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Effect of ozone gas exposure time and ozone cycle on starch content of wheat (*Triticum aestivum*) during bulk storage

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Abstract
The purpose of the current study was to determine how bulk storage of the wheat variety GW 496 affected its starch content as a result of exposure to gaseous ozone. Gaseous ozone treatment is used to control insects and pests during the bulk storage of wheat grain in metal silos. Because it leaves no residue and is environmentally friendly, ozone gas is the preferred and most practical method for treating grains while they are being stored. In this study, the application of ozone gas to wheat grain during bulk storage and evaluation of impact of various ozone exposure time and ozone cycle on wheat starch content. A pilot-scale ozone disinfestation system for wheat grains was developed. The two-factorial experimental design on the influence of the parameters of the technological process of ozone treatment on the physicochemical qualities of wheat seeds was carried out. Wheat grains were treated by gaseous ozone with various time durations (0 min, 30 min, 60 min, 90 min and 120 min) and at various frequency cycles (7 days, 14 days, 21 days). The starch content of wheat during storage was significantly different after every 30 day interval up to 120 days of storage. A decrease from (68.50% to 68.34%) in starch content was observed for the control sample for the storage period up to 120 days, whereas a decrease from (68.29 to 66.66%) in starch content was observed in ozone-treated sample for the storage period of 30 days to 120 days. On the other hand, excess ozone can also cause some negative effects on starch content. This study provided new insights into how stored wheat grain responds to ozone treatment and highlighted the role of ozone exposure time and frequency of cycle for the starch content of stored wheat.

Keywords: Ozone, wheat, starch content, bulk storage, ozone exposure time, ozone cycle, carbohydrate

1. Introduction
The economy of the nation is significantly impacted by grain storage. Grain storage has been a practise since the dawn of humanity. India's second-most important food crop, after paddy, is wheat. Nearly one-third of the total production of food grains comes from wheat. India is the second-largest producer of wheat in the world. As per GAIN Report USDA, India is heading for third consecutive record wheat harvest with marketing year (MY) 2019/20 (April/March) production forecast at 100 million metric tons (MMT). Largest wheat growing states in India are Uttar Pradesh, Punjab, Haryana, Madhya Pradesh, Rajasthan, Bihar and Gujarat. Maintaining threshold temperature, proper humidity within the storage environment are the important problems faced in bulk storage structure. Main objective of bulk storage is to prevent stored grain from deterioration of quality. Insects consume large amounts of stored grain and cause other deteriorating factors that will affect the economic value of the stored grain. Due to the infestation of insects and pests, the reduction in starch content of wheat grain occurs. Global population growth inevitably results in increase in food needs. At the identical time, agricultural land has been decreased drastically because of the rapid urbanization and industrialization which severely affects the land availability for the growers. The traditionally followed chemical methods for insecticide during storage have major limitations including being environmentally unhealthy, time-consuming, and are labor-intensive.
Ozone is a strong oxidant that has been widely utilized in gas form and mixed with water as a decontaminant in the food and beverages, medical and water supply industry. Ozone is used as an alternative for pest control and reduction of microorganisms including mold and mycotoxin in stored grain (Mendez *et al.*, 2003) [12]. Artificially produced ozone can decompose rapidly therefore it cannot be accumulated or transported. Thus it should be continuous generated (Miller *et al.*, 1978) [13]. Ozone may easily generated onsite where it's required, by either corona discharge (CD), ultraviolet (UV) or electrolysis of water (Kim *et al.*, 1999) [6].

Chemical insecticides give more production compare to control treatment (kumar *et al*., 2011) [7] but have many side effects after consuming. Ozone offers several safety advantages over chemical pesticides. There are not any repositories of toxic chemicals, nor risks of residual pesticides from chemical mixing or disposal, additionally no packaging waste being produced. Ozone has the short half-life so that it reverts to naturally occurring oxygen and not leaving any residue on the product. The main objective of this study was to determine the effect of ozone on starch content of ozone treated bulk stored wheat. But the chemical insecticide have their own disadvantages on human body. So the ozone is best preferred to prevent losses during storage of wheat grains.

Wheat's economic importance and contribution to human diets cannot be denied. Wheat is often viewed primarily in terms of energy as starch 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. The body prefers carbohydrates and starches for energy due to the ease with which they can be metabolized into glucose, or blood sugar, in the body. At maturity, the wheat grain contains 85% (w/w) carbohydrates, of which 12% are polysaccharides found in the cell walls and 80% of which are starch (Stone and Morell, 2009) [19]. Wheat flour was determined to have 84.5% carbohydrates, 10% protein, and 58% starch by Nandini and Salimath (2001) [14]. Fraser *et al.* (1956) [2] determined starch content 67.4% in flour of wheat. Clegg (1956) [1] investigated starch content of 58.8% and sugar content 2.7% in wheat. Typically, wheat is turned into wheat flour, which has a starch content of 65-73%. (Park *et al*., 2009) [16]. The main component of wheat endosperm, wheat starch, is known to have an impact on structure by creating a rigid network in baked goods during the gelatinization process (Maningat *et al.*, 2009) [9]. As a result, the starch content of flour significantly affect the quality of wheat and its end products.

Hubbard *et al.* (1950) [4] determined that there was significant decrease in starch digestibility with the increase in infestation of grains caused by insects. Reduction was to the extent of 68% (wheat) at 75% infestation level caused by *R. dominica*. Starch is an easier target for *R. dominica* due to the feeding behaviour of this pest. Jood *et al.* (1996) [5] worked on available carbohydrates of cereal grains as affected by storage and insect infestation. They discovered that On the other hand, after 4 months of storage there was a noticeable decrease (9-14%) in the level of starch. Starch content of wheat was decrease from 60.55% to 52.13% up to 4 month of storage. A marginal increase in the levels of sugars may be attributed to break down of starch during storage as was also observed earlier (Sudhakar and Pandey, 1981) [21].

After exposure to ozone, changes in starch polymers and the emergence of S-S linkages in dough were noticed (Sandhu *et al*., 2011) [17]. Ozone may inhibit the development of microorganisms and oxidise yellow pigments, improving the whiteness of noodle sheets, according to (Li *et al*., 2012). Limited data is available regarding the impact of ozone gas on the starch characteristics of wheat flour, despite prior research reporting the effects of ozone on dough. The goal of this study was to ascertain the starch content of wheat that had been exposed to ozone gas for varying amounts of time and cycle up to 4 months. Ozone gas used in this study might weaken the starch granule structure and increase the starch damage. Mei *et al.* (2016) [11] investigated that ozonization damaged the wheat endosperm starch granule and increase degradation of wheat starch. They noticed reduction of starch content with the treatment time was 1 h or less. Ozone treatment times between 1.5 h and 2.0 h showed a higher oxidation rate and further degradation of starch. Reduced starch levels are prejudicial to the quality of wheat products, promoting enzymatic degradation and the rate of water absorption during dough making. Straumite *et al.* (2021) [20] investigate that white flour have 76.2% starch content. Ozone gas influence the starch content of 64.15% with ozone treatment whereas without ozone the starch content was 65.36%.

Mason *et al.* (2006) [10] worked on the controlling stored grain insects with ozone fumigation. They concluded that fumigation with ozone does not appear to be detrimental to starch content, starch content was not affected by continuous and repeated treatment with ozone. Savi and Scussel (2014) [18] worked on effects of ozone gas exposure on toxigenic fungi species. Ozone exposure time (40, 60, 90, and 120 min) was varied by them. They resulted that the application of O_3 gas in concentrations of 60 mg/L up to 120 min did not affect the quality attributes of the wheat grains, such as isolated starch oxidation. Wang *et al*. (2016) [23] investigated the effect of exposure time to ozone on wheat DON detoxification. The exposure time was for 0, 30, 60, or 90 min and temperature at 25°C under 75% RH. No significant differences ($p > 0.05$) were found in starch content of ozone-treated samples. Nickhil *et al.* (2021) [15] recently studied on the effect of gaseous ozone (500–1000 ppm) on the chickpea grains. There was some minor degradation of intracellular cell wall and distribution of starch in the ozonated sample.

2. Material and Methods

The freshly harvested cleaned wheat grains (GW 496) were collected from Junagadh, Gujarat, India. The unwanted materials present in wheat grains, if any, were removed. The samples having initial moisture content of 10.40% (wb). The moisture content was determined using the AOAC-935.29 method.

In the bulk storage, the wheat grain samples (20 kg) were filled in bins and subjected to different ozone exposure time (30, 60, 90, 120 min) and ozone frequency cycle (7, 14, 21 days). The conditions, at which maximum mortality was achieved, were considered for the final treatment.

Starch, the principal constituent, has been the subject of endless attention. The total carbohydrate content of wheat contain starch, sugar pentosans, and cellulose. The various methods evolved have been compared and contrasted from time to time by different workers. The method finally selected as the most suitable for the present use was the phenol sulphuric acid method as described by Hodge and Hofreiter (1962) [3]. Starch is an important polysaccharide. It is the storage form of carbohydrate in plants abundantly found in roots, tubers, stems, fruits and cereals. Starch, which is composed of several glucose molecules, is a mixture of two types of components namely amylose and amylopectin. Starch is hydrolysed into simple sugars by dilute acids and the quantity of simple sugars is measured colorimetrically.

Plate 3.12: Starch content measurement

The sample was homogenised in hot 80% ethanol as the first step to get the sugars out. centrifuged after that, keeping the residue. The residue was then repeatedly cleaned with hot 80 percent ethanol. For the residue, drying was done over a water bath. Add 6.5mL of 52 percent perchloric acid and 5.0mL of water to the residue. Extract for 20 minutes at 0 °C. Save the supernatant after centrifugation. Fresh perchloric acid should be used to repeat the extraction. Make up to 100mL of the supernatant by centrifuging it. Glucose is dehydrated to hydroxymethyl furfural in a hot acidic medium. With phenol, this produces a green product with a peak absorption at 490 nm. With phenol, this produces a green product with a peak absorption at 490 nm. Pipette out various volumes of the working standard—0.2, 0.4, 0.6, 0.8, and 1 mL into test tubes. In two different test tubes, pipette out 0.1 and 0.2mL of the sample solution. Water should be added to bring the volume in each tube to 1mL. Use 1mL of water to create a blank. Each tube should contain 1mL of phenol solution. Shake well after adding 5mL of 96 percent sulfuric acid to each tube. After 10 minutes, shake the contents of the tubes, then submerge them in a water bath set at 25 to 30 °C for 20 minutes. At 490 nm, read the colour. Utilizing the common graph, determine the total amount of starch that is present in the sample solution.

2.1 Statistical analysis

For determination of accurate effect of ozone on seeds, two factorial experiment was established. All observation of starch percentage were analyzed using analysis of variance (ANOVA). The main results were expressed in terms of mean standard deviation and $p < 0.05$ were considered statistically significant.

Table 1: Influence of ozone on starch content of stored wheat (%)

	Storage period (Starch Content %)			
	30 days	**60 days**	**90 days**	**120 days**
Control	68.50	68.44	68.39	68.34
Exposure time(T)				
30 min	68.07^{c}	67.95^{b}	67.70^{b}	67.65^{b}
60 min	67.95^{bc}	67.83^{b}	67.59^{b}	67.54^{b}
90 min	67.78^{ab}	67.33^{a}	67.44^{ab}	67.39^{ab}
120 min	67.57^{a}	67.44^{a}	67.23^{a}	67.17^{a}
CD	0.264	0.265	0.265	0.266
SEM	0.085	0.085	0.085	0.085
Ozone Cycle (C)				
7 days	67.46	67.32	67.08	67.02
14 days	67.91	67.57	67.57	67.52^{a}
21 days	68.15	68.03	67.83	67.77^{a}
CD	0.229	0.230	0.230	0.230
SEM	0.073	0.074	0.074	0.074
T*C				
CD	N/A	0.459	N/A	N/A
SEM	0.147	0.147	0.148	0.148
C.V.%	0.307	0.308	0.309	0.308

3. Result and Discussion

In laboratory of CAET, Junagadh Agricultural University, Junagadh, an experiment was set to identify the effect of various time of exposures of ozone and frequency cycle of ozone on bulk storage of wheat. Monthly 26 wheat samples were collected and analysed for starch content of wheat during storage.

The result of the experiment on the effect of ozone gas on the starch content of wheat seeds of GW 496 is presented in table 1. The starch content was estimated as per the method given. It can be seen from table, there was a major change in the starch content of untreated wheat grains and ozone-treated samples. A decrease from (68.5% to 68.34%) in starch content was observed for the control sample for the storage period of 30 days to 120 days whereas a decrease from (68.29 to 66.66%) in starch percentage was observed in ozone-treated sample for the storage period of 30 days to 120 days. The loss in starch content of sample may be attributed to the hidden infestation in addition to grain's own metabolic activity. Starch content convert to sugar during storage. There might be some eggs, which hatched over the period of storage and damaged grain over the period of storage and at room temperature. Sugar levels may have slightly increased, which may be due to starch breaking down while being stored, as was also shown earlier (Sudhakar and Pandey, 1981) [21].

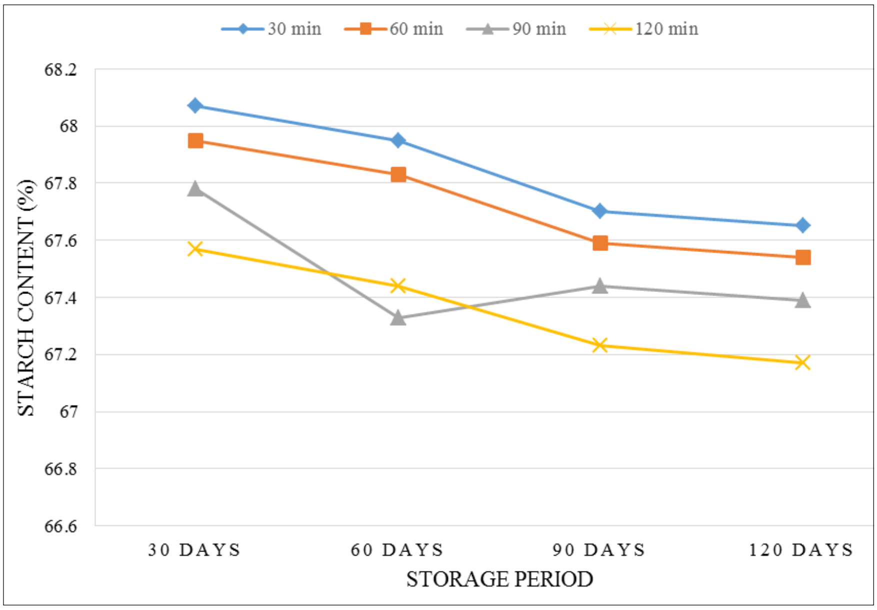

Fig 1: Effect of ozone exposure time (min) on starch content of wheat up to 120 days of storage

Starch content of wheat during storage were significantly differed after every 30 days of interval up to 120 days of storage due to effect of ozone exposure time. Significant effect of 30 min and 90 min exposure time were at par for the storage period after 90 days, 120 days storage period. In general trend, starch content decrease with increasing ozone exposure time. Interaction effect of exposure time and ozone cycle were non-significant for every 30 days interval of storage up to 120 days except after 60 days of storage. Mason *et al*. (2006) [10] worked on the controlling stored grain insects with ozone fumigation and recorded that starch content was not affected by continuous and repeated treatment with ozone. Savi and Scussel (2014) [18] applied ozone exposure time (40, 60, 90, and 120 min) on wheat grain and resulted that the application of O3 gas in concentrations of 60 mg/L up to 120 min did not affect the quality attributes of the wheat grains, such as isolated starch oxidation. Wang *et al.* (2016) [23] resulted that there was no significant differences (p> 0.05) were found in starch content of ozone-treated samples at the exposure time was for 0, 30, 60, or 90 min and temperature at 25 °C under 75% RH.

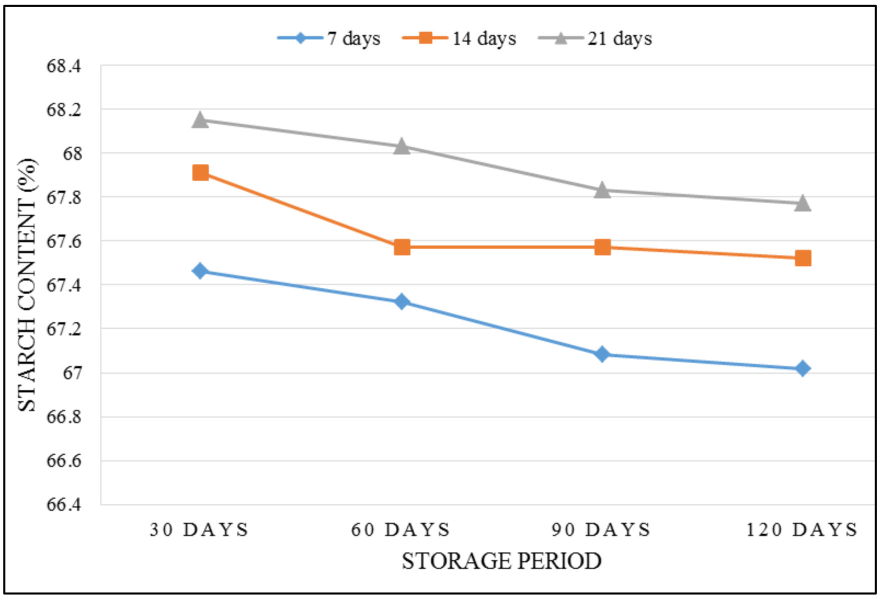

Fig 2: Effect of ozone cycle (days) on starch content of wheat during 120 days of storage

Starch content of wheat during storage were significantly differed after every 30 days of interval up to 120 days of storage due to effect of ozone cycle. Effect of ozone cycle of 14 days and 21 days were at par after 120 days of storage period. The starch content was increase with 7 days of ozone cycle to 21 days of ozone cycle after every 30 days interval up to 120 days of storage period. The starch content was significantly maximum for 21 days of ozone cycle after every 30 days interval up to 120 days of storage period. Interaction effect of exposure time and ozone cycle were non-significant for every 30 days interval of storage except after 60 days of storage. Nickhil *et al*. (2021) [15] resulted that there was some minor degradation of intracellular cell wall and distribution of starch in the ozone treated at 500–1000 ppm on the chickpea grains.

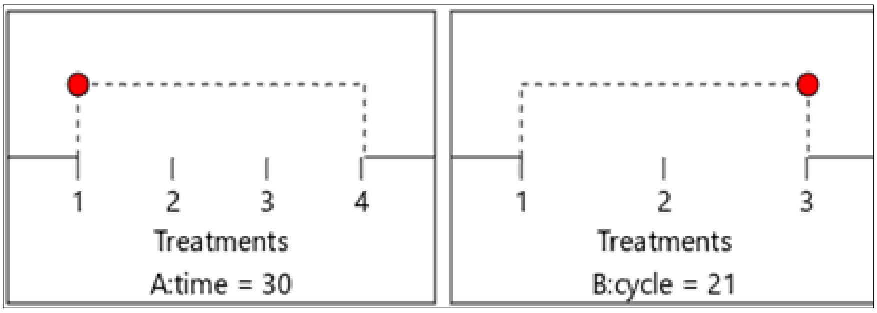

Fig 3: Optimization parameter of ozone treatment for maximum starch content of wheat up to 120 days of storage

Thus, the result of experiments performed treating wheat seed with ozone makes it clear that ozone firmly decrease the starch content of wheat with storage periods. As shown in fig. 3, optimal parameters given for treating wheat grains with ozone to stimulate starch content should be exposure time of 30 min and an ozone cycle of 21 days recommended.

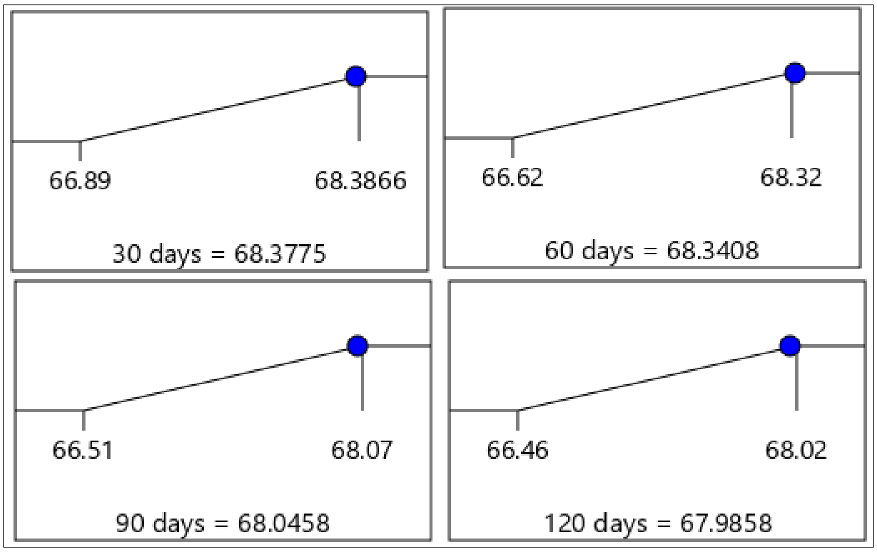

Fig 4: Maximum starch content (%) at optimum parameter of ozone treatment after 30 days, 60 days, 90 days and 120 days in bulk storage of wheat

At the recommended optimal parameters for treating wheat grains with ozone, continuous observation taken after 30 days, 60 days, 90 days and 120 days. The result shows that exposure time 30 min and ozone cycle at 21 days gives best result of starch content. As per given in fig.4, after 30 days of applied optimum condition of ozone, 68.37% starch content were found. After 60 days of storage the starch content start to decrease slowly and found 68.34% starch content in wheat grains. After 90 days of storage, starch content slightly decrease from above month and reach up to 68.04%. At the end of 120 days, the starch content was 67.98% at desirability of 1 which gives best result in compare to other treatment of ozone exposure.

4. Conclusion

The experiments conducted in this study demonstrate the effect of ozone on wheat grain starch content. Ozone has potential for use in storage insect control because it can be generated at site of application, it does not leave toxic residue after treatment and rapidly control the insect pest infestation. The starch content of wheat seeds reach up to its peak point and then it starts to reduce. A decrease from (68.50% to 68.34%) in starch content was observed for the control sample for the storage period up to 120 days, whereas 30 min of ozone exposure time and 21 days of ozone cycle give maximum starch content from (68.29% to 67.92%) in the ozone-treated sample for the storage period up to 120 days. However, compared to ozone-treated grains, storage insects seen inside the control sample and resulted in damaged grains inside control sample. Considering the maximum starch content, ozonation of 1000 ppm concentration at exposure time of 30 min and ozone cycle of 21 days can be recommended as a non-toxic disinfectant for wheat grains.

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