**Book Chapter:**

**Title: Ozone-based grain storage: a green technology with great potential for improving food safety.**

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**Abstract:**

Ozone technology is increasingly being used in the grain storage sectors. When grain is stored in bulk in metal silos, insects and other pests cause a significant difficulty. 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 shed light on the reactions of stored grain to ozone treatment and underlined the importance of treatment durations and ozone cycle frequency on various properties of grains like insect population (adult/kg), germination percentage, moisture content, carbohydrate, starch, color and odor of various food grains (e.g., 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.

**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. As per fourth advance estimates for 2021-22, the production of food grains in the country is estimated at 315.72 million tonnes. The production during 2021-22 is higher by 25 million tonnes than the previous five years' (2016-17 to 2020-21) average production of food grains. The storage of grain is practiced from the era of 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. Therefore, grain storage attracts the attention of our policy maker, scientists, development worker, grower and marketing agencies in terms of production and productivity are concerned.

An FAO estimate of world-wide annual losses in store has been given as 10% of all store grain. More than 70 insect pests have been identified which attacked store grains and cereal products in store houses. The overall damaged caused by these insect pests, worldwide it is estimate to be 10-40% annually. As a result, maintaining store quality and effective administration is urgently necessary (Upadhyay and Ahmad, 2011). Increased demand of grains are 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 problems of Stored-product insects like beetles, moths, indian meal moth, almond moth, angoumois grain moth and psocoptera (several species) in grain storage have been a great threat to quality of grains. Economies of developed and developing nations depend directly or indirectly on storage of cultivated grains since they are related to the several millions of people. The main problem with bulk storage structures is maintaining optimum temperatures and humidity in the storage environment (Sawant *et al*., 2012). Primary aim of storage is simply to prevent deterioration of the quality of stored grain.

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. Damaged kernels, which can affect how well-received the grain is generally, the encouragement of the growth of other deteriorating organisms like mold, and the contamination of the grain with their excrement, shed skin, and other byproducts of their life cycle, which can give off unpleasant odors, are a few of these factors. Spores of molds are microorganisms that tend to grow in stored grain. They settle on the grain surface, in the air and in storage structures. Eggs and larval developmental stages of insects are usually not directly affected by contact of insecticides. Fumigation influences these developmental stages but is also problematic because it has high toxicity and great potential for the development of resistant strains of harmful insects (Rees, 1996). Methyl bromide use was prohibited in 1995 as a result of an international agreement linking this fumigant to a reduction in the ozone layer found 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.

**Traditional fumigants used in storage**

Fumigants are commonly used in stored grains as remedial treatments. The fumigants are gaseous poisons that kill insects as the gas enters their bodies through the spiracles. 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. Azadirachtin was found to be successful for the control of S. oryzae, R. dominica, and T. confusum by Athanassiou et al. (2005), but at dose rates that were significantly greater than the currently employed grain protectants. Daglish *et al*. (2014) investigated that phosphine resistance problem was increasing and insect populations with resistant individuals can be common and newer or stronger types of resistance were emerging. Kaur and Nayak (2015) investigated that on estimated time to population extinction of strongly phosphine resistant *C. ferrugineus* was 10 days at 2 mg L−1 (1400 ppm) and 25 °C. Athanassiou *et al.* (2015) investigated methyl bromide efficacy under laboratory conditions. They showed that eggs were more tolerant than adult to control psocids in 48 h fumigations in the absence of significant amounts of grain. Methyl bromide application reduced due to its status as an ozone-depleting substance.

**Toxic effects of fumigants**

Different diseases may be brought on by the acute or long-term toxic effects of fumigants. Boon *et al*. (2008) concluded that the organophosphate, organochlorine and related pesticides disrupt nerve function, resulting in paralysis and may cause death. Insecticides may cause acute symptoms that include salivation, meiosis, urine, diarrhea, diaphoresis, and excitement of the central nervous system, according to research by Mishra *et al.* (2014). Damage to the central and peripheral neurological systems, cancer, allergies and hypersensitivity, reproductive problems, and immune system disruption are only a few of the specific impacts of pesticides. In accordance with Neme and Satheesh (2016), 34% of the 380 samples of cereal grains tested in Poland contained pesticide residues.

**Nature of ozone and ozone generating and supplying system**

Pure ozone is a poisonous, blue, unstable, and potentially explosive gas that occurs naturally 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. n 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 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) reported 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 is converted to oxygen due to its short half life time and not leave any chemical residue on 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** |
|  | Rice Weevils (Sitophilus oryzae) | Small, reddish-brown beetles | Rice, wheat, barley, and oats | 1.0–2.0 g/m3 and exposure time of 2–6 h.  | Srivastava, 2021 |
|  | Lesser grain borer (*Rhyzopertha dominica)* | dark reddish brown  | Wheat, corn, rice and millet.  | 2.5g m−3 for 8h. | Mishra *et al.,* 2019 |
| 0.428 g/m³ (200 ppm) ozone for 6 h  | [Abdelfattah](https://www.researchgate.net/profile/Nilly-A-H-Abdelfattah?_sg%5B0%5D=3YJny1U1_EFsHA0lxzPNkmOAj_5zaQQrODoBNwzLt8bchoFXpH_1Y6oduUzbAklCOviX2U4.KyFTnoG7fECOF_atusQW4UQ3ojW3oGMXwDF3qGyDcomeJcHYOTtk8xG82-K63xAf9aG1dRExXEAa2TTkUL0fww&_sg%5B1%5D=p9Y-o9ufJxhB-1Ppql7ImFRMowhkBHuKc_2zSmtr6QPjhT3CFxhGaVikX-AAUwbSBzqyU7w.hOkI666kN79nEistxcbjnCln0c7X2bTkez6NBx6I9cE_a1PhoEaPodlColOBwMKu7hDfzsho0JxRDMj2XoPzBg) *et al.,* 2023 |
| 0.42 g/m3 for 72 h  | Xinyi *et al.,* 2018 |
|  | 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 *et al*., 2019 |
|  | 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 | Seyedabadi *et al*., 2021 |
|  | Sawtoothed Grain Beetles (Oryzaephilus surinamensis) | Small, brown beetles | Stored products, including cereals, flour, and dried fruits. | 5 ppm for 15 to 90 min  | Sadeghi *et al*., 2017 |
|  | 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 *et al*., 2001 |
|  | Red Flour Beetles (Tribolium castaneum) | Widespread pests | Flour, cereals, and other grain-based products. | 50ppm ozone for 3d | Kells *et al.,* 2001 |

**Generation of ozone**

Splitting a diatomic oxygen molecule is the first step in the production of ozone. By combining with another diatomic oxygen, the resulting free radical oxygen can then be used to create the tri-atomic ozone molecule. However, a significant amount of energy is needed to break the O-O bond. Ozone can be created by using corona discharge techniques and ultraviolet light with a wavelength of 188 nm to start the synthesis of free radical oxygen. Corona discharge is typically utilized to produce ozone at commercially viable quantities. (Rice *et al.,* 1981). There are two electrodes in corona discharge, one is the high tension electrode and the other is the low tension electrode. Narrow discharge gap was provided in between electrodes (fig.1). A portion of these collisions take place and each oxygen atom can produce an ozone molecule when the electrons have enough kinetic energy to split the oxygen molecule. If air is passed through the generator as a feed gas, 1–3% ozone can be produced, however, using pure oxygen allows yields to reach up to 6% ozone (Rice *et al.,* 1981). Ozone gas cannot be kept because it spontaneously breaks down into oxygen atoms, according to Coke's (1993) research.



**Fig 1. Schematic diagram of ozone generation by corona discharge method (Rice *et al.,* 1981).**

**Mechanism of ozonation on pest control**

Ozone is a very unstable molecule and rapidly decays into O2 releasing a single oxygen atom that is highly reactive. This single oxygen molecule reacts with bacteria or viruses' cell membranes, damaging cellular components and interrupting normal cellular activity. If ozone contacts a volatile organic compound the free oxygen atom reacts with it, removing the odor. (Mason *et al.,* 2006). Ozone causes oxidative tissue damage even at low concentrations resulting in DNA strand breaks, alteration of pulmonary function, bronchial responsiveness, membrane oxidation or mutations in vivo. (Liu *et al*., 2007).

**Effect of ozonation on various properties of grains:**

**Effect of ozone on insect population (adult/kg) during storage**

The main aim of ozone application in storage was to achieve high mortality rate and almost no quality change of stored grain. Ozone is used in the grain processing sector as a substitute for existing fumigants like methyl bromide and phosphine for the control of storage pests.. According to research by Sousa *et al.* (2008), stored-grain pests like *Tribolium castaneum, Rhyzopertha dominica, Oryzaephilus surinamensis, Sitophilus oryzae,* and *Ephestia elutella* are all killed by ozone when used as a fumigant. 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. There were many research was done base on lower ozone concentration and higher exposure time but the most appropriate treatment was high ozone concentration and low exposure time for more economical and energy saving. The effectiveness of ozone fumigation in a corn grain mass (about 9 tons) against adult insects like the red flour beetle, maize weevil, and larvae from the Indian meal moth was assessed by Kells et al. (2001). They have found that 50 ppm ozone for 3 d resulted in 92–100% mortality of adult. He has found that the lower dose was also significantly efficient but led to a lower insect mortality (77–99.9%) depending on the insect species. The ozone concentration range from 0 to 2000 ppm was used by Maier *et al.* (2006). From their research work, they have been investigated that after three days of exposure at 50 ppm of ozone to grains, 100 % insect mortality gain. At the high concentration, 100% mortality of adult red flour beetles, confused flour beetles and maize weevils, as well as greatly reduced emergence (Mason *et al.*, 2006)

**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 |

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 was 2.75 after 2 months of storage, 7.62 after 4 months of storage and 20.43 after 6 month of rice storage. (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).

**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 lo 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. Sudhakar *et al.* (2011) reported that the injection of low doses of O3 in tomato seeds (*Lycopersicon esculentum*) increases the germination rate and produced seedlings with longer roots. The reason behind good root growth in ozone treated seedlings could be attributed to the increased production of jasmonic acid (Violleau *et al*. 2008). Mason (2006) has resulted that seed germination are not affected by continuous and repeated treatment with ozone. Well germinated seedlings were calculated out of 100 seed as per given formula.

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

The few work 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 O3 may stimulate DNA-repair mechanisms and antioxidant activity or dormancy breaking effects in hydrated seeds. Application of ozone in enhancing the germination of winter wheat seeds revealed that besides improving the germination, the germinating energy of the seeds are also enhanced (Avdeeva *et al.* 2018). The sorghum seed germination percentage is 100 % after 6 months at -5 °C temperature when 25-30 °C storage temperature decrease the germination percentage up to 37 %. The higher grain moisture and temperature negatively correlate with grain germination percentage as observed by many researchers (Owolade *et al*., 2011). O3 gas causes degradation of chemical constituents present in the grains. Seed germination loss may occur from excessive use of O3 gas. (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) treated maize seed with ozone (20 g/m3) for 6.8 or 20.5 minutes. Germination tests were started immediately or 48h after treatment. They discovered that ozone-treated seeds germinate more frequently than untreated ones. They also observed faster start of germination for treated samples than untreated one. Because of this early germination, treated samples had more seeds with large roots at 4 and 5 days than untreated ones. 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. They resulted that storage duration is directly influenced to the germination percentage of wheat. They got germination percentage of wheat grain decreased up to 65% with respect to the storage duration.

As shown in table 2, Shingala AM and MN Dabhi, (2022) reported the effect of ozone on wheat germination. 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. After all of these advantages, ozone gas treatment enhances the germination percentage of wheat seeds up to its peak point and then it start to reduce. The reduction rate also very slow compares to control treatment.

**Influence of ozone gas on wheat moisture content**

During storage, high moisture grain, ozone gas may not be effective against the internal insects. On the other hand, at less moisture content (10% wb), ozonation will have a higher lethal effect on the internal insects. At higher moisture content (14% wb) the absorption of ozone gas on the surface of grains may be high due to its reactive nature. (Nickhil *et al.*, 2021).

**Influence of ozone gas on total carbohydrate of wheat**

Grain’s economic importance and contribution to human diets cannot be denied. Grains is often viewed primarily in terms of energy as carbohydrate and certainly plays vital role in that respect. The grain is also rich in protein and fiber as well as lipids, vitamins, minerals and phytochemicals which may promote healthy diet. Cereals and breads are the main source of energy for all age group. Including sugar, starch, and fiber in the total carbohydrates content is subject to the labeling of carbohydrates as a whole.

Ozone treatment led to higher carboxylic group concentrations and lower overall carbohydrate content. During storage, changes in carbohydrates lead to damage. Carbohydrates degrade into CO2 extremely slowly during storage, particularly when humidity approaches 14% (Oessoe *et al.*, 2014). Rehman and Shah (1999) reported that total soluble sugars increased by 12% after six months of storage.

According to Sandhu *et al*. (2012), ozone treatment increases the concentration of carboxylic groups while lowering the total carbohydrate content in the amylopectin fraction. As a result of ozone treatment of flour and isolated starch, there was partial depolymerization of high molecular weight amylopectins, which caused the production of low molecular weight starch polymers and amylose. Visual analysis via scanning electron microscopy indicated no effects from ozone treatment. Similarly, X-ray diffraction and change in gelatinization from DSC both indicate that starch granule crystallinity was not affected by ozone treatment.

As shown in table 2, demonstrate the effect of ozone on wheat grain carbohydrate content (Shingala AM and MN Dabhi, 2022). Ozone gas treatment has negligible effect on carbohydrate content of grain. The total carbohydrate percentage of wheat seeds reach up to its peak point and then it starts to reduce. The reduction rate also high compared to control treatment. Considering the maximum carbohydrate percentage, ozonation at exposure time of 30 min and ozone cycle of 21 days can be recommended as a non-toxic disinfectant for wheat grains.

**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). Wheat flour have 84.5% carbohydrates, 10% protein, and 58% starch content (Nandini and Salimath, 2001) whereas 67.4% reported by Fraser *et al*. (1956) and 58.8% reported by Clegg (1956).

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.

**Influence of ozone gas on colour and odor:**

The insect consuming large amounts of stored grain will also cause other deteriorating factors. The growth of deteriorating organisms like mold and contamination of grain with their shed skin, excrement can produce off-odors. (Pedersen, 1992). Off-odors found in grain were mainly related to spoilage cause by mold, insect secretions, rodent or bird excrements or contaminants. The U.S. Federal Grain Inspection Service (FGIS) categorizes off-odors in grain as musty, sour, and commercially objectionable foreign odor (COFO) that if present in a grain lot, it will be categorize as sample grade (USDA-FGIS). The whiteness of flour samples increased with the ozone exposure time. Color b value of wheat flour decreased as exposure time to ozone increased (Lee *et al.*, 2017).

**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 main reasons for applying O3 in grain are for pest and insect control, degradation of pesticide residues. As O3 does not leave any residues in the treated food, there is no need for an aeration step after treatment, and also due to the excellent results of ozonation, it has been considered a “green alternative” to improve food grain quality. 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 conditions used, O3 may lead to positive changes in the grain such as, increase of germination percentage, protein content, gluten content, bread and cake volume, increase of strength. Ozonation can be used commercially by grain processors, farmers, and seed producers as a non-chemical alternative for stored grain protection. Due to the adaptable characteristics of ozone, ozone treatment has the potential to save large amounts of operational costs and increase reliability and production up-time.

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