

**Novel Technologies for Food Processing and Shelf Life Extension**  
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**Lecture – 46**  
**Disinfestation Grains**

Hello everybody. In the earlier class we studied grain storage; now in this class let us study Disinfestation of Grains.

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**Methods of disinfestation**

Disinfestation

- Physical (High operational cost)
- Chemical
  - Contact insecticides
  - Gas fumigation
- Biological (Not so useful for bulk storage)

**Disadvantages of chemical insecticides/fumigants**

- Effective on fully developed insects; have little or no effect on eggs or larvae.
- Insects became resistant when used repeatedly.
- Some residues of the product, though not highly toxic, may linger in foods.

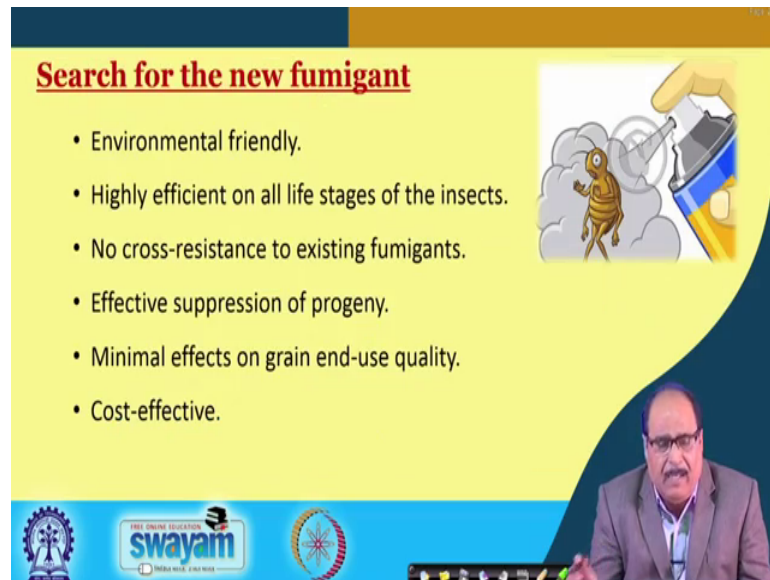
The slide also features a grid of icons on the right side, including a dollar sign, a hand holding a leaf, a hand holding a magnifying glass, a hand holding a leaf, a hand holding a leaf, a hand holding a leaf, a hand holding a leaf, and a hand holding a leaf. At the bottom, there are logos for IIT Kharagpur, Swayam, and a circular logo with a gear and a leaf.

There are different ways by which grains can be disinfested like physical methods are there, chemical methods as well as biological methods. The physical methods generally are costlier process, biological methods are not very useful for bulk storage of grains, they can be done for small quantity grains etcetera.

So, the most commonly used methods of disinfestation of grains include chemical methods, that is here the contact of the grain infested grain with the some chemicals insecticides or it may be gas fumigation. But, these chemical methods also they have certain disadvantages like they are effective on fully developed insects, they have very little or almost no effect on the eggs or larvae of insects. Insects become resistant to the use of chemicals particularly that insects insecticide etcetera upon repeated use.

So, these were the later stage or later on it may be after long time sometime the chemicals effectiveness of the insecticide to that particular insect may get reduced, also some residues of the product though not highly toxic may linger on in foods for some time.

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**Search for the new fumigant**

- Environmental friendly.
- Highly efficient on all life stages of the insects.
- No cross-resistance to existing fumigants.
- Effective suppression of progeny.
- Minimal effects on grain end-use quality.
- Cost-effective.


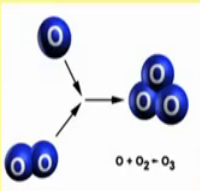
The slide features an illustration of a hand holding a blue and yellow spray nozzle, spraying a cloud of grey smoke that contains a brown insect. At the bottom of the slide, there is a video feed of a man with glasses and a mustache, wearing a brown jacket, speaking. Below the video feed are logos for 'swayam' (Free Online Education) and 'INDIA RISE, PROGRESS'.

So, these are some of the so drawbacks of chemical insecticides or fumigants and therefore, there is a continuous hunt for new fumigants which are environmental friendly, which are highly efficient and all the stages of insects, which have no cross resistance to existing fumigants, which are effective suppression of progeny. They result, they cause effective suppression of all the forms of insects whether it is a larvae, egg, pupae and so on and the process should have minimal effect on the grain end use quality and ultimately its should be cost effective.

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**Ozone**

- Ozone, or trioxygen, is an inorganic molecule with the chemical formula  $O_3$ .
- Ozone is created in the atmosphere when the sun's rays split oxygen molecules into single atoms.
- These atoms combine with nearby oxygen to form a three-oxygen molecule, called **ozone**.



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So, in this regard ozone becomes a very very potential fumigant for use in grain for the killing of insects are for disinfestation of grains. Ozone I hope you all know is a trioxygen, it is a inorganic molecule with the chemical structure  $O_3$ .

Ozone is created in the atmosphere when the sun's rays is split oxygen molecule into single atom and this single atom oxygen molecule are single oxygen, it combines with the oxygen to form a three oxygen molecule which is called ozone as you can see here in this figure that is single oxygen. It combines with two oxygen's and ultimately ozone having three oxygen molecules are formed three I mean three oxygen's are formed.

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**Why ozone?**

- Most powerful oxidizing agent
- Instantly destroys microbes and insects
- Environmental friendly
- Higher oxidation-reduction potential (2.07 V) than that of chlorine (1.36 V)
- Does not affect product taste
- No harmful by-products

O3

The slide features a molecular model of ozone (O<sub>3</sub>) and a small inset image of a man in a suit. The bottom of the slide includes the Swayam logo and navigation icons.

So, why ozone or why it is been promoted? Because it is a one of the most powerful oxidizing agent, it instantly destroyed microbes and insects, it is environment friendly, it has higher oxidation reduction potential than that of general chemical oxidants like chlorine etcetera. It does not affect product taste and it is not harmful to anyone does not give harmful by products.

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**Milestones in ozone treatment of food products**

- 2001 Dec. 21: The US Department of Agriculture's Food Safety and Inspection Service (USDA/FSIS) approved the use of ozone in contact with meats and poultry
- 2001 June 26: Food and Drug Administration (FDA) approved the use of ozone as an Antimicrobial Agent for the Treatment, Storage and Processing of Foods in Gas and Aqueous Phases
- 1939: Discovered to prevent the growth of yeast and mold during the storage of fruits
- 1909: A food preservative for the cold storage of meats
- 1893: Used as a disinfectant in drinking water

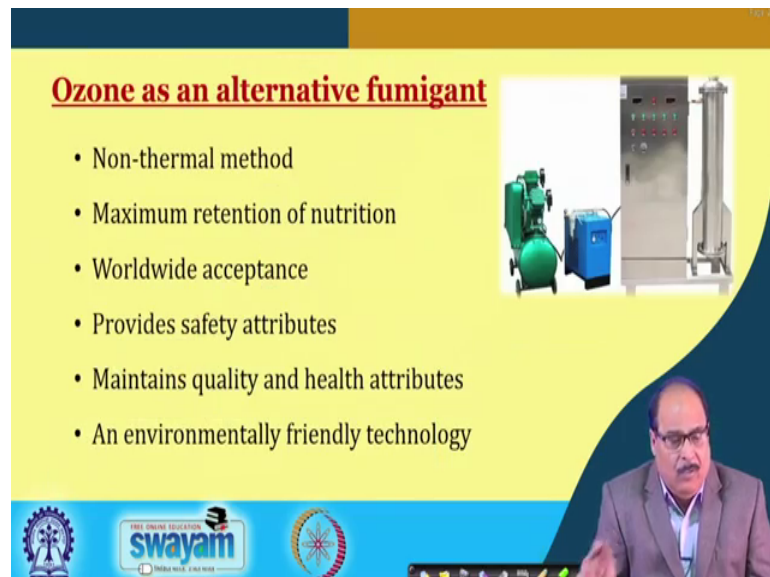
The slide features a staircase graphic with images of various food products and a small inset image of a man in a suit. The bottom of the slide includes the Swayam logo and navigation icons.

So, in this slide I have just tried to give you the milestones in ozone treatment of food products. In the year 1893 ozone was first time used as a disinfectant in drinking water,

in 1909 it was used as food preservative for the cold storage of meat. In the year 1939 it was discovered that ozone prevents the growth of yeast and mold during the storage of fruits.

In June 2001 food and drug administration of the United States approved the use of ozone as an antimicrobial agent for the treatment in storage and processing of foods in gas and aqueous phases. Same year in the month of December the US Department of Agriculture Food Safety and Inspections Service which is commonly called as USDA FSIS, it approved the use of ozone in contact with meats and poultry.

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**Ozone as an alternative fumigant**

- Non-thermal method
- Maximum retention of nutrition
- Worldwide acceptance
- Provides safety attributes
- Maintains quality and health attributes
- An environmentally friendly technology

The slide features a yellow background with a dark blue curved border on the right. An image of industrial ozone generation equipment is shown on the right side. At the bottom, there is a blue banner with logos for 'THE OPEN UNIVERSITY', 'swayam', and 'INDIA'S OPEN UNIVERSITY'. A small inset video of a man speaking is visible in the bottom right corner.

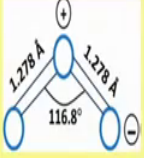
So, ozone is now seen as a very very effective alternative to chemical and other fumigants in the grain and other four materials because it is considered to be a non thermal method, it results into the maximum retention of nutrition that is disinfestation by the ozone does not have any significant effect on the nutritional components present into the food.

It has worldwide acceptance provides good safety attributes to that on the treated materials, maintains quality and health attributes and of course, more importantly it is considered to be an environmental friendly environment friendly technology, that does not cause any problems of pollutions etcetera to the environment.


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**Structure and properties of ozone**

- Ozone( $O_3$ ) is a tri-atomic oxygen formed by addition of a free radical of oxygen to molecular oxygen.
- It is partially soluble in water and the solubility varies with temperature.
- The half-life of ozone is about 20-30 min in distilled water at 20 °C.
- It is a potent antimicrobial agent against bacteria, fungi, viruses, protozoa and also against bacterial and fungal spores.



Chemical structure of ozone

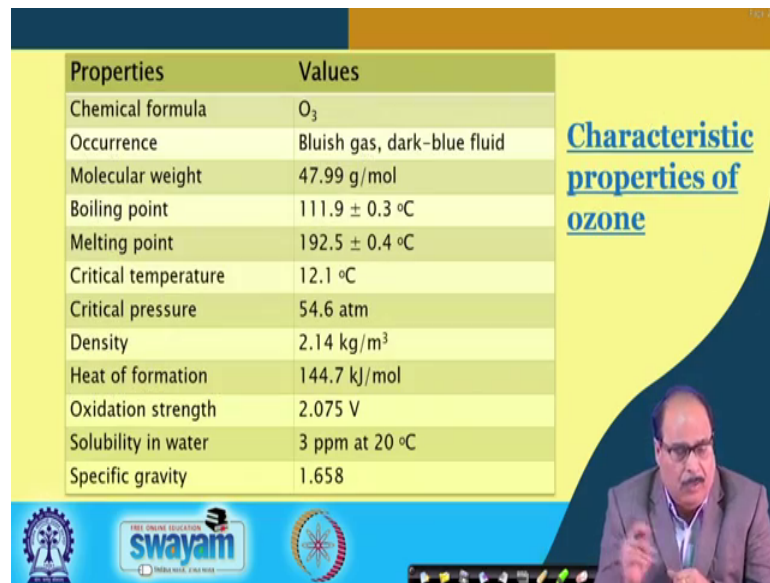


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So, before I come to the application aspects of ozone in grain storage or other food processing, let us consider that properties of the ozone or structure of the ozone as you can see here in this figure that is a the ozone is a tri atomic oxygen formed by addition of a free radical of oxygen to a molecular oxygen.


It is partially soluble in water and its solubility varies with temperature that is the solubility in water varies with temperature the half life of ozone is about 20 to 30 minute in distilled water at 20 degree Celsius. It is a potent antimicrobial agent against bacteria, fungi, viruses, protozoa and also it is effective against the bacterial and fungal spores. So, all sorts of microorganism include on there is spores can be in activator or can be destroyed by ozone.

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Properties	Values
Chemical formula	O <sub>3</sub>
Occurrence	Bluish gas, dark-blue fluid
Molecular weight	47.99 g/mol
Boiling point	111.9 ± 0.3 °C
Melting point	192.5 ± 0.4 °C
Critical temperature	12.1 °C
Critical pressure	54.6 atm
Density	2.14 kg/m <sup>3</sup>
Heat of formation	144.7 kJ/mol
Oxidation strength	2.075 V
Solubility in water	3 ppm at 20 °C
Specific gravity	1.658

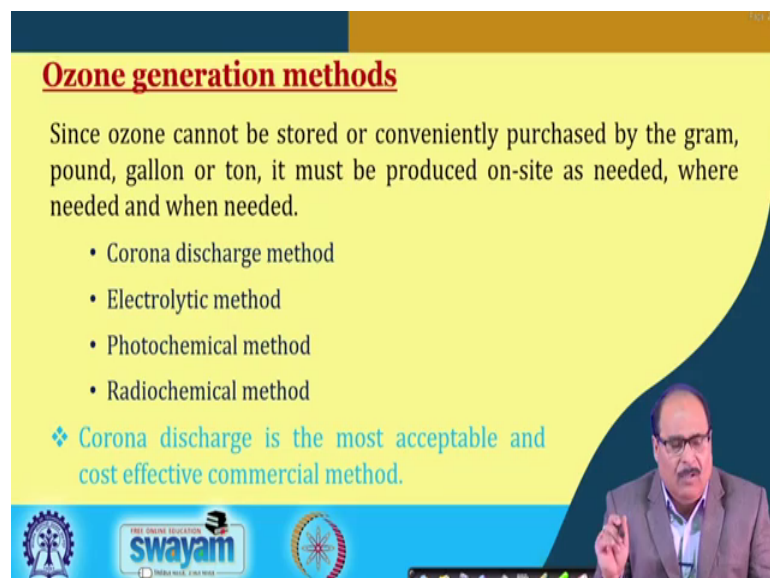
**Characteristic properties of ozone**



In this table detailed characteristics or properties of the ozone is given like, it is a bluish gas or dark blue fluid it is molecular weight is around 47.99 gram per mol, boiling point about 111.9 plus minus 0.3 degree Celsius, it has melting point of 192.5 plus minus 0.4 degree Celsius.

It's a critical temperature is 12.1 degree Celsius, critical pressure is 54.6 atmosphere and then density heat of formation oxidation strength etcetera. Its solubility is water at 20 degree Celsius, it is a 3 ppm a soluble and it has a specific gravity of 1.658

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**Ozone generation methods**

Since ozone cannot be stored or conveniently purchased by the gram, pound, gallon or ton, it must be produced on-site as needed, where needed and when needed.

- Corona discharge method
- Electrolytic method
- Photochemical method
- Radiochemical method

❖ Corona discharge is the most acceptable and cost effective commercial method.



So, this now the how the oxygen can be generated? And since in the its properties is decide that it has a very less half life that is about 20 to 30 minutes. So, and also it cannot be stored ozone cannot be stored or conveniently purchased by gram, pound, gallon or tons etcetera like other gasses, you can store oxygen in a cylinder carbon dioxide etcetera in the cylinder, but ozone cannot be done like that. So, it must be produced on site as needed where needed and when needed?

So, there are different methods for the ozone generation or production of ozone and those include corona discharge method, electrolytic method, photochemical method and radio chemical method. And among all this the corona discharge is the most acceptable and cost effective method which is used commercially for generation of ozone for various food and other applications.

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**Photochemical method of ozone formation**

The natural production of ozone is by either lightning or UV radiation.

$$3\text{O}_2 \longrightarrow 2\text{O}_3$$

1. UV radiation strikes an oxygen ( $\text{O}_2$ ) molecule, splitting off the O atoms.

2. Each O atom collides with an  $\text{O}_2$  and a third molecule to produce two  $\text{O}_3$  molecules. The third molecule is required to remove excess energy associated with the collision.

Ozone formation naturally from Lightning.

Lightning, and electrical spark will split the oxygen molecule into atomic oxygen. These oxygen atoms will quickly bind to oxygen molecules to form ozone.

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The photochemical method of in ozone formation you can see here in this picture. In fact, it is the natural production of ozone either by lightning or by UV radiations, that is the solar light that is the UV rays etcetera of the solar radiations they split oxygen molecule you can see here in this figure that is  $\text{O}_2$  into  $\text{O}$  plus  $\text{O}$ .

And these  $\text{O}$  acts are combines with another  $\text{O}_2$  and gives the  $\text{O}_3$  molecule and then new  $\text{O}$  is formed this new  $\text{O}$  again this gives similarly this by lightning etcetera also that is a they may cause a splitting they may split the  $\text{O}_2$  into  $\text{O}$  plus  $\text{O}$  and this  $\text{O}$  individual



O they can combine with O<sub>2</sub> and give O<sub>3</sub>. So, this is how this photochemical method results into the formation of ozone naturally.

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**Corona discharge method**

- Dust-free, oil-free air or an oxygen-containing gas mixture, or O<sub>2</sub> itself, is passed through the space of a high-energy electrical field between two electrodes separated by a dielectric material, usually glass.
- One electrode is a grounded medium, and the other is a dielectric one.

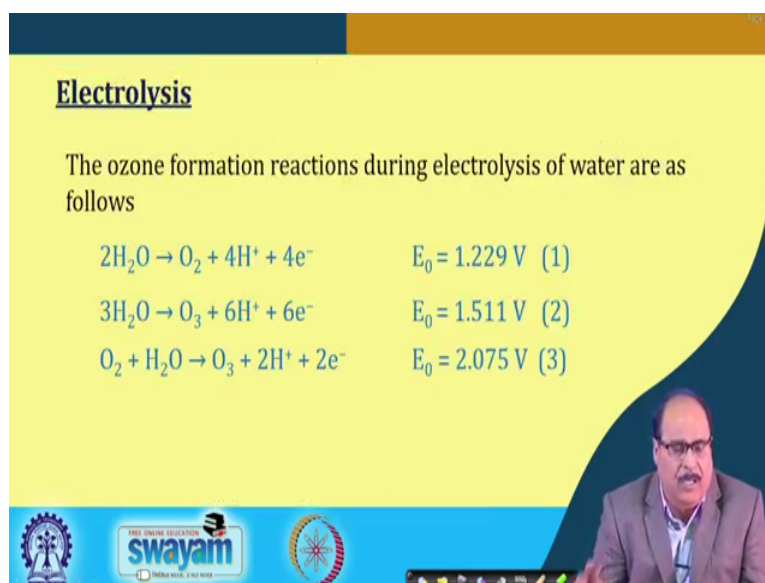
Schematic of corona discharge technology

In the corona discharge method in fact, as you can see here in this figure, that is the dust free, oil free or an oxygen containing gas mixture or even pure oxygen is passed through the space of a high energy electrical field between two electrodes and these two electrodes are separated by a dielectric material and which is usually a glass.

So, the one electrode which is high tension electrode, the other electrode is a low tension electrode and there is a tube through which the oxygen is passed and discharged. And the dielectric this high tension electrode and low tension electrode they dielectric material glass is provided here you can see here.

So, this O<sub>2</sub> in this discharge tube is converted into O<sub>3</sub> and the heat released is dissipated from the system. So, this is in fact, one electrode is a grounded medium and the other is a dielectric one that you can see here in this picture, so in this way the ozone is generated from the O<sub>2</sub> gas or pure gas mixtures.

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**Electrolysis**

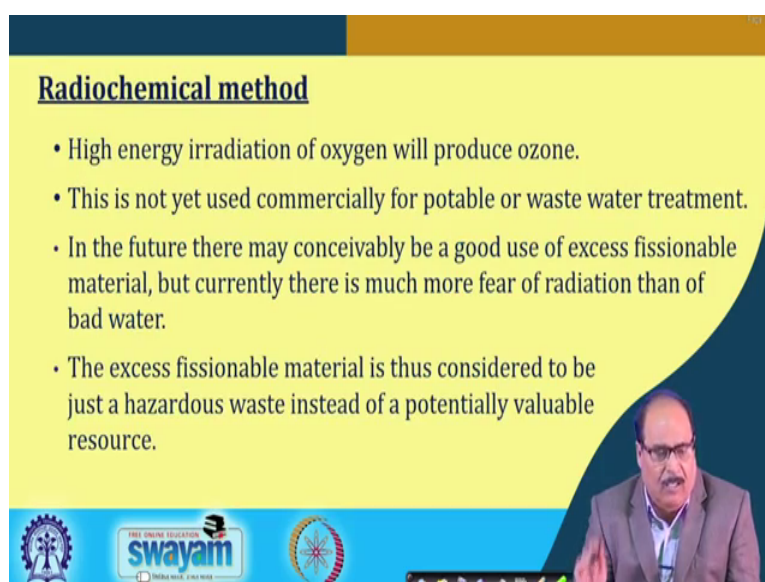
The ozone formation reactions during electrolysis of water are as follows

$$2\text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4\text{e}^- \quad E_0 = 1.229 \text{ V (1)}$$
$$3\text{H}_2\text{O} \rightarrow \text{O}_3 + 6\text{H}^+ + 6\text{e}^- \quad E_0 = 1.511 \text{ V (2)}$$
$$\text{O}_2 + \text{H}_2\text{O} \rightarrow \text{O}_3 + 2\text{H}^+ + 2\text{e}^- \quad E_0 = 2.075 \text{ V (3)}$$

The slide features a yellow background with a blue header and footer. The footer contains the Swayam logo and a small image of a presenter in a brown jacket and glasses.

The ozone formation reaction during electrolysis of water can be seen here like water when electricity is passed into this, then it gives O<sub>2</sub> and hydrogen ions. This a 3 molecules of water they give O<sub>3</sub> plus 6 hydrogen ions. H<sub>2</sub>O plus O gives O<sub>3</sub> plus 2 hydrogen ions. So, in this way that is by electrolysis or electrolytic is splitting of water ozone is generated.

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**Radiochemical method**

- High energy irradiation of oxygen will produce ozone.
- This is not yet used commercially for potable or waste water treatment.
- In the future there may conceivably be a good use of excess fissionable material, but currently there is much more fear of radiation than of bad water.
- The excess fissionable material is thus considered to be just a hazardous waste instead of a potentially valuable resource.

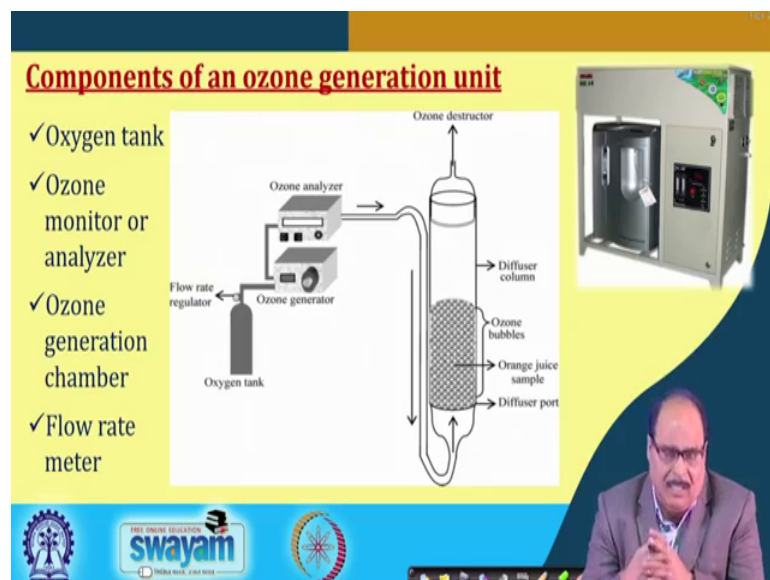
The slide features a yellow background with a blue header and footer. The footer contains the Swayam logo and a small image of a presenter in a brown jacket and glasses.

The radio chemical method has high energy irradiation of oxygen which produces ozone, this is not yet used commercially for potable or waste water treatment. In the future

there may be conceivably a good use of an excess fissionable material. But, currently there is much more fear of irradiation than that of bad water that is the some fissionable materials are required for production of ozone in this process.

So, the excess fissionable material is thus considered to be just a hazardous waste instead of a potentially valuable resource. Maybe in the days to come this can become popular when the radiations hazard etcetera are taken care of or it is made sure that by radio chemical methods, that is a ozone will be generated and the generated ozone is safe from radio activity point of view.

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So, the components of an ozone generation unit as you can see here in this figure, it contains an oxygen tank, ozone monitor are analyzer, ozone generator chamber and then a flow rate meter. So In fact, this is a set of where ozone generated from this system it is allowed to pass through in a assembly or in a column which contains orange juice or some other samples.

So, that this type of set up can be made in the laboratory for the ozone treatment of the food material, we have also in our laboratory similar set up that is the ozone generator in our lab that we have the photograph of that you can see here this. So, towards the later part of this lecture I will show you some of the results, which we have done conducted on the treatment of ozone ozonisation of wheat grains and its effect on infestation

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### How ozone works?

- Unstable third oxygen can combine with organic & inorganic molecules to destroy them through oxidation.
- Respiratory tracts of insects are destroyed when they inhale ozone.

Rice grains observed with a stereoscopic microscope subjected to (A) atmospheric air, and (B) ozone gas for different periods of exposure

	12h	24h	36h	48h	60h
A					
B					

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So, let us see the how ozone works? How it does its disinfection or its effect on microorganism or microbial killing or inactivation? So, the  $O_3$  that is  $O_2$  and  $O$ ; so,  $O$  which is attached in  $O_3$  to our  $O_2$  to form  $O_3$  that is in fact, still is not very stable the attachment of  $O$  with  $O_2$  is a quite unstable.

So, what happens when the ozone comes in contact with some other biological material may be organic or inorganic molecules etcetera, then this  $O$  part of the  $O_3$  which is quite unstable it combines with the other organic or inorganic molecule with which it comes in contact and causes the oxidation of that molecule. And this oxidation causes the distraction as you can see here in this figure that is this ozone the  $O$  part that is one  $O$  part of this attaches itself to the bacterial cell right and the  $O_3$  reverts to  $O_2$ .

So,  $O_2$  is generated warm and  $O$  part of the  $O_3$  gets itself attached with the microbial cell, it rather dictates the physiological and other processes and may be it causes the that is the lysis of the cell components etcetera and the physiological process is affected and ultimately after sometime the cell dies may be lysis of the cell takes place. Similarly when the insects etcetera when they inhale ozone their respiratory tracts are destroyed and because of this the insects etcetera get destroyed or they die.

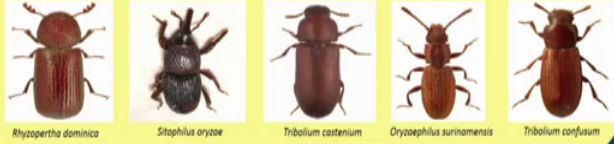
Here in this picture you can see the rice grain these observed with a stereoscopic microscope subjected to one atmospheric air in the case of A and then subjected to ozone

gas for different periods of exposure time and you can see the effect ultimately in these pictures.

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**Effectiveness of ozone in pest control**


Scientists have evaluated the efficiency of ozone in controlling a variety of insects in stored grains.



*Rhizopertha dominica*      *Sitophilus oryzae*      *Tribolium castaneum*      *Oryzaephilus surinamensis*      *Tribolium confusum*

Agent	Oxidation potential
Ozone	208
Peroxides	178
Hyper chlorides	148
Free chlorine	136

Comparison of oxidizing power of ozone

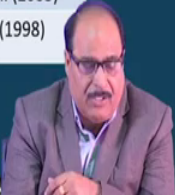


The ozone has been reported to be effective against almost all varieties of insects which are problematic in food grains which cause infestation of food grains like wheat, paddy rice, maize etcetera etcetera. And this as earlier also is a this ozone it has a very very high oxidizing power, it has much more oxidation potential, then those of the common chemical oxidants pro oxidants are oxidants like peroxides, hyper chlorides, free chlorine etcetera and because of this high oxidation potential it is effective in the microbial inactivation and as well as insects killing.

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**Dose and exposure time of ozone for disinfestation of major food grains**

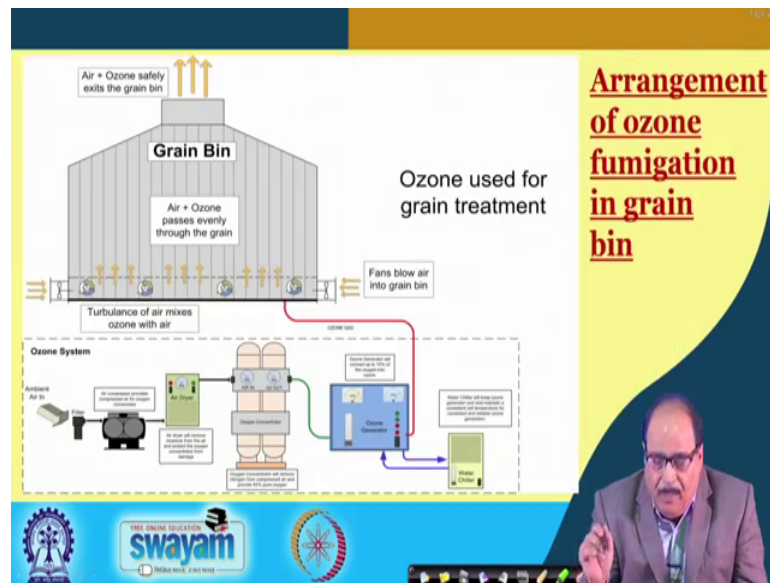
Insect	Grain	Dose (ppm)	Time (Days)	Mortality (%)	Source
<i>Rhyzopertha dominica</i>	Wheat	120	1.2	94	Qin et al. (2003)
<i>Tribolium confusum</i>	Corn	50	3	92	Kells et al. (2001)
<i>Tribolium castaneum</i>	Paddy	120	1.2	88	Qin et al. (2003)
<i>Oryzae surinamensis</i>	Wheat	70	4	67	Bonjour et al (2011)
<i>Sitophilus zeamais</i>	Paddy	120	1.2	100	Qin et al. (2003)
<i>Plodia interpunctella</i>	Corn	50	3	100	Strait (1998)



In this slide different researcher have worked and reported that like for example, Qin et al in 2003 reported that 120 ppm of ozone in 1.2 days resulted in 94 percent mortality of *Rhyzopertha dominica* in wheat grain. The same researchers reported that 120 ppm of ozone in 1.2 days resulted in 88 percent mortality of *tribolium castaneum* in paddy.

So, the various researcher have reported that doses from 50 to around 120 ppm of ozone from may be 1.2 to 4 days resulted in the mortality of various insects from about 67 to 100 percent in the grains like wheat, corn paddy etcetera. Means the different insects even can be treated successfully and disinfested successfully the different grains can be disinfested successfully by optimizing or by using different combinations of ozone. And this other factors the grain, that is the moisture content of the grain and all the thing that also, so and one can get desired mortality.

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Here it is the a system proposed by it is taken from the literature where some researchers they have given a model that is how that is in the grain storage bins etcetera in the problem, how this ozone can be applied? So, in the lower part of the picture you can see there is a ozone generation unit. So, this ozone it is connected that is generated in this system and then this through pipe it is sent to the storage bin in the bottom of the bin.

And from both two sides in this bin the air are blown, so this turbulence of air mixes the ozone well with the air and then this air and ozone mixture passes evenly through the grain and ultimately that it disinfest the insect etcetera in a kills the insect etcetera and then ozone and air safely exists the grain bin. So, this type of set ups can be used or can be effectively utilized for disinfestation of grains, in the larger storage bins are silos etcetera.

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**Ozone treatment of wheat : A case study**



(Mishra et al. 2016)

$$\text{Mortality}(\%) = \left( \frac{N_0 - N_f}{N_0} \right) \times 100$$

$N_0$  = Initial number of insect infested  
 $N_f$  = Final number of insect after treatment



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So, this is a case study we are showing in the results in our laboratory we have done infestation of wheat grain as I told you earlier using ozone we have studied different combinations and permutations insects also 2 3 common insects which grow on wheat, we have used. So, insects of different stages of these insects all the eggs, pupae, larvae and adults in different concentrations they were used is that is the grains they were first conditioned to wearing moisture content and in this conditioned grains we are infested with known number of the different life insects of different life cycle.

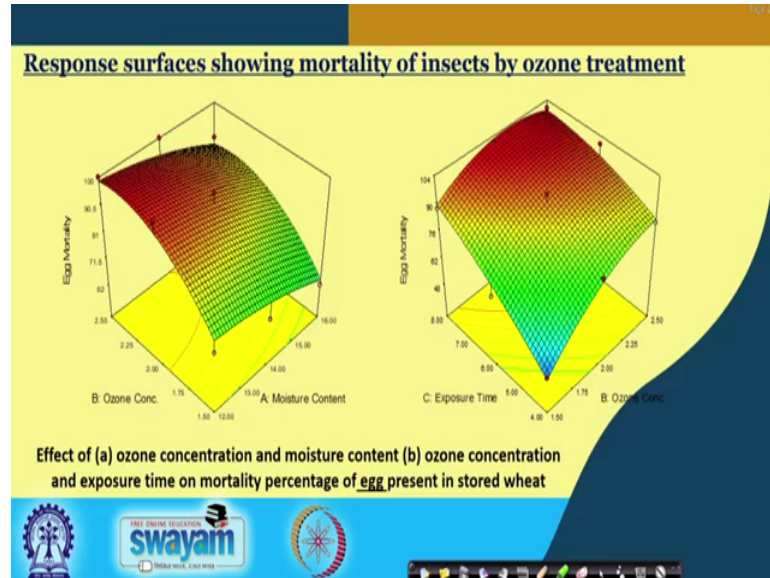
Then it was stored for some time for allowing the insects to grow alright and once means that is in this way samples of under controlled conditions infested grains samples were created and of course, the by counting the number of insect etcetera, by appropriate methods, the level of infestation was known. That is what are the number of insects initially present in the grain in the known weight of known quantity of a wheat grain? So, this now wheat grain sample infested wheat grain samples known quantity of with known level of infestation were treated with the ozone gas.

You can see here in this bottle this infested grain samples are kept and the ozone it is connected through pipe, the ozone is generated and this it is purely experimental set up. So, it was first known quantity of ozone was fast with this grain for different period of times and data were collected. And the mortality rate finally, was calculated from  $N_0$  minus  $N_f$  by  $N_0$  into 100 that is the percent mortality, where  $N_0$  is the initial number of



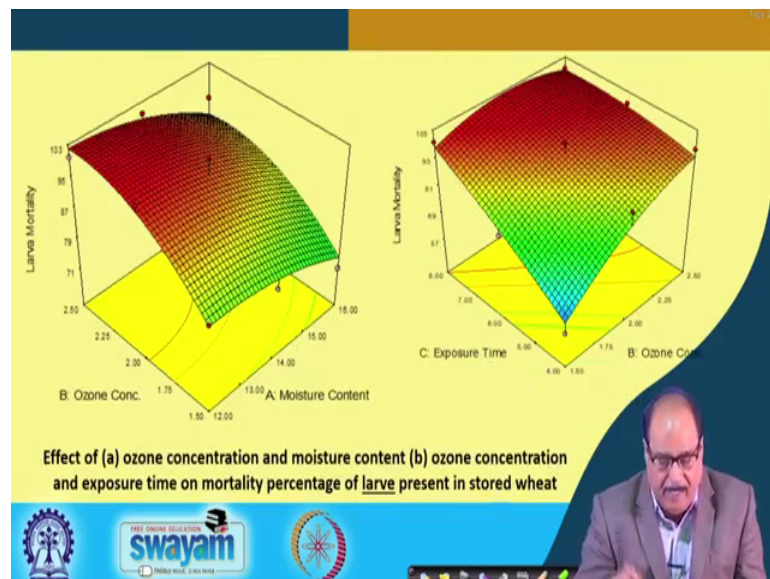
insects present in the infested grain sample and  $N_f$  is the final number of insect after treatment.

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And these are the response surface graph that is in the this case a that is it is the effect of ozone concentration and moisture content of the grain and egg mortality where exposure time and ozone concentration on egg mortality. So, these response says that the moisture content as well as ozone concentration whether exposure time all this they have significant effect on the mortality of the egg.

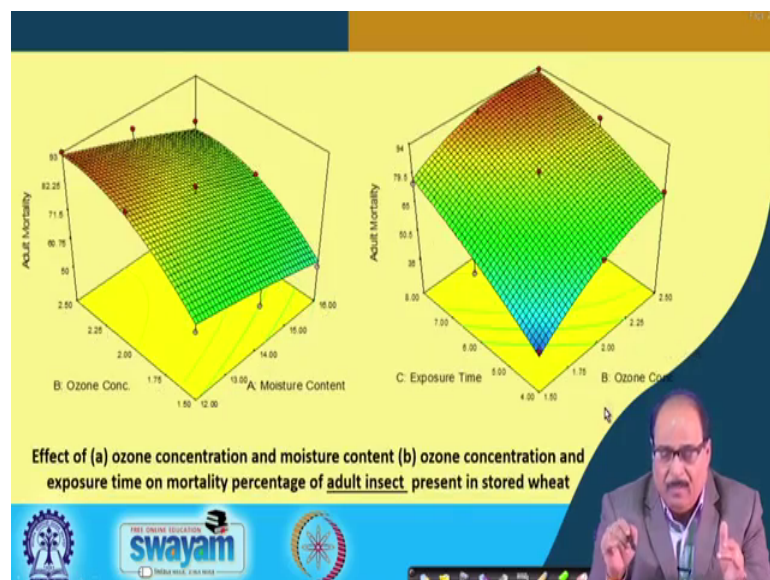
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Similarly similar effects that is a ozone concentration, moisture content of the grain, exposure time right have significant effect on the larvae mortality and even mortality ranging from different percentage that is a even up to 100 percent of the mortality ranging from 71 to 100 percent in this case where obtained in other case 57 to 93 or 99 percent.

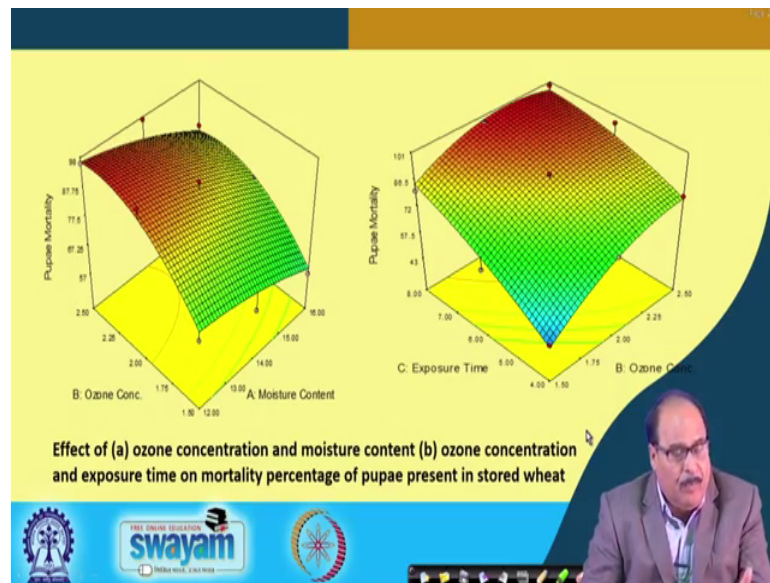
So, depending upon the conditions of the grain it is a temperature, moisture content, concentration of ozone and exposure time varying degree of are that is even up to 100 percent mortality where found out in our case.

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This graphs response surface graph shows the effect of oxygen, ozone concentration, moisture content of the grain exposure time on adult mortality, that is adult insect mortality.

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And similarly, the on the pupae mortality this response surface shows that how the pupae mortality varied with the various an in ozone concentration, moisture content and exposure time and the wheat grain?

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**Regression models developed for prediction of responses of ozone treatment**

Responses (%)	Regression models	R <sup>2</sup>	Adjusted R <sup>2</sup>	CV (%)
Adult mortality	$-200.333+1.611*A+182.888*B+22.083*C-1.250*A*B+0.250*A*C-4.583*B*C-0.166*A^2-28.666*B^2-0.708*C^2$	0.971	0.956	5.42
Pupae mortality	$-277.388+10.722*A+195.500*B+23.972*C-0.750*A*B+0.375*A*C-5.333*B*C-0.541*A^2-33.333*B^2-0.916*C^2$	0.966	0.948	5.28
Larvae mortality	$-258.768+9.263*A+191.888*B+24.152*C-0.166*A*B+0.437*A*C-6.333*B*C-0.527*A^2-32.444*B^2-0.861*C^2$	0.961	0.941	5.17
Egg mortality	$-201.222+12.41*A+151.833*B+12.333*C-0.666*A*B+1.000*A*C-0.758*B*C-0.708A^2-22.000*B^2-0.500*C^2$	0.928	0.891	5.29

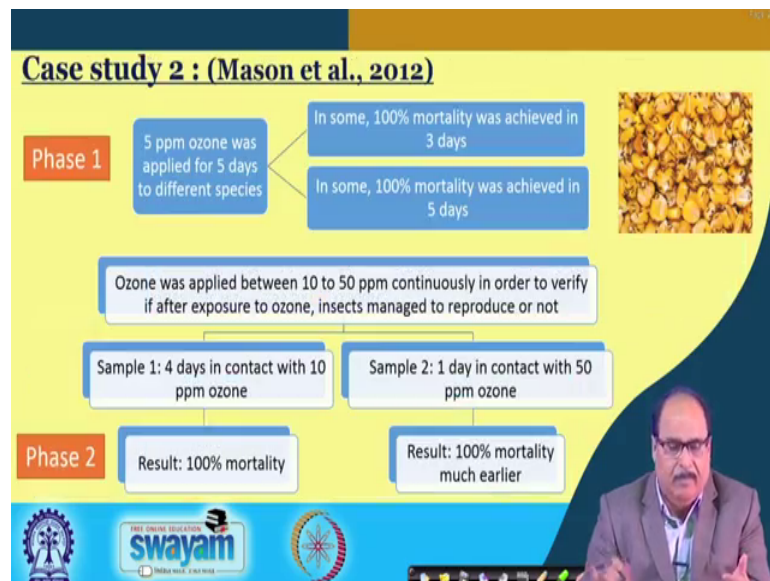
**Validation of the regression models developed for the treatment of ozone for disinfestation of all life stages of *R. dominica***

Responses (%)	Predicted values	Experimental values	Residuals
Adult mortality	98.99	97±1	1.99
Pupae mortality	101.90	100±2	1.904
Larvae mortality	100.46	99±1	0.462
Egg mortality	102.94	100±0	2.939

So, these data response surface data where use to develop regression models for the production of responses like mortality, adult mortality, pupae mortality, larvae mortality and egg mortality and the R square value or adjusted R square value obtained in this shows that the model is a best field.

In fact, these models were used to predict the values and then under the optimized conditions, the experiment was conducted and you can see here in the table below that for all the cases of that is whether it is a adult mortality percentage, pupae mortality percent, larvae and egg the predicted values are close with the experimental value or experimental values are very close. In fact, residuals are very less in fact, ranging from 0.4 to 2.9 maximum. So, this shows that the models are highly accurate and they have given their prediction level is very high.

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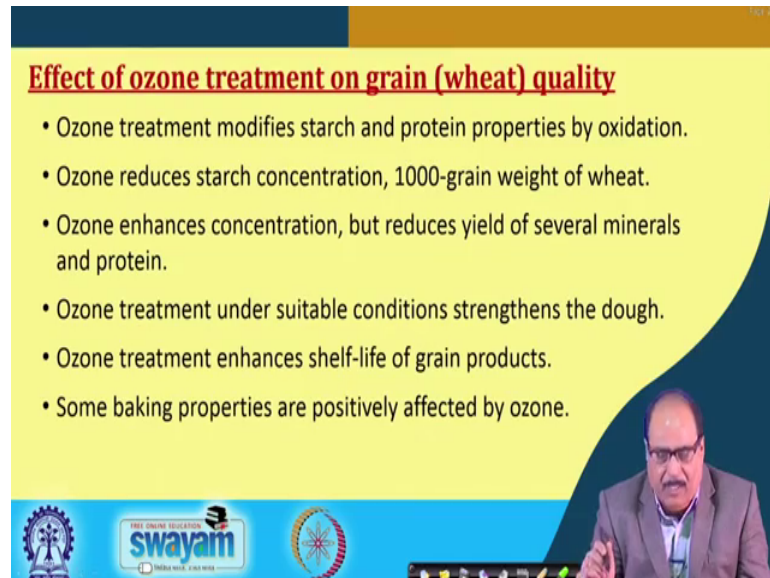
Another case study that is taken from the literature or Mason et al. in 2012 they reported, that 5 ppm ozone was applied for 5 days to different species of insects in maize. In some time 100 percent mortality was achieved in 3 days in some case, even 100 percent mortality was achieved in 5 days depending upon of course, level of infestation grain, conditions and type of the insect's life stages.

Ozone was applied between 10 to 50 ppm continuously in order to verify it after exposure to ozone, in order to verify it after exposure to ozone where insects manage to reproduce or not and in sample 1 that is 4 days in contact with 10 ppm ozone. It resulted hundred percent mortality means the insects were not able to reproduce once after they were treated with ozone.

Similarly in other case also that is in sample and second sample one day in contact with 50 ppm; 50 ppm of ozone resulted is hundred percent. So, it was very very effective

ozone treatment of grains whether it is maize grain, whether it is a wheat grain and it was found to be effective in disinfecting them.

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**Effect of ozone treatment on grain (wheat) quality**

- Ozone treatment modifies starch and protein properties by oxidation.
- Ozone reduces starch concentration, 1000-grain weight of wheat.
- Ozone enhances concentration, but reduces yield of several minerals and protein.
- Ozone treatment under suitable conditions strengthens the dough.
- Ozone treatment enhances shelf-life of grain products.
- Some baking properties are positively affected by ozone.

The slide features a yellow background with a dark blue header and footer. The footer contains logos for 'swayam' and 'INDIA RISE, INDIA RISE' along with a small video inset of a man in a suit speaking.

So, the in our study also from the literature people have studied they reported the effect of ozone treatment on grain quality we have analyze the grain quality or our results indicated that. In fact, ozone treatment little bit modified the starch and protein properties because it results it might cause the oxidation of starch and protein etcetera.

However the effect are not very significant as far as the quality of the baking characteristics are other characteristics of the wheat is concerned in fact, in some baking properties are positively affected by ozone. Ozone reduces the starch concentration; it results in to the lowering of 1000 grain weight of wheat.

However, it enhances the concentration of several minerals and vitamins etcetera. Ozone treatment under suitable conditions strengthens the dough, means the protein quality etcetera may be this oxidation of protein results has some maturation effect on the protein. So, baking properties etcetera has improved. Ozone treatment enhances the self-life of the grain products, with this I thank you for your patience hearing.

Thank you.