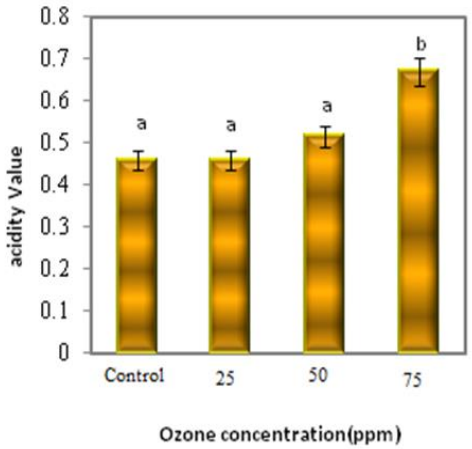

Fig 6 ozonation concentration effect on the acidity value (P<0.05).

3-4- The effect of ozone on the changes of corn starch

Table 4 shows the mutual effect of different drying conditions on the amount of carboxyl formation in corn starch. Statistical analysis of

the results*Expressing the significant impact of the effects of the main factors*Concentration is the timing factor and the interaction effect of concentration and time (P<0.05).

Table 4 Effect of ozonation time and concentration on the Carboxyl content of corn seed (%).

Ozonation time (day)				
7	5	3	1	Ozone concentration(ppm)
0	0	0	0	25
0.058±0.01[a]	0	0	0	50
0.091±0.017[c]	0.082±0.025[b]	0.079±0.024[b]	0.061±0.015[a]	75
0				(Control)

*Numbers with different letters indicate significant differences in the 5% level of probability.

The test comparing the averages showed that although carboxyl was formed from day 1, its increase and changes at different times up to day 5 were not significant compared to the control (Figure 7).

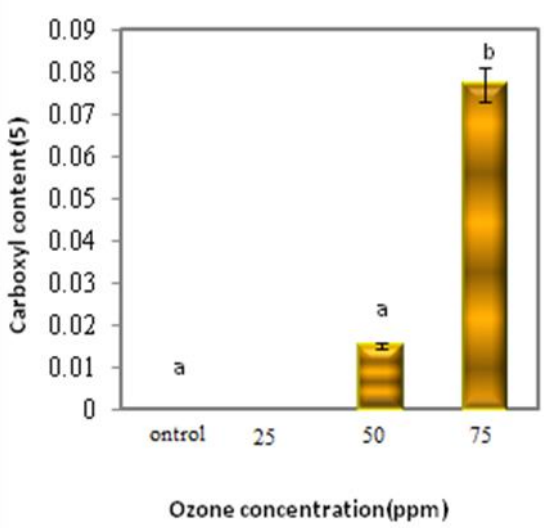

Fig 7 Ozonation time effect on the Carboxyl content (P<0.05).

With the increase of ozone concentration from zero to 50 ppm, the amount of carboxyl did not change significantly (P<0.05). The results of the mean comparison test showed that ozone gas at a concentration of 75 ppm significantly caused the formation of carboxyl compared to the control sample, so that its amount was 0.078%. has arrived (Figure 8). It should be noted that the increase in incubation time had no significant effect on the amount of carboxyl until the 5th day of incubation (P<0.05). The results clearly show that concentrations higher than 50 ppm along with times higher than 5 days can cause oxidative changes of starch in corn kernels. The concentration of 25ppm has no effect on the amount of carboxyl in all life times and its value is zero. At the concentration of 50 ppm, carboxyl was not formed by increasing the aging time until 5 days, and its value is zero.

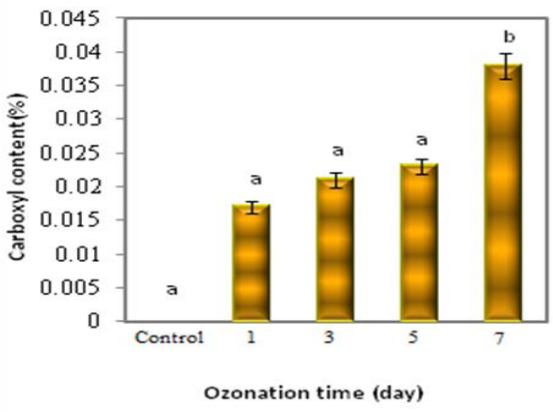

Fig 8 Ozonation concentration effect on the Carboxyl content (P<0.05).

No significant change was observed in the concentration of 75 ppm compared to the control until 1 day, and there was a significant increase only in 3, 5 and 7 days (P<0.05).

In other words, the effect of high ozone concentrations and long exposure times has caused the oxidative change of starch. so It can be concluded that the use of ozone at a concentration of 25 ppm at all times and at a concentration of 50 ppm up to 5 days and at a concentration of 75 ppm up to 1 day can be applied in terms of the absence of formation of carboxyl groups in corn starch.

After prolonged exposure to ozone, the gas may penetrate the grain starch and cause its oxidation. Since corn grain has a relatively high amount of starch, the oxidation of this part of the grain is a possible side effect of ozone gas, which causes a decrease in grain quality [6]. The results of Vanir et al. (2012) did not show a significant change in the crystallization of starch in different drying conditions, and the carboxyl changes were not significant [11]. Savi et al. (2014) also reported the changes in the amount of carboxyl and carbonyl of wheat

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starch after applying 60 to 120 micromol/mol of ozone in the range of 0 to 0.079 and 0.198 to 0.523%, respectively, which is a confirmation in line with The results of this research are [8].

5-3- The effect of ozone on the amount of corn grain protein

The interaction effect of different moisture conditions on the amount of corn grain protein changes in different moisture conditions compared to the control sample is shown in Table 5. As can be seen, protein changes were in the range of 10.41 to 9.24% in different living conditions (P<0.05).

Table 5 Effect of ozonation time and concentration on the Protein content of corn seed (%).

Ozonation time (day)				
7	5	3	1	Ozone concentration(ppm)
10.40±0.29[a]	10.39±0.4[a]	10.41±0.35[a]	10.40±0.21[a]	25
10.36±0.31[a]	10.35±0.40[a]	10.37±0.56[a]	10.38±0.36[a]	50
9.24±0.31[c]	9.63±0.51[b]	10.20±0.44[a]	10.31±0.38[a]	75
10.44±0.30[a]				(Control)

*Numbers with different letters indicate significant differences in the 5% level of probability.

The results of the investigation of the life time factor also showed that with the increase of the life time, the changes in the amount of protein compared to the control were not significant (P<0.05). The average comparison test showed that there was no significant difference between the aging times from 1 to 7 days (Figure 9).

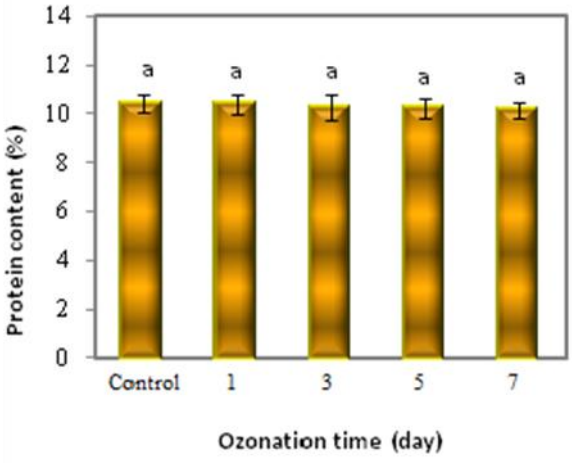

Fig 9 Ozonation time effect on the Protein content (P<0.05).

Examining the process of protein changes affected by the ozone concentration factor alone showed that with increasing ozone concentration from zero to 75 ppm, protein changes are small. The average comparison test showed that the changes in the amount of protein up to the concentration of 50 ppm were not significant compared to the control sample, and at the concentration of 75 ppm, the amount of protein reached 9.86% and it decreased by 0.56% compared to the control (Figure 10).

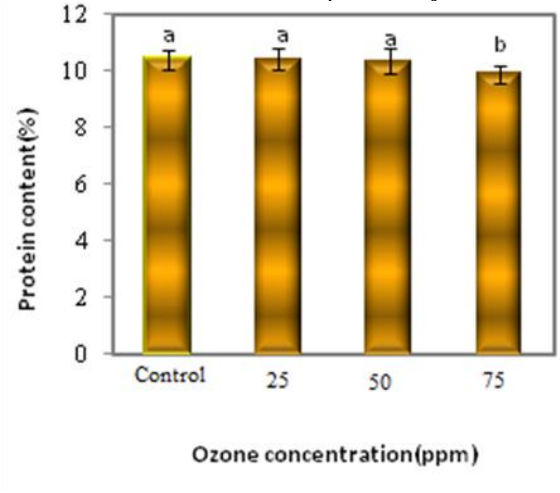

Fig10 Ozonation concentration effect on the Protein content (P<0.05).

Proteins, as one of the dominant components in corn, can be attacked by oxygen free radicals, and high concentrations of ozone increase the rate of protein decomposition [18]. Wang et al. (2008) showed that at low concentrations of ozone gas (less than 50 ppm) there is no difference between the amount of protein in the irradiated samples compared to the control corn, which is in line with the results of this research [17].

4 - Conclusion

Ozone gas in high concentrations can cause oxidation or decomposition of chemical compounds in corn seeds. According to the results of this research, ozone gas is effective in inhibiting the spread of fungi and fungal toxins in corn seeds, and no significant change in the physico-chemical properties of corn seeds has been achieved at the optimal concentration and time.

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6- Resources

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ارزیابی تاثیر گاز ازن بر ویژگی‌های کیفی و میکروبی دانه ذرت

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چکیده

تولید ذرت بعد از گندم مقام دوم را در بین غلات داشته و معمولاً بخش زیادی از آن بلافاصله پس از برداشت مورد استفاده قرار نمی‌گیرد، بلکه برای استفاده تدریجی در فصول دیگر و یا به‌منظور صادرات به سایر مناطق، در انبارها نگهداری می‌شود. گسترش انواع قارچ‌ها و به‌دنبال آن تولید سم‌های قارچی به‌خصوص در مناطق با رطوبت بالا، علاوه بر خسارت کمی و کیفی به محصولات انبار شده سبب افزایش ضایعات و به‌مخاطره انداختن سلامت جامعه می‌شوند. در راستای جایگزینی روش‌های کم‌خطر افزایش عمرانبارمانی، در این تحقیق تاثیر گاز ازن با دو متغیر غلظت ازن (۲۵، ۵۰ و ۷۵ ppm) در مدت زمان ازن‌دهی (۱،۳، ۵ و ۷ روز) بر دانه ذرت غالب منطقه گلستان (رقم سینگل‌کراس۴۰۷) ارزیابی شد. همچنین ویژگی‌های میکروبی به لحاظ کنترل گسترش قارچ‌ها و تغییرات خصوصیات کیفی دانه بررسی شد. نتایج نشان داد، افزایش غلظت ازن تا ۵۰ ppm و زمان ازن‌دهی۳ روز بر کاهش رشد قارچ‌ها و تولید آفلاتوکسین معنی‌دار بوده است ($P<0/05$). همچنین نتایج حاکی از آن بود استفاده از غلظت ۷۵ ppm در زمان‌های۱، ۳، ۵ و ۷ روز سبب تغییرات اکسیداتیو معنی‌داری در خصوصیات روغن (افزایش شاخص اسیدیته) و نشاسته (افزایش شاخص کربوکسیل) دانه ذرت نسبت به نمونه شاهد شده است. همچنین شرایط مختلف ازن‌دهی تا غلظت ۵۰ ppm در زمان‌های مختلف تاثیر معنی‌داری بر میزان پروتئین دانه ذرت نداشت.