

OZONE-BASED GRAIN STORAGE: A GREEN TECHNOLOGY WITH GREAT POTENTIAL FOR IMPROVING FOOD SAFETY

Abstract

Ozone technology is increasingly being used in the grain storage sectors. Insects and other pests pose a serious problem when grain is kept in metal silos in bulk. Ozone gas is the preferred and most feasible approach for treating grains while they are stored in bulk since it leaves no residue and is environmentally beneficial. This chapter addresses the effects of ozone treatment on grain that has been stored and highlights the significance of treatment durations and ozone cycle frequency on a variety of grain properties, including insect population (adult/kg), germination percentage, moisture content, carbohydrate, starch, colour, and odour of various food grains (such as corn, wheat, rice, and other minor grains). It becomes clear that ozone has a high capacity to preserve grain storage quality while guaranteeing food safety.

Keywords: Ozone, ozone exposure time, ozone cycle, bulk storage, grain storage, germination, starch, carbohydrate.

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I. INTRODUCTION

India's economy is heavily reliant on agriculture. Agriculture and related economic activities employ 54.6 percent of the total workforce. (Census 2011). This rapid economic growth is expanding and diversifying the demand for food grains. According to the fourth advance projections for 2021–22, the nation is likely to produce 315.72 million tonnes of food grains. The production of food grains in 2021–2022 is 25 million tonnes higher than the average production during the preceding five years (2016–2020). The storage of grain is an old age practice since the beginning of civilization. Major food grains (rice, jowar, bajra, maize, ragi, small millets, wheat, barley) growing states in India are Karnataka, Andhra Pradesh, Telangana, Maharashtra, Uttar Pradesh, Madhya Pradesh, Tamilnadu, Bihar, Gujarat and Haryana. As far as production and productivity are concerned, grain storage garners the interest of policy makers, scientists, development workers, growers, and marketing agencies.

An FAO estimate of world-wide annual losses in store has been given as 10% of all store grain. Threats posed by more than 70 insect pests to grains and cereal products kept in storage facilities have been identified. These insect pests are thought to harm the grain yield on a yearly in the range of 10% to 40%. As a result, maintaining store quality and effective administration is urgently necessary (Upadhyay and Ahmad, 2011). Increased demand of grains is a natural consequence of increasing global population. Due to increased urbanization and industrialization, agricultural land has substantially diminished. The traditional chemical insecticide used in storage has highly dangerous for health also ecologically unsound. Infestation of pests and insects during bulk storage of grains has compelled us to investigate non-chemical alternatives to disinfestation during bulk storage of grain. Ozone gas disinfestation technique is non-chemical green alternative of chemical disinfestation technique.

The presence of stored-product insects such as beetles, moths (including Indian meal moths and almond moths), angoumois grain moths, and various species of psocoptera has posed a significant challenge to maintaining the quality of stored grains. The economies of both developed and developing countries are reliant, either directly or indirectly, on the storage of cultivated grains due to their connection to millions of people. The main problem with bulk storage structures is maintaining optimum temperatures and humidity in the storage environment (Sawant *et al.*, 2012). The fundamental goal of storage is to effectively safeguard the quality of stored grain, primarily by preventing its deterioration.

Insects not only consume a significant portion of stored grain, but they also contribute to other degrading conditions that reduce the grain's market value. Several factors contribute to the overall quality of the grain, including damaged kernels that can impact its reception, the promotion of the growth of other deteriorating organisms such as mold, and contamination of the grain with their excrement, discarded skin, and other byproducts of their life cycle, resulting in the emission of unpleasant odors. Spores of molds are microorganisms that tend to grow in stored grain. They can be found on the surface of the grain, in the air, and within storage structures. In general, pesticide exposure does not have a direct effect on larval developmental stages in insect eggs. While fumigation affects these developmental stages, it also poses challenges due to its high levels of toxicity and potential for the emergence of resistant harmful insect species (Rees, 1996). Methyl bromide use was prohibited in 1995 as a result of an international agreement, linking it to a depletion of the ozone layer in the Earth's

stratosphere (Johnson *et al.*, 1998). There is a need for innovative insect control techniques because there are few options for pest control in storage and a high risk of pesticide resistance (Zettler *et al.*, 1989). The use of ozone, which has shown promise in earlier experiments, is one potential strategy.

II. TRADITIONAL FUMIGANTS USED IN STORAGE

Fumigants are often used as a treatment in stored grains. These fumigants are airborne chemicals that enter insects' body through their spiracles and kill them. According to Kenkel *et al.* (1991), many administrators of silos applied fumigant based more on habit or convention than on actual proof of the need for better storage. Bell (2000) worked on phosphine and methyl bromide fumigants and concluded that both have issues of continued development of resistance and restricted access. Athanassiou *et al.* (2005) discovered that Azadirachtin effectively controlled *S. oryzae*, *R. dominica*, and *T. confusum*, but the required dosage was considerably higher than that of the currently used grain protectants. Daghli *et al.* (2014) investigated that phosphine resistance problem was increasing and insect populations containing resistant individuals can be common, and there were indications of the emergence of more potent or novel forms of resistance. Kaur and Nayak (2015) studied the estimated duration it would take for a highly phosphine-resistant *C. ferrugineus* population to become extinct, finding that it was 10 days at 2 mg/L (1400 ppm) and 25 °C. Athanassiou *et al.* (2015) investigated methyl bromide efficacy under laboratory conditions. It was found that during 48-hour fumigations in the absence of substantial grain quantities, eggs exhibited greater resistance to psocid control compared to adults. The use of methyl bromide decreased due to its classification as a substance contributing to ozone depletion.

III. TOXIC EFFECTS OF FUMIGANTS

Different diseases may be brought on by the acute or long-term toxic effects of fumigants. Boon *et al.* (2008) determined that organophosphate, organochlorine, and similar pesticides interfere with nerve function, leading to paralysis and potentially fatal outcomes. Insecticides may cause severe symptoms that include salivation, meiosis, urine, diarrhea, diaphoresis, and excitement of the central nervous system, according to research by Mishra *et al.* (2014). Pesticides can lead to various specific effects, including harm to the central and peripheral nervous systems, the development of cancer, the onset of allergies and heightened sensitivity, reproductive issues, and disturbances in the immune system, among others. In accordance with Neme and Satheesh (2016), 34% of the 380 samples of cereal grains tested in Poland contained pesticide residues.

IV. NATURE OF OZONE AND OZONE GENERATING AND SUPPLYING SYSTEM

Ozone in its pure form is a volatile, potentially explosive, blue gas that is toxic in the atmosphere. The strong oxidant properties of ozone gas has been widely utilized for decontamination of food grains, beverages, medical and water supply industry. In order to eliminate microorganisms in stored grain, such as mold and mycotoxin, ozone is employed as an alternative to pesticides (Mendez *et al.*, 2003). Ozone created artificially decomposes quickly, making it impossible to store or transport. Therefore, it needs to be continuously produced (Miller *et al.*, 1978). Utilizing corona discharge (CD), ultraviolet (UV), or water electrolysis, ozone can be created fast on-site when necessary (Kim *et al.*, 1999). Kumar *et al.*

(2021) observed that chemicals are effective on pest of storage grains but dangerous for health. Comparing ozone gas to chemical pesticides, there are various safety benefits. Ozone transforms into oxygen because of its brief half-life and does not leave behind any chemical residue on the surface of food. This chapter's major goal is to investigate how ozone affected certain food grain qualities while they were being stored in bulk. However, chemical insecticides have drawbacks for the human body as well. In order to avoid losses when storing food grains, ozone is suggested.

Table 1: Summary of Major Stored Grain Insects and its Ozone Treatment

Sr. No.	Insects	Description	Infest	Ozone doze	References
1.	Rice Weevils (Sitophilus oryzae)	Small, reddish-brown beetles	Rice, wheat, barley, and oats	1.0–2.0 g/m ³ and exposure time of 2–6 h.	Srivastava, 2021
2.	Lesser grain borer (Rhyzopertha dominica)	dark reddish brown	Wheat, corn, rice and millet.	2.5g m ⁻³ for 8h.	Mishra <i>et al.</i> , 2019
				0.428 g/m ³ (200 ppm) ozone for 6 h	Abdelfattah <i>et al.</i> , 2023
				0.42 g/m ³ for 72 h	Xinyi <i>et al.</i> , 2018
3.	Granary Weevils (Sitophilus granarius)	Granary weevils infest a wide range of grains	Stored wheat and other cereal crops	0.002 ppm, equaling 300 mg (5 mg/L) and 2 hours	Lemic <i>et al.</i> , 2019
4.	Indian Meal Moths (Plodia interpunctella)	Common pantry pests	Stored grains, as well as flour, cornmeal, nuts, and other food products	6 ppm and 50 min	Syedabadi <i>et al.</i> , 2021
5.	Sawtoothed Grain Beetles (Oryzaephilus surinamensis)	Small, brown beetles	Stored products, including cereals, flour, and dried fruits.	5 ppm for 15 to 90 min	Sadeghi <i>et al.</i> , 2017
6.	Maize Weevils (Sitophilus zeamais):	small, brown weevil has four reddish-brown spots on the wing covers	Corn and maize-based products.	50 ppm ozone for 3d	Kells <i>et al.</i> , 2001
7.	Red Flour Beetles (Tribolium castaneum)	Widespread pests	Flour, cereals, and other grain-based products.	50ppm ozone for 3d	Kells <i>et al.</i> , 2001

1. Generation of Ozone: Splitting a diatomic oxygen molecule is the first step in the production of ozone. When it combines with another diatomic oxygen molecule, the resultant oxygen free radical can be utilized to generate the three-atom ozone molecule. Nonetheless, a substantial quantity of energy is required to disrupt the O-O bond. The production of ozone can be initiated through corona discharge methods and ultraviolet light of 188 nm wavelength to initiate the formation of free radical oxygen. Corona discharge is typically utilized to produce ozone at commercially viable quantities. (Rice *et al.*, 1981). In corona discharge, there are two electrodes: one is the high-voltage electrode, and the other is the low-voltage electrode.

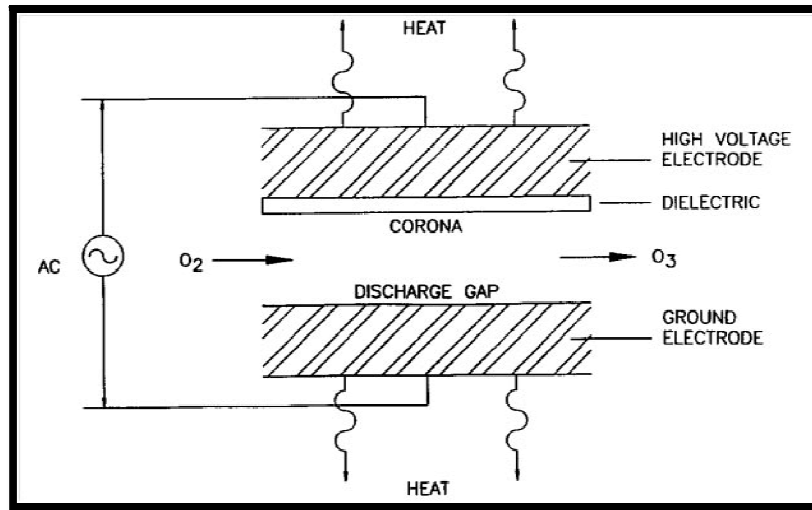


Figure 1: Schematic Diagram of Generation of Ozone by Corona Discharge Method (Rice *et al.*, 1981).

Narrow discharge gap was provided in between electrodes (Fig.1). Some of these collisions occur, and when the electrons possess sufficient kinetic energy to break the oxygen molecule, each oxygen atom can generate an ozone molecule. When air is introduced as the feed gas into the generator, it can produce ozone at a concentration of 1-3%. However, employing pure oxygen as the feed gas can increase the ozone yield to as much as 6%. (Rice *et al.*, 1981). Ozone gas cannot be kept because it spontaneously breaks down into oxygen atoms, according to Coke's (1993) research.

V. MECHANISM OF OZONATION ON PEST CONTROL

Ozone is an exceedingly unstable molecule and quickly breaks down into O₂, liberating a highly reactive single oxygen atom. This lone oxygen atom interacts with the cell membranes of bacteria or viruses, causing harm to cellular components and disrupting their regular cellular functions. If ozone contacts a volatile organic compound the free oxygen atom reacts with it, removing the odor. (Mason *et al.*, 2006). Even at low concentrations, ozone leads to oxidative damage to tissues, resulting in DNA strand fractures, changes in pulmonary function, heightened bronchial reactivity, membrane oxidation, or mutations in vivo (Liu *et al.*, 2007).

Effect of Ozonation on Various Properties of Grains

1. Effect of Ozone on Insect Population (adult/kg) During Storage: The primary objective of using ozone in storage was to attain a substantial mortality rate among pests while minimizing any alterations in the quality of stored grains. Ozone is employed within the grain processing industry as a replacement for conventional fumigants such as methyl bromide and phosphine in order to manage storage pests. According to research by Sousa *et al.* (2008), ozone as a fumigant can be effective to kill stored-grain pests like *Tribolium castaneum*, *Rhizopertha dominica*, *Oryzaephilus surinamensis*, *Sitophilus oryzae*, and *Ephestia elutella*. From the Grain Inspection Handbook (2004), “infested” designation recommended by FGIS on the basis of insects per Kg of grain. Various ozone treatments were used based on various insects of storage and grain. Many studies have investigated the use of lower ozone concentrations for extended exposure durations. However, it was found that the most effective and efficient approach is to employ high ozone concentrations for shorter exposure periods, leading to cost savings and energy efficiency.

Kells *et al.* (2001) evaluated the efficiency of ozone fumigation in a corn grain bulk of approximately 9 tons against adult insects such as the red flour beetle, maize weevil, and Indian meal moth larvae. They reported that exposing the grains to 50 ppm ozone for 3 days resulted in mortality rates ranging from 92% to 100% for adult insects and a lower dose was found to be effective, albeit with slightly lower insect mortality rates, which ranged from 77% to 99.9% depending on the species of insect. Maier *et al.* (2006) explored ozone concentrations ranging from 0 to 2000 ppm. Their research demonstrated that exposing grains to 50 ppm of ozone for three days resulted in a 100% mortality rate among insects. Mason *et al.* (2006) observed that at elevated concentrations, adult red flour beetles, confused flour beetles, and maize weevils exhibited a mortality rate of 100%, and there was also a significant decrease in their emergence.

Life stage of insects also affect the efficacy of ozone on them. Eggs and larva stages are susceptible to ozone as compared to pupa and adult stage. Pupa and adult stage of insects are active stage during their life span. That’s why ozone effectively affect those stages of insects. Bonjour *et al.* (2011) concluded from ozonation during wheat storage that eggs of insects were not affected by ozone at all exposure periods. Throughout the first two months of storage, the insects population remained largely constant. Infested wheat samples started to produce more debris and have more damaged kernels starting in the third month. The fact that *S. granarius* takes 30–40 days to develop from egg to adult is the cause of population stasis (Keskin and Ozkaya, 2015). Insect population per kg of sample after 2, 4 and 6 months of storage of rice storage was 2.75, 7.62 and 20.43, respectively. (Seadh *et al.*, 2015). Kalsa *et al.* (2019) detected number of live insect per kg sample of wheat storage in metal silo at the end of 2, 4, 6 months. They measured only live *R. dominica* adult species detected through the 6 month duration which were 11 insects/kg after the 2 months, 1 insects/kg after the 4 months and 89 insects/kg after the 6 months in metal silo of 100 kg size. For *C. maculatus* insects, treatment with 1000 ppm ozone for 5 days straight resulted in 100% insect mortality (adult and egg) (Nickhil *et al.*, 2021).

Table 2: Influence of Ozone on Wheat Germination

(Shingala AM and MN Dabhi, 2022, Shingala *et al.*, 2022¹, Shingala *et al.*, 2022²)

Storage period		30 days			60 days			90 days			120 days		
Grain properties		Germination %	Total carbohydrate %	Starch content (%)	Germination %	Total carbohydrate %	Starch content (%)	Germination %	Total carbohydrate %	Starch content (%)	Germination %	Total carbohydrate %	Starch Content (%)
Control		99.50	78.94	68.50	96.50	78.88	68.44	94.50	78.76	68.39	93.50	78.75	68.34
Ozone Exposure time (T)	30 min	98.17	78.73	68.07	98.66	78.59	67.95	97.50	78.33	67.70	96.66	78.10	67.65
	60 min	99.17	78.54	67.95	99.50	78.41	67.83	98.50	78.19	67.59	97.33	77.94	67.54
	90 min	97.66	78.18	67.78	98.33	78.005	67.33	97.16	77.74	67.44	96.50	77.42	67.39
	120min	97.5	77.70	67.57	98.16	77.45	67.44	96.66	77.14	67.23	95.66	76.77	67.17
	CD	0.867	0.401	0.264	0.63	0.448	0.265	0.86	0.382	0.265	0.86	0.317	0.266
	SEM	0.276	0.129	0.085	0.20	0.144	0.085	0.27	0.123	0.085	0.27	0.102	0.085
Ozone Cycle (C)	7 days	97.37	77.95	67.46	98.00	77.68	67.32	97.00	77.30	67.08	96.37	76.89	67.02
	14days	98.12	78.34	67.91	98.75	78.18	67.57	97.12	77.93	67.57	95.87	77.61	67.52
	21days	98.87	78.57	68.15	99.25	78.47	68.03	98.25	78.39	67.83	97.37	78.18	67.77
	CD	0.74	0.34	0.22	0.55	0.388	0.230	0.746	0.331	0.230	0.74	0.274	0.23
	SEM	0.239	0.111	0.073	0.177	0.176	0.074	0.239	0.106	0.074	0.239	0.088	0.07
T*C	CD	N/A	N/A	N/A	N/A	N/A	0.459	N/A	N/A	N/A	N/A	N/A	N/A
	SEM	0.479	0.223	0.147	0.354	0.249	0.147	0.479	0.212	0.148	0.479	0.176	0.14
C.V.%		0.68	0.342	0.307	0.506	0.380	0.308	0.694	0.352	0.309	0.701	0.310	0.30

2. Influence of ozone gas on Germination %: When a natural seed aging event happens, the time of seed storage can be one of the factors that causes seed deterioration. Temperature and storage period made a highly significant influence in the percentage of germination. Joa-Abba and Lovato (1999) observed that protracted storage periods resulted in low germination and subsequent seedling growth. As a result, directly affect the agronomic characteristics of the seed, since farmers want high-quality seed that enables high rates of germination and uniformity of seedlings under specified conditions. Temperature during storage also affect the germination percentage of grain. Cold storage of grain have higher germination rate compare to room temperature. The seed coating of insecticide give better result during storage and give 92% germination in wheat after 14 month storage (Chaturvedi *et al.*, 2021).

Ozone is utilized in agriculture as a germination booster and a powerful antibacterial agent. In small amounts, ozone improves seed germination. According to Sudhakar *et al.* (2011), administering small amounts of ozone to tomato seeds (*Lycopersicon esculentum*) resulted in an enhanced germination rate and the development of seedlings with elongated roots. The improved root growth observed in ozone-treated seedlings might be linked to the increased production of jasmonic acid (Violleau *et al.* 2008). Mason (2006) found that continuous and repeated ozone treatments did not impact seed germination. The calculation of well-germinated seedlings was based on a formula applied to 100 seeds.

$$\text{Germination (\%)} = \frac{\text{seeds germinated}}{\text{total seeds}} \times 100$$

The few works were done by scientists in the field of ozone application in seeds for detecting germination change. Savi *et al.* (2014) reported positive effect of ozone on seed germination maximum up to 120 min of ozone exposure time. They also mentioned that the O₃ therapy had an impact on wheat germination 180 minutes after exposure, with a reduction of up to 12.5%. Lower or no germination was the result of the storage period's elevated temperatures (Freeman, 1980). The ozone gas exposure time increased the seed germination in alpine plants (Abeli *et al.*, 2016). The ozone has the potential to activate DNA repair mechanisms, boost antioxidant activity, or initiate dormancy-breaking processes in hydrated seeds. The utilization of ozone to improve the germination of winter wheat seeds not only enhances their germination rate but also augments their germinating energy (Avdeeva *et al.* 2018). The sorghum seed germination percentage is 100 % after 6 months at -5 °C temperature when 25-30 °C storage temperature decreases the germination percentage up to 37 %. Several researchers have noted that there is an inverse relationship between elevated grain moisture levels and temperature with the percentage of grain germination (Owolade *et al.*, 2011). Excessive use of ozone gas can lead to a decrease in seed germination as it causes the degradation of chemical constituents within the grains (Tiwari *et al.*, 2010). Gaseous ozone treatment in barley had not significant effect on germination energy up to 30-min ozone exposure time. The largest decreases of 61% to 53% in germination with increased exposure time after 30 min. (Kottapalli *et al.*, 2005). 96% germination percentage was found in barley grain that was highly infected with *F. graminearum*, which caused a drop in germination (Beattie *et al.*, 1998). From their result it could be proven that the response of ozone and fungal infection was responsible for causing reduced germination energy. Violleau *et al.* (2008)

subjected maize seeds to ozone treatment at a concentration of 20 g/m^3 for either 6.8 or 20.5 minutes. Germination assessments were initiated either immediately or 48 hours following the treatment. They also noted that seeds exposed to ozone exhibited a higher germination rate compared to untreated seeds, and the treated samples began germinating more quickly than the untreated ones. Due to this accelerated germination, the treated samples had a greater number of seeds with substantial roots at the 4- and 5-day marks compared to the untreated samples. Ozone is a potent antibacterial and germination-enhancing agent. If applied sparingly (Pandiselvam *et al.*, 2020), ozone can improve seed germination rates. According to Chattha *et al.* (2015)'s research on wheat grain storage, the quality of the grain changed as it was kept in a cement bin. Their findings revealed that the duration of storage has a direct impact on the germination rate of wheat, with the germination percentage of wheat grains decreasing by as much as 65% as the storage duration increased.

Shingala and Dabhi, (2022) reported the effect of ozone on wheat germination (As shown in Table 2). Ozonation at 60-minute and 21-day exposure intervals has been proposed as a non-toxic treatment for wheat grains. Ozone has the potential to be used in storage insect control because it can be generated at the site of application, leaves no toxic residue after treatment, and controls insect pest infestations quickly. Despite all these benefits, ozone gas treatment boosts the germination rate of wheat seeds up to its maximum point, after which it gradually begins to decline. Moreover, the rate of reduction is notably slower compared to the control treatment.

- 3. Influence of Ozone Gas on Wheat Moisture Content:** During storage of grain with high moisture levels, ozone gas might not be as effective against internal insects. Conversely, when the moisture content is lower (around 10% w.b.), ozonation exhibits a more potent lethal effect on internal insects. In situations with higher moisture content (around 14% w.b.), the absorption of ozone gas on the grain surface can be significant due to its reactive properties. (Nickhil *et al.*, 2021).
- 4. Influence of Ozone Gas on Total Carbohydrate of Wheat:** Grains are economically significant and a crucial part of human diets. They are primarily seen as a source of energy in the form of carbohydrates, but they also provide essential protein, fiber, lipids, vitamins, minerals, and phytochemicals that contribute to a healthy diet. Cereals and breads serve as the primary energy source across all age groups. The categorization of carbohydrates as a whole, including sugar, starch, and fiber, is a key aspect of carbohydrate labeling.

Ozone treatment led to higher carboxylic group concentrations and lower overall carbohydrate content. Throughout the storage period, alterations in carbohydrates result in deterioration. The degradation of carbohydrates into CO_2 occurs at an exceedingly slower rate during storage, especially when the humidity level reaches approximately 14% (Oessoe *et al.*, 2014). According to a study by Rehman and Shah (1999), there was a 12% rise in the total soluble sugars content following six months of storage.

Sandhu *et al.* (2012) reported the application of ozone leads to an increase in the concentration of carboxylic groups while reducing the overall carbohydrate content within the amylopectin fraction. Consequently, when flour and isolated starch undergo

ozone treatment, there is a partial depolymerization of high molecular weight amylopectins, resulting in the generation of low molecular weight starch polymers and amylose. Visual examination via scanning electron microscopy did not reveal any discernible effects resulting from ozone treatment. Likewise, both X-ray diffraction analysis and differential scanning calorimetry (DSC) indicate that there were no alterations in starch granule crystallinity due to ozone treatment.

Shingala and Dabhi, (2022) demonstrated the effect of ozone on wheat grain carbohydrate content (Table 2). The use of ozone gas treatment minimally impacts the carbohydrate content of grains. The total carbohydrate content in wheat seeds reaches its highest point before gradually decreasing. The rate of reduction is also relatively higher compared to the control treatment. To maintain the maximum carbohydrate content, it is advisable to use ozonation with a 30-minute exposure time and an ozone cycle of 21 days as a non-toxic disinfection method for wheat grains.

- 2. Influence of ozone gas on starch content:** The starch content of wheat flour, which is typically prepared from wheat, ranges between 65-73% (Park *et al.*, 2009). According to Nandini and Salimath (2001), wheat flour contains 84.5% carbohydrates, 10% protein, and 58% starch content. This differed from the findings of Fraser *et al.* (1956), who reported a carbohydrate content of 67.4%, and Clegg (1956), who reported a carbohydrate content of 58.8%.

Alternation in polymers of wheat starch and S-S linkages reported after treated grains with ozone (Sandhu *et al.*, 2011). Ozone gas may make starch more damaged and degrade the structure of the granules. According to Mei *et al.* (2016), ozonization caused damage to the starch granules in the wheat endosperm and increased starch breakdown. Within 1hr of ozone treatment, starch content reduction was reported by them. After 1 hr., if ozone continuously exposed then it increased oxidation rate and degrade starch quality. Mason *et al.* (2006) reported negligible effect of ozone on starch content after repeated treatment. Savi and Scussel (2014) studied how exposure to ozone gas affected different types of toxic fungi and reported that 60 mg/L concentrations of ozone for 120 min didn't affect starch content. Wang *et al.* (2016) reported that ozone up to 90 min not significantly affect starch content. The influence of ozone up to 1000 ppm caused minor reduction in starch reported by Nickhil *et al.* (2021). As shown in table 2, the starch content of stored wheat is given by Shingala *et al.* (2022²) up to 120 days of bulk storage with ozone treatment with slightly alteration in starch content.

- 3. Influence of Ozone Gas on Colour and Odor:** Insects that consume substantial quantities of stored grain can lead to additional detrimental effects. This includes the growth of deteriorating agents such as mold and the contamination of grain with their excrement and discarded skin, resulting in the emission of unpleasant odors. (Pedersen, 1992). The presence of unpleasant odors in grain is primarily associated with spoilage caused by factors such as mold, insect secretions, rodent or bird droppings, or contaminants. The U.S. Federal Grain Inspection Service (FGIS) classifies these grain odors into categories including musty, sour, and commercially objectionable foreign odor (COFO). If any of these odors are detected in a grain lot, it is classified as sample grade according to USDA-FGIS standards. Lee *et al.* (2017) observed that the whiteness of

flour samples increased with longer ozone exposure times. Additionally, the color b value of wheat flour decreased as the exposure time to ozone increased.

VI. CONCLUSION

The major problem in bulk storage of grain is a loss in both quality and quantity of food grains because of pests, insects and other microorganisms. The growth of these microorganisms is influenced by the environmental factors. The primary purposes of utilizing ozone (O₃) in grain treatment are pest and insect management, as well as the breakdown of pesticide residues. Ozone treatment is advantageous because it doesn't leave behind any residues in the treated food, eliminating the need for post-treatment aeration. Additionally, due to its excellent outcomes, ozonation has been regarded as an environmentally friendly alternative for enhancing the quality of food grains, often referred to as a "green alternative." The chemicals used in traditional spraying methods like phosphine and methyl bromide which are toxic and carcinogenic. Ozone treatment replaces these chemicals. Depending on the parameters employed, the application of ozone can result in beneficial alterations in grain properties. These changes may include an elevation in germination percentage, protein content, gluten content, as well as an increase in the volume of bread and cakes, and enhanced strength. Ozonation can be adopted on a commercial scale by grain processors, farmers, and seed producers as a non-chemical method for safeguarding stored grain. Thanks to its adaptable nature, ozone treatment has the potential to yield substantial savings in operational costs while enhancing reliability and production uptime.

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