To study the effect of plasma treatment on storage fungi of Mung bean (*Vigna radiata)*

A dissertation report
submitted in partial fulfilment of the requirement for
the award of degree of

Master of Technology
In
Biotechnology

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CANDIDATE'S DECLARATION

I hereby declare that the work being presented in the M.Tech dissertation entitled "**To study the effect of plasma treatment on storage fungi of Mung bean (Vigna radiate)**" in partial fulfilment of the requirements for the award of Degree of Master of Technology, Department of Biotechnology, Thapar University, Patiala is my own laboratory work during the period of **July 2015 to January 2016**, under the conception and supervision of Dr. Sanjai Saxena (supervisor), Professor, Department of Biotechnology, Thapar University, Patiala and from **January 2016 to June 2016** under Dr. Sunita Mishra (co-supervisor), CSIR-CSIO, Chandigarh. I have not submitted the matter embodied in this thesis for the award of any other degree.

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CERTIFICATE

This is to certify that the thesis entitled "**To study the effect of plasma treatment on storage fungi of Mung bean (*Vigna radiata*)**" being submitted by **Ms. Saru** in partial fulfilment of the requirements for the award of Degree of Master of Technology in Biotechnology, Thapar University, Patiala is a bonafide work carried out under the esteemed supervision and conception of **Dr. Sanjai Saxena** and **Dr. Sunita Mishra**. The report has not been submitted for the award of any other degree.

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I dedicate this thesis to my beloved family who are an ever supporting and encouraging with their great patience.

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Abbreviations

S.No	Abbreviation	Full form
1.	ACN	Acetonitrile
2.	AD	Alzheimer's dementia
3.	COX	Cyclooxygenase
4.	DNA	Deoxyribonucleic acid
5.	dNTP	Deoxynucleotide triphosphate
6.	EA	Ethyl acetate
7.	EDTA	Ethylene diamine tetra acetic acid
8.	ITS	Internal transcribed spacer
9.	L	Litre
10.	M	Molar
11.	mAU	Milli-absorbance unit
12.	mM	Milli-molar
13.	mg	Milli-gram
14.	mL	Milli-litre
15.	mm	Milli-metre
16.	nm	Nanometer
17.	NO	Nitric oxide

18.	PCR	Polymerase Chain Reaction
19.	ppm	Parts per million
20.	µg	Microgram
21.	µL	Microlitre
22.	R_f	Retention factor
23.	rpm	Rotation per minute
24.	CDA	Czepak Dox agar
25.	CDB	Czepak Dox broth
26.	CMA	Corn Meal Agar
27.	GLA	Grape leaves agar
28.	HPLC	High performance liquid chromatography
29.	MEA	Malt Extract Agar
30.	PDA	Potato Dextrose Agar
31.	RESV	Resveratrol
32.	SIRT 1	Sirtuin 1
33.	SNA	Synthetischer nahrstoffarmer agar
34.	TAE	Tris acetate EDTA
35.	TE	Tris EDTA
36.	TFA	Tri-fluro acetic acid
37.	TLC	Thin layer chromatography
38.	WA	Water Agar

Executive Summary

This study focuses on the inactivation of *Aspergillus flavus* from the surface of the mung bean by using the low pressure cold plasma. Before decontamination process, the sample is inoculated with the 10^6 spores of *Aspergillus flavus*. The plasma treatment of the sample is carried out by using the SF_6 gas for the duration of 2-10min. There is 1 log reduction of the spores after 2 minute treatment and further increases to 2-Log reduction after 5 min of plasma treatment.

Complete decontamination of the spores from the mung bean surface is achieved within 10 minutes of treatment. Various biochemical parameters of the mung bean sample are also investigated to study the effect of plasma treatment on the nutritional value of the sample. The cooking time of the mung bean sample is decreased from 35 to 30 minutes due to increase in the water uptake ratio of the plasma treated sample. Ash content of the mung bean predicts the mineral present inside the mung bean and it is found to be increased after plasma treatment. Proximate analysis of the sample has been also investigated and it is notice that the protein, fat and carbohydrate content of the mung bean is increased from 27% to 30 %, 1.30 %to 1.80%, and 1.186 to 1.1935µg/ml respectively.

FTIR and SDS-PAGE of the treated and untreated sample is compared and there is no change in the peaks of FTIR and the protein bands of the SDS-PAGE. Antioxidant activity of the untreated and plasma treated mung bean sample is studied and it results in the increase of the total phenolic and flavonoid content of the mung bean. Radical scavenging activity of the sample is also carried out by the FRAP and DPPH assay and it is observed that scavenging activity of the mung bean sample enhanced. Tetrazolium staining was performed and it is observed that the 98% of the seeds are viable after plasma treatment.

Chapter 1

Introduction

1. INTRODUCTION

Post harvest loss is the measure of the qualitative and quantitative loss of the food. These losses occur at the time of threshing, harvesting, storage, primary processing, secondary processing and packaging of the food. It is estimated that 12 to 16 million metric tons post harvest loss of grains occurs per year in India that cost around 5,00,000 million rupees (Singh 2010) Mung bean is a seasonal grain and need to be stored as it is consumed by the large population for the whole year and get spoiled due to improper storage conditions. Food safety is a critical issue in food industry. Long term storage of grains produces toxic compound by the storage fungi. Fungal contamination is one of the major causes of the grains spoilage (Pitt et al. 1997) contamination of the cereal grains occurs due to grain moisture content, storage conditions (time and temperature), dust, water, soil having animal faeces, conditions of transport and processing which leads to high economic loss and risk to human and animal health through the synthesis of mycotoxin (MacDonald et al. 2004).

Spoilage of the grains with the fungal species is due to the improper handling and storage conditions. Most commonly found toxin produced by the fungus is deoxynivalenol, ochratoxins, fuminisin, zearalenone and aflatoxin. Majorly two types of fungal contamination is found- Field fungi and Storage fungi. Field fungi are those which affects the cereals before harvest while storage fungi contaminate the cereals at the time of the storage. The original source of contamination in both the categories is due to field fungi. The field fungi such as *Fusairum graminearum. F. moniliforme* and *Aspergillus flavus* that grows on the plants under stressed conditions. Storage fungi *like Penicillium verrucosum* and *A. ochraceous* also contaminates the seed from the soil and grows under favourable conditions at the time of storage (Singh et al. 2013).

1.1 Strategies to remove post harvest loss

Fungal contamination and production of aflatoxin from it cannot be avoided at the time of production and storage because of variable environmental conditions. Therefore an effective, economical and reliable method must be selected which can remove fungus before the toxin is produced at the time of storage. Various treatments were used for the

decontamination of the fungus. An adequate method must have the following properties:

- It should properly kill the mycotoxin
- It should not be toxic for food
- It should not change the physical properties, taste, or nutritional value of the food (Beaver 1991).

Methods which are used for suppression of fungal growth and mycotoxin production are divided into three categories are physical, chemical and biological method. Physical method includes thermal inactivation (heat treatment), irradiation, aeration, moisture control and modified atmosphere conditions. In chemical method, the sample is treated with chemicals but it leaves the toxic chemical residue behind which have the negative effect on human. Lastly, the biological method which uses the microorganisms for the treatment of the sample but all this methods have the disadvantages which cannot be neglected. Therefore, plasma treatment came into origin as it is eco- friendly and the internal properties of the sample are not affected (Calado et al. 2014).

1.2 Plasma treatment

A plasma is an ionized gas which contains equal number of positive ion and negative electron (Niemira et al. 2012). It is also known as the fourth state of matter as it is produced on applying energy such as heat, radiation, electricity and laser light to the gas results in the breakdown of the intermolecular and intra-atomic structures which ultimately releases free electrons and ions. On recombination of these active particles, UV and visible light produced that reacts with food substrate and releases energy which targets the mycotoxin.

Two types of Plasma are known- thermal and non thermal Plasma. Thermal plasma is also known as hot plasma which contains electrons, neutrals and ions at same temperature while non thermal or cold atmospheric plasma contains electrons at higher temperature than neutrals and ions. The non thermal plasma can be produced by plasma needle, dielectric discharge, atmospheric pressure plasma and plasma pencil (Hoffmann et al. 2013). The plasma which is used in our project is the low or atmospheric pressure plasma jet.

1.3 Applications of Plasma Treatment

Plasma treatment is widely used method for enhancing the surface quality of many industrial products. This method removes the foreign material from the surface of component and results in highly improved surface properties. Some of the industrial application of plasma treatment is listed below as (Lieberman and Litchenberg 1994):

a) Automotive Industry: In automotive industry, Plasma treatment are performed to obtain highly finished surface and also to improve the wear resistant properties of components.

b) Transport Industry: The primary motive of plasma treatment in transport industry is to reduce the component weight while it also enhances the surface quality of composites and polymers.

c) Plasma treatment is basically a surface modification technique used for the surface cleaning purpose and also been used earlier for the decontamination of the fungal species from the grain/legumes, from nut surfaces (Basaran et al. 2008).

d) Plasma treatment also increases the nutritional value and antioxidant activity of the food items hence, increases the shelf life of the food. Hence the present study was undertaken to assess the effect of low pressure cold plasma treatment on nutritive, surface and biochemical properties of mung bean (Amini and Ghoranneviss 2016).

Chapter 2

Review of Literature

2. REVIEW OF LITERATURE

Legumes are rich in protein, vitamins and minerals thus acts as the substitute of meat for the vegetarian people also they are cholesterol and fat free. Among legumes, mung beans (*Vigna Radiata*) have high lysine content which is good for the patients suffering from viral disease. Mung bean grains also exhibit high level of antioxidant activity due to the presence of phenolic and flavonoid content. Antioxidant have lots of benefits on human body such as prevention of oxidative injury caused by heat stress, cardiovascular diseases, cataract development, decreases fat absorption, lower the incidence of influenza infection and increases the energy expenditure (Tang et al. 2014).

2.1. Post harvest losses

Measurement of the food loss either in numbers or the quality of food from the field at the time of harvesting to the retail market is known as post harvest loss. As the population of the world is increasing results in an increase by approximately 60% to satisfy the food demand in the next 10 years. This demand can be fulfilled by increasing the productivity and reducing the food loss after harvest. It is estimated that around 13, 00,000 tons of food get wasted every year globally. Post harvest loss estimation in India has been shown in the table 1. Reduction of the food losses results in increase the availability of the food.

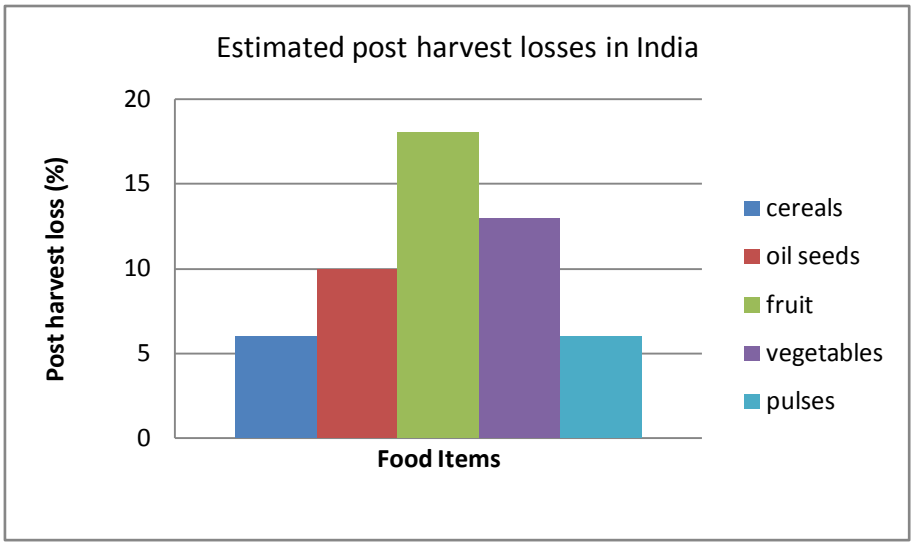

Figure 1 Estimation of post harvest loss in India

2.1.1 Factors affecting post harvest loss

Post harvest loss of food supply is due to various factors such as moisture, weather, pests and infrastructure of storage at the time of harvesting, food storage, processing and packaging is shown in table 1.

Stages of food supply	Moisture	Weather	Pests	Infrastructure
Harvesting	✓	✓	✓	✗
Food storage	✓	✓	✓	✓
Processing	✓	✓	✓	✓
Packaging	✗	✗	✗	✓

Table 1: Factors affecting post harvest loss

2.2 Storage fungi

Storage fungi have negative impact on the crops due to synthesis of mycotoxin. Previously it has been reported that four different fungi belonging to 3 different genera were isolated from 20 different samples of mung bean. The fungus which were isolated are *Aspergillus flavus, Aspergillus niger*, *Fusarium* spp. and *Penicillium* spp. and population of the fungus isolated was 7.0-17.50%, 4.0-11.50 %, 7.25 to 19.50% and 4.00 to 8.50% respectively. Therefore, it can be concluded that *Aspergillus* spp. is the main storage fungi in the mung bean which is producing the aflatoxins (Barua et al. 2007).

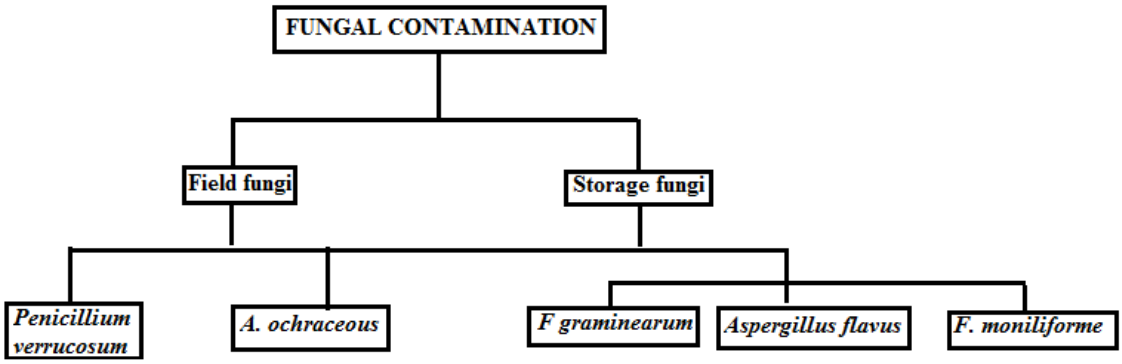

Figure 2: Schematic view of the fungal contamination and its categories

2.3 Strategies to prevent the storage fungi

Detoxification process helps in preventing the mycotoxin production. Detoxification process can be achieved by removing the toxins present in food substance. It can be divided into three categories are as follows:

2.3.1 Physical method: This method includes thermal inactivation (heat treatment), irradiation (UV, gamma, microwave etc), aeration, moisture control and modified atmosphere conditions.

- **UV Radiations**- Murata et al. 2008 have studied the effect of UV radiations on the wet and the dry conditions and confirmed that UV radiations were effective to both the conditions of the grains. Fungus was taken of quantity 30mg/kg in the Solid form ZEN or DON was dissolved in the 1g of buffered solution and dried on filter paper. Filter paper was treated with UV radiations of power 24 mW cm^{-2} and found that total reduction of ZEN and DON was done at 15 min and 20 min respectively. It been reported earlier that U.V. radiations have the adverse effect on the crops as they affects the chlorophyll content in most of the crop species. Hence, UV radiations ultimately effect the yield of the crops (Kakani et al. 2003).
- **Gamma Radiations**- Ghanem et al. 2008 reported that gamma radiations are effective for degradation of storage fungi from food crops. They have inoculated the food crops with 10^{-7} spores of the *Aspergillus flavus* which produces the aflatoxin B1 and incubate for 10days at 27°C. Gamma radiations at different doses such as 4, 6, and 10 kGy results in the reduction of spores which vary with the sample. Gamma radiation

is widely used method for the decontamination (F.L. Ferreira-Castro et al. 2007) but this method reduces plant germination, plant life, plant height and also the grain number or grain weight (Khah et al. 2015).

- **Microwave radiations:** It has been reported that microwave cooking adversely affect the composition and nutritional quality of legumes (Hefnawy et al. 2011). The treatment of wheat seedlings with microwave radiations reduces their germination and vigor index (Gaurilcikiene et al. 2013).

Irradiation was considered as the most effective method but it has some disadvantages which are listed below

I. Irradiation cannot be applied to all the food items as many vegetables, milk and dairy products lose their nutritional value and firmness after irradiations.
II. Irradiation also results in the degradation of fat/water soluble vitamins, increase in the oxidation compounds and radiolytic substances which is hazardous.
III. Irradiation can create the mutation on the microorganism and produce the more toxic strain (Calado et al. 2014).

2.3.2 Chemical methods

Chemical method involves the treatment of the sample with chemicals. Phosphine gas is used for the protection of grains from the insects and mites. Phosphine is a toxin gas use for the suppression of fungus like *Aspergillus flavus* nad *Aspergillus parasiticus* ultimately reduces the mycotoxin. Fungicides are also used for the removal of mycotoxin from the agricultural products. Fungicides such as diazinon, malathion, landrin and dichlorvos are used for the removal of aflatoxin B1 from the grains. Various Herbicides includes Glufosinate-ammonium are used for the reduction of mycotoxin such as aflatoxin produced by the fungus *Aspergillus flavus.* By using this method, the chemical residues are left behind which imparts negative impact on the human health and animals (Juodeikiene et al. 2012).

2.3.3 Biological methods

This method involves the usage of microbial strain for the removal of mycotoxins (Kabak et al. 2006). Biological methods include the use of fermentation techniques which can

reduce the toxicity produced by mycotoxin. Beer fermentation helps in the reduction of ZEN and converts it into *β*-zearalenol which is less toxic and ethanol fermentation can also reduce the mycotoxin from the food. There are some strains such as, *Streptococcus sp* and *Lactobacillus* which reduce the mycotoxin like ochratoxin and Aflatoxin B1 from the milk.

2.4 Plasma treatment

Plasma is the fourth state of matter which is produced by providing energy to the gas and convert it into ionized gas which have the free radicals, UV–Visible radiations and neutrals and ions. Figure 2.1 represent the plasma production. Plasma is a gas of which a fraction of its constituents are no longer electrically neutral. Instead, the atoms/molecules are ionized, that is, they have lost (or gained) one or more electrons and moreover free electrons are also present in the plasma.

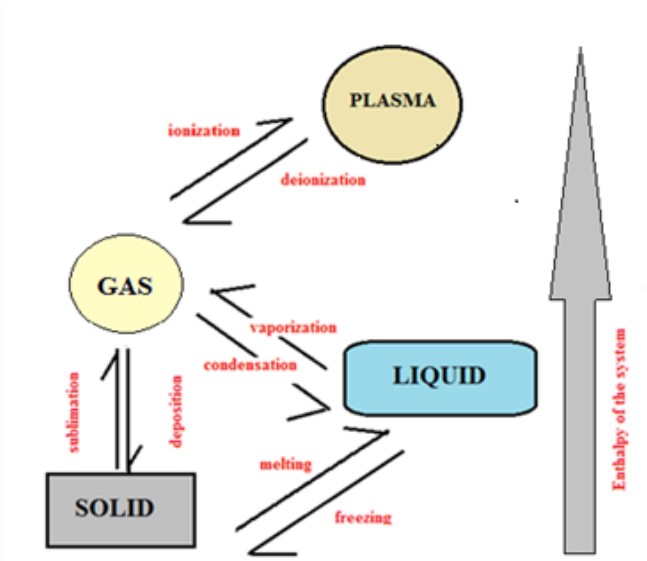

Figure 3: Plasma is an ionized gas

2.5. Plasma treatment for Decontamination

2.5.1. Fungal decontamination by plasma treatment

Pervin Basaran et al. 2008: They have used the SF_6 and O_2 gas for the disinfection of *Aspergillus parasiticus* on the nut surfaces for different duration up to 20 minutes. On treatment of sample for 5minutes results in the 1 log reduction of aflatoxin and further 5 min results in the additional 1 log reduction. There was a 50% reduction in the aflatoxin

using the air gas and only 20% reduction on using using the SF_6. After decontamination, morphology and organoleptic behavior of the nuts were examined and it was found that there is no visual change in the surface of the nut samples and even the taste of the nuts remains same after treatment.

Selcuk et al. 2008: Cold plasma treatment is used for the decontamination of *Penicillum and Aspergillus spp.* from the surface of grains and legumes. Cold plasma treatment used was self designed and two gases were used i.e. Air and SF_6 gas for 30s-30 min. The base and running pressure of plasma reactor was 100 mTorr and 500 mTorr respectively. The applied power of the reactor was 300 Watts. It was seen that there is 3 g reduction after 15 minutes for both the species. After decontamination, seed germination or growth conditions were studied and there was no significant change in the germination of wheat and beans. Nutritional value, cooking quality, Wheat gluten content , gluten index and sedimentation were also studied. Cooking quality was deteriorated, water uptake of seeds and cooking yield was same after plasma treatment.

Dasan et al. 2015: They have decontaminated the hazelnuts which are artificially inoculated with the species of *Aspergillus flavus* and *Aspergillus parasiticus*. The operating gases which were used in the plasma reactor was O_2 and N_2 having range of 100-70% voltage and frequency 18-25 kHz and the flow of the gas was 1000–5000 L/h. Dry air gas was used for the decontamination of both the species for duration of 5 minutes at varying plasma conditions. There is 4.5 and 4.19 log reduction of the *Aspergillus flavus* and *Aspergillus parasiticus* respectively after 5 minutes exposure of plasma. The decontamination of both the species after increase in the voltage and frequency of the plasma reactor. The damage caused by the plasma reactor on the *Aspergillus flavus* and *Aspergillus parasiticus* was observed using SEM.

2.5.2. Bacterial decontamination by plasma treatment

Ziuzina et al. 2015*:* illustrated inactivation of internalized bacteria and biofilms for *Salmonella, Escherichia coli,* and *L. monocytogenes* by using the plasma treatment. Lettuce broth was prepared and inoculated with bacterial suspension of concentration 1 X 10^7 CFU/ml. Decontamination was performed using plasma air gas of 80kV power was used for 30 s at normal atmospheric pressure conditions reduced the microorganism

populations. Reduction of spores also depends on storage conditions, type of bacteria and biofilm age. Biofilm populations was reduced to 1 X 10^5 on 300sec of treatment.

Butscher et al. 2015: they have performed the inactivation of *Bacillus amyloliquefaciens* endospores on wheat. The gas flow rate was 15 nlm (norm lire per minute) using gas such as argon and oxygen with varying concentration and the aeration gas flow was 3 nlm. The plasma power was also varying continuously and the best result was shown at 900 watt than the lower power of plasma at 700watt. The most effective treatment time which observed was 30s and results in 2 log reduction of the spores. Dough texture and flour was analyzed for the treated and untreated sample with the farinograph, it was found that the water absorption was remained same but the dough softness was decreased a little which shows a better gluten quality after plasma treatment.

Reineke et al. 2015: illustrated the inactivation of the *Bacillus* spores by using the mixture of gases in the plasma reactor. In this plasma, argon was used as the reference gas and O_2 and N_2 gas admixture was added continuously until the unstable plasma ignition was visible. Three different parameters were optimized firstly using pure argon, secondly is the argon+ O_2 (0.135 vol %) and thirdly is the argon+ O_2 (0.135 vol%)+ N_2 (0.2 vol%) were optimized. The mixture of argon + O_2 (0.135 vol%) + N_2(0.2 vol %) produces 4 times more UV photon in comparision to the pure argon gas. The inactivation of the *Bacillus* spores was not affected by the mixture of gases. The effect of the plasma treatment using pure argon for the decontamination of *Bacillus subtilis* and *Bacillus atrophaeus* was 2.4 $\log_{10}$ and 3.1 $\log_{10}$ respectively. It was found that the highest UV-C photon spectral intensity was emitted by the mixture of argon + O_2 (0.135 vol %) + N_2 (0.2 vol%) and pure argon gives no UV-C photon.

Butscher et al. 2016: decontamination of bacterial endospores present on the surface of wheat and polypropylene model substrate by plasma treatment with power supply of 10kHz for 5min – 15 min and 5 kHz-15 kHz for 10 min but the pulse voltage and length of plasma were constant i.e. 500 ns and 10 kHz respectively. Firstly, the wheat grains and polypropylene was artificially inoculated with the *Geobacillus stearothermophilus*. Decontamination of the wheat grain is little difficult due to deep furrow present on the surface of the wheat but with long treatment time, fast pulse

frequency and high voltage improves the decontamination of the bacterial spores from the wheat grains. It was also found that the reason for decontamination was not due to the mechanical, electrical and thermal factors but only because of the reactive species produced after plasma treatment. The SEM confirmed the decontamination of the bacterial endospores from the wheat grains.

2.5.3. Effect of plasma treatment on the biochemical characteristics

Proximate analysis

Proximate Analysis is a partitioning of compounds in a feed into six categories based on the chemical properties of the compounds. The six categories are moisture, ash, crude protein (or Kjeldahl protein), crude fat, crude fibre, and nitrogen-free extracts (digestible carbohydrates).

Chen et al. 2014: They have illustrated the improvement in nutrition of brown rice using plasma reactor. Brown rice were exposed to plasma ranging from 1kV to 3kV for 10 minutes. After treatment of brown rice with the plasma results in the increase in the water uptake, germination percentage and also seedling length measured by using vernier calliper after germination. The best results were shown by the 3kV plasma for the duration of 10 minutes. Various nutritional properties of the rice were studied after plasma treatment. The α- amylase activity of sample was increased after plasma treatment and results in the rapid germination of the sample. Gamma aminobutyric acid (GABA) content which is good for the brain and central nervous system also increases after plasma treatment by 19 to 28 mg/100 g approximately. The most important anti-oxidant activity of the rice also increases after plasma treatment.

Thirumdas et al. 2015: Studied the effect on the cooking quality and the physiochemical properties of the basmati rice using low temperature plasma processing. Atmospheric air is used as the reference gas in the plasma reactor and working pressure for the plasma generation, radiofrequency power supply of the electrodes was 0.15 mbarr, was 13.56 MHz. Different power supply of the plasma for different time durations was used i.e 30W for 5-10 min duration and 40W for 5-10 min duration of time were analyzed. Proximate composition of the basmati rice was studied i.e. moisture, ash, fat,

carbohydrate, amylose and protein were determined. Cooking quality of the basmati rice was also analyzed by checking the cooking time, cooking loss and water uptake of the basmati rice. Contact angle, moisture uptake, texture profile analysis and microstructure of the rice for checking the morphology of the rice by SEM was also performed. The moisture content of the rice was decreased from the rice by 10.28 ±0.02 to 7.55 ±0.02 and ash content was increased from 0.345 to 0.365 g/100g. The protein content was increased from 5.43g/ 100g to 5.55g/100g . It has been observed that there is decrease in the contact angle and increase in the surface energy which makes the sample more hydrophilic as a result which ultimately results in the change of the cooking properties in a positive manner i.e. cooking time got decreased from 20 min to 13 min as the water uptake capacity of the rice after plasma treatment got increased. The hardness and stickiness of the rice was also decreased using plasma treatment.

Ling et al. 2015: Increase in the germination of the oilseed rape (*Brassica napus* L.) under drought stress after cold plasma treatment was reported. Two species were taken Zhongshuang 7 and Zhongshuang 11 under both the conditions i.e. drought and well watered. Germination power was more significantly increased in case of Zhongshuang 7 than Zhongshuang 11. The oil rapeseed variety Zhongshuang 7 under drought stress conditions result in the increase of the germination rate, vigor index and germination index after plasma treatment by 6.25% , 6.89% and 29.58% respectively while the Zhongshuang 11 was increased by 4.44%, 19.64% and 4.77% respectively. The contact angle of the plasma treated seeds Zhongshuang 7 and Zhongshuang 11 were decreased by the 30.38% and 16.91% respectively. Total protein content was also estimated before and after plasma treatment of both the varieties Zhongshuang 7 and Zhongshuang 11, the increase of the total protein content by 10.90% and 6.04% respectively but the malondialdehyde content in seedling decreases after plasma treatment. The most important factor that increases after plasma treatment is the drought tolerance due to improvement in the antioxidant enzyme activity, lipid peroxidation adjustment and osmotic adjustment product especially of the drought tolerant variety.

2.5.4 Effect of plasma treatment on the protein bands by SDS-PAGE

Pal et al. 2015: The effect of non thermal plasma treatment on the short and long grain rice and physio-chemical properties, pasting, amino acid composition and protein characteristics were evaluated. This non thermal plasma reactor consist of dielectric barrier discharge having two aluminum electrodes powered from the step-up transformer for the duration of 5 and 10 minutes two discrete voltages were applied across the electrodes. Ozone is generated by the dielectric barrier discharge as an active agent and it was found that the ozone liberated for 5 and 10 min was same for both the varieties at 60kW was 120 ± 20 ppm and 220 ± 20 ppm respectively. Non thermal plasma treatment results in the increase of asparagine, serine, histidine, thymine, glutamic acid, tryotophan, isoleucine, phenylalanine and proline etc. Amylose content was also increased by the non thermal plasma treatment of both the varieties. Swelling power is measured at different temperature i.e. 60℃, 70℃ and 90℃ of both the varieties. The swelling power of the long rice is more in comparison to the short rice before and after plasma treatment. SDS – PAGE do not show any change in the bands of both the species total protein after non thermal plasma treatment for the duration of 5 and 10minutes. The transmittance was also studied for both the treated and untreated samples and was found that untreated sample show lesser whiteness in comparison to the treated rice samples.

2.5.5. Effect of plasma treatment on the anti-oxidant properties

Antioxidants are those substances which inhibit the oxidation of other substance and oxidation produces free radicals. Antioxidant addition to foods has become important for increasing the shelf life of food. Antioxidant vitamins are required in the diet for the good health as they have the anti disease activity Various natural sources are used for the production of Anti-oxidative substances such as grains/cereals/beans, fruits, roots, vegetables, oilseeds, leaf waxes, spices, bark, seaweeds and roots are preferred over others (Duh et al. 1997). Mung beans contain high levels of amino which are used as antioxidants and they fight against cancer. Mung beans show anti-tumor activity that prevent the cell mutation and also protect DNA from damage. It has been already studied that the antioxidant activity of the mung bean antioxidant activity is due to two protective flavanoids i.e. vitexin and isovitexin that have high free-radical scavenging abilities which lowers the oxidative stress and prevent cancer formation (Li, 2012).

Kim et al. 2015: Studied the effect of atmospheric dielectric barrier discharge plasma treatment on the antioxidant activity of the naringin. Naringin is a flavonoid found in the citrus species having the bitter taste. The sample was prepared by dissolving pure naringin in the methanol and plasma treated for 20 minutes. The conversion products after treatment were analyzed by the HPLC and found that the antioxidant activity in the DPPH radical and peroxynitrite assays got increased when compared to the control. After purification of the treated sample by column chromatography led to the isolation of new flavanone derivatives i.e. narinplasmin A (2) and B (3) which were not present in the control sample. Using spectroscopy the known compounds were verified using the previous literature data of those compounds. It was found that the narinplasmin A (2) was having the higher antioxidant activity after plasma treatment confirmed by DPPH and $ONOO^-$ assays.

Amini et al. 2016: Investigated the cold plasma influence on the antioxidant activity and shelf life of walnut. Microbial growth inhibition was studied during storage of the walnut. They have proved that the 11min of plasma treatment with the argon gas was effective for the full decontamination of the *Aspergillus flavus*. TPC and antioxidant activity of the walnut was increased after 15 min of plasma treatment.

Chapter 3

Aim of the study

3. AIM OF STUDY

The Aim of the present investigation is

1. Decontamination of the *Aspergillus flavus* from the surface of mung bean *(Vigna radiata).*

2. Studies on biochemical parameters of mung bean after plasma treatment.

Chapter 4

Materials & Methods

4. MATERIAL AND METHODS

4.1 Sample collection and procurement of test pathogen

Mung bean (*Vigna radiata L.)* variety SML-668 was procured from the Punjab Agriculture University, Ludhiana (Punjab), India. The collected samples were kept in sterile zip pouches and preserved at 4°C.

Aspergillus flavus (MTCC 871) was procured from Microbial Type Culture Collection (MTCC), (IMTECH), Chandigarh in the lyophilized form.

4.2 Activation of pathogenic culture

The procured test pathogen was revived by adding 200µl of sterile saline in the lyophilized ampoule of culture followed by spreading 20µl of this culture suspension on to Potato dextrose agar (PDA) plates under aseptic environment. Further, plates were incubated at 26 ± 2˚C for 7-10 days. *Aspergillus flavus* was identified by observing various micrometric and microscopic features such as colony color, appearance, growth rate and spore morphology. The pure culture was then transferred on PDA slants supplemented with 10% (v/v) glycerol for long term preservation.

4.3 Preparation of spore suspension

To the actively growing pure culture of *A. flavus*, 5 ml of sterile saline-Tween 80 (0.1%) solution was added and spores suspension was collected in a sterile centrifuge tube under aseptic conditions. The collected spore suspension was centrifuged at 10,000 rpm for 10 min. After centrifugation, the supernatant was drained off and spore pellet was re-suspended in 0.1% sterile saline solution. The optical density of the spore suspension was measured at 530 nm and cell density was aseptically adjusted to $1x10^6$ spores/ml in physiological saline- Tween 80 (0.1%) solution.

4.4 Preparation of Mung bean for plasma treatment:

4.4.1 Surface sterilization

Mung bean (3g) sample was surface sterilized by soaking in 0.5% sodium hypochlorite solution for 2 min followed by rinsing with double distilled water three times. The grains

were then allowed to dry over a filter paper under aseptic conditions. The dried seeds were then further subjected to *Aspergillus flavus* infection.

4.4.2 Inoculation of spore suspension

The sterilized dried mung beans were inoculated with 600 µl of spore suspension ($1x10^6$ spores mL^{-1}) inverted gently for uniform distribution of spores. The inoculated samples were incubated at 30˚C for 15-16h in an incubator. The initial concentration of spores was confirmed by plating the infected grains onto PDA plates and CFU/g was determined.

4.5 Plasma Treatment

Plasma treatment was carried out for the decontamination of the infected mung bean sample. The *Aspergillus flavus* infected mung beans were placed inside Low pressure Plasma reactor, PICO model (Diener Electronic, Germany). The plasma reactor consisted of matching network, vacuum pump, stainless steel chamber with rotary drum inside. SF_6 gas supplied to the plasma reactor for 10 mins and gas supply was controlled needle valve and mass flow controllers fitted inside plasma reactor chamber. The powered electrode was fixed inside the chamber whereas the outer chamber was grounded. The pressure was maintained at 0.3 mbar and temperature inside was adjusted to 27°C. The pressure and temperature of the system was maintained by using the appropriate pressure gauges (pirani sensors and baratron) and thermocouples respectively (Figure 4).

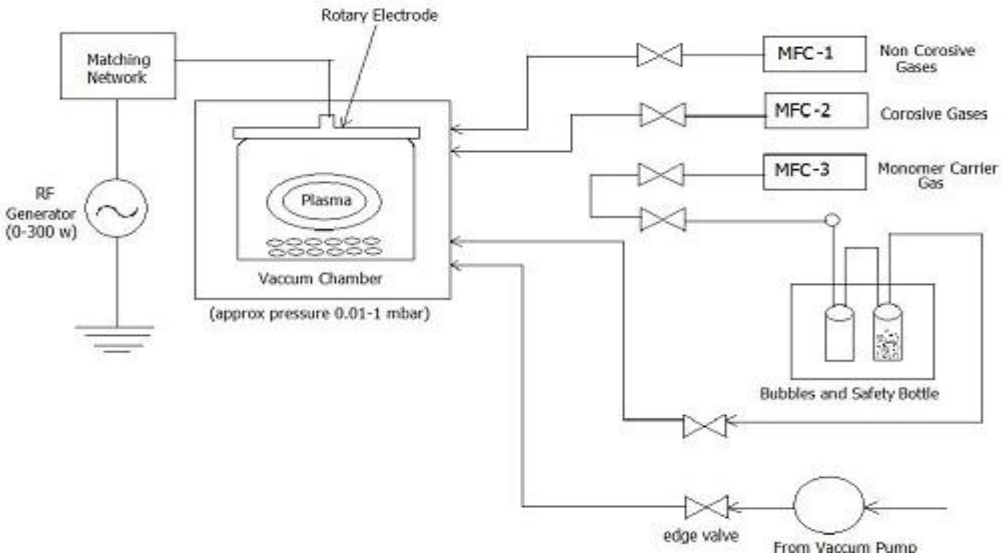

Figure 4: Schematic view of the plasma treatment system

4.6 Evaluation of the plasma treated spore survivors

To evaluate the cell viability of plasma treated and untreated mung bean samples, different dilutions ranging from 10^{-1} to 10^{-5} cells/ml of the plasma treated and untreated sample were prepared. 100 µl of each dilution was spread uniformly onto Czapek Dox Agar plates followed by incubation at 30°C for 3-4 days. After incubation, number of colonies were counted with the help of colony counter and the results were expressed as log CFU/g.

4.7. Effect of plasma treatment on cooking properties of Mung bean:

4.7.1. Cooking time

The cooking time of plasma treated and untreated samples were performed as per the method described by (Thirumdas et al. 2015) Briefly describing, 2 g of plasma treated and untreated mung bean sample were taken in a test tube followed by addition of 20 ml of distilled water. The test tubes were kept at boiling water bath and grains were removed after different time intervals and was pressed against two glass plates to ensure optimal cooking time.

4.7.2. Water uptake of mung bean

The water uptake capacity of plasma treated and untreated mung bean sample was determined by following the method of (Thirumdas et al. 2015). Briefly, 2g of plasma treated and untreated mung bean sample was cooked in a test tube for an optimal cooking period in a boiling water bath. Thereafter, the cooked sample was placed on a filter paper to drain off excess water from sample surface. Subsequently, the weight of the sample was noted to estimate the water uptake ratio.

4.8 Estimation of the antioxidant activity:

4.8.1. Extraction of Mungbean samples

2g of plasma treated and untreated mung bean samples were crushed to a very fine powder in the pestle and morter followed by addition of 10 ml of acetone/water solution (50:50, v/v). Further, the contents were transferred to a sterile centrifuge tube, vortexed for 3 min and then centrifuged at 10,000 rpm for 15 min. After centrifugation, the pellet

was discarded and the supernatant was collected into fresh centrifuge tube. The supernatant was further stored at 4°C till further use (Xu and Chang 2007).

4.8.2 FRAP Assay

The Ferric Reducing Antioxidant Activity (FRAP) assay was performed as described previously by (Benzie and Strain 1996). It is mostly employed for the detection of antioxidant components in dietary poly-phenols. This method is based upon the conversion of potassium ferricyanide and ferric chloride in the presence of antioxidant into potassium ferrocyanide and ferrous chloride. Briefly, 3 ml of FRAP reagent (10ml of 300 mM acetate buffer of pH 3.6, 1ml of 10mM of TPTZ in 50mM HCl, 1ml of 20mM $FeCl_3.6H_2O$) was added to 100 µl of each crude extract. The samples were incubated at 37˚C for 15 min in dark. Then the absorbance was measured at 593nm at UV-VIS spectrophotometer (Varian co.). Control reaction was set up using ascorbic acid. FRAP value was calculated and expressed as ascorbic acid equivalent (AA/g) of mung bean sample.

4.8.3 DPPH free radical scavenging assay

The antioxidant potential of Plasma treated and untreated samples were determined by previously described method of (Aoshima et al. 2004). To 100µl of the plasma treated and untreated mung bean extract, 2.9 ml of DPPH reagent was added followed by vigorous vortexing. Further, the samples were incubated at 37˚C for 30 min to observe the discoloration of DPPH reagent. Methanol was used as control. The absorbance was recorded at 517 nm using UV-Vis spectrophotometer. The percentage inhibition was calculated by:

Inhibition % = $[S_{control} - S_{sample\ extract} / S_{control}] X 100$

Where, S (control) - absorbance of DPPH reagent + methanol, S (sample extract) - absorbance of DPPH reagent + sample extract.

4.8.4 Estimation of total phenolic content

The total phenolic content (TPC) was estimated by following the procedure of Folin–Ciocalteu (Singleton et al. 1998). To 0.5ml of crude extract of plasma treated and untreated mung bean samples, 2.5 mL of Folin-Ciocalteu reagent (10 %) was added followed by 2.5 ml of 7.5% $NaHCO_3$. All samples were vortexed and incubated at 45˚C

for 45 minutes in dark. After incubation, absorbance of all samples were measured at 765 nm. Gallic acid was used as standard. A standard curve of gallic acid was prepared by using different concentrations (10ppm – 200ppm). The phenolic content of samples were calculated by plotting the values in gallic acid standard curve and expressed as gallic acid equivalent (mg of GAE/g).

4.8.5 Estimation of total flavonoid content

The total flavanoid content (TFC) of plasma treated and untreated mung beans were carried out using aluminium chloride colorimetric assay with minor modifications (Zhishen et al. 1999). Briefly describing, 1 ml of crude extract was diluted with 4 ml of sterile distilled water in a test tube followed by addition of 0.3 ml of 5% $NaNO_2$. The mixture was incubated at 37˚C for 5 min and 0.3 ml of 10% $AlCl_3$ was added in test tube followed by incubation for another 5 min. Further, 2 ml of 1N sodium hydroxide was added into it and volume of the mixture was brought to 10 ml with distilled water. The absorbance was measured at 510 nm using a UV-Visible Spectrophotometer against the blank containing distilled water instead of sample extract. Catechin was taken as control and a standard curve of catechin was prepared by using different concentrations ranging from 10ppm to 250ppm. All the tests were performed in triplicates and the flavonoid content of the samples were expressed as micrograms of catechin equivalents (mg of CAE/g).

4.9 Fourier transform infrared (FTIR) spectrometry

FTIR is employed for the identification of functional groups of various compounds present in the seed flour. Plasma treated and untreated mung beans were crushed to very fine powder in a mortar and pestle followed by addition of KBr into the sample in the ratio of 99:1. The contents were mixed properly and a very fine layer of this mixture was placed under a pressure of 110 psi in hydraulic pump for 2 min. After 2 min, the pressure was released and the pellet was taken out and kept in desiccator for 5-10 min. The prepared KBr pellets of samples were placed in FTIR Varian 600 IR series apparatus to detect any changes in absorption spectrum of plasma treated and untreated samples. From absorption spectrum, the absorption bands and the wave number (cm^{-1}) of dominant

peaks were analyzed for the amide, carboxylic acids, aldehyde and ketonic groups (Amir et al. 2013)**.**

4.10 Proximate analysis of the plasma treated mung bean:

4.10.1. Protein estimation

The protein content of the plasma treated and untreated mung bean samples was estimated by the protocol of AOAC, 2010. The nitrogen content of the mung bean sample was carried out by Kjeldahl method. Kjeldahl method involves three steps i.e. digestion, distillation and titration. Briefly, 2 g of finely grounded plasma treated and untreated mung bean samples were added into a round bottom conical flask and further 15 ml of concentrated sulfuric acid (H_2SO_4) was added. Subsequently, Potassium sulfate and copper sulfate was added in ratio 8:1 and flask was kept for 2 hours on the digestion unit for acid digestion. After acid digestion, samples were allowed to cool at room temperature. Further, sample was diluted to 140 ml using Millipore water followed by addition of 50% concentrated sodium hydroxide (NaOH) solution with a few glass beads. The solution was distilled using Kjeldhal distillation unit (Model unit B- 45). The Liberated ammonia is entrapped in 4% boric acid solution with methyl red indicators. Finally, the solution was titrated against 0.01 N HCl. Blank reaction was set up without sample.To calculate protein content, nitrogen conversion factor of 6.25 was applied.

$$\% \text{ of Nitrogen} = \{(S-B) * 1.4007 * 0.1\} / \text{Weight of sample}$$

$$\% \text{ of Protein content} = 6.25 \text{ X N } (\%)$$

Where, S = sample burette reading from titration, B = blank burette reading from titration

4.10.2 Fat estimation

The fat content of plasma treated and untreated mung bean samples were done as per the method described by AOAC, 2010. Briefly, 2 g of treated and untreated mung bean sample was crushed and kept in thimble with a top glass wool. To the pre-weighed (W_1) round bottom flask, 5-10 glass beads were dispensed and then 300 ml of petroleum ether was added into the flask. Simultaneously, thimbles containing the samples were placed in glass jacket kept onto pre-labeled extraction flasks. A condenser is fitted over the glass jacket for reflux. Cold tap water was percolated through the condenser for condensation

purpose which promote in refluxing the ether in the thimble back, the unit was turned on and was allowed to reflux for 6 hours. After switching off the system, the remaining solvent was extracted from the sleeve in the flask. Further the flask was incubated at 130˚C in oven for 3.30 h, allowing the ether to evaporate out leaving behind the extracted oil at the bottom of the flask. Flasks were placed in the desiccators to cool down and the weight was taken as W_2. The percentage fat content was calculated by using the formula.

% Fat content = $(W_2-W_1)/ S X 100$

Where, W_1 = Weight of empty round bottom flask, W_2 = Final weight of the flask after experiment, S = weight of the sample.

4.10.3 Carbohydrate Estimation

Carbohydrate content estimation of plasma treated and untreated samples were done by using Anthrone method (Yemm and Willis 1954). Different concentrations of glucose (Stock- 1mg/ml) ranging from 0.2 mg/ml to 1mg/ml were prepared. To 1ml of each dilution of plasma treated and untreated sample extract, 4ml of anthrone reagent was added. Further, the tubes were vortexed and incubated in boiling water bath for 10 minutes followed by cooling at room temperature for 2 min. The absorbance was noted at 620nm. Test tube containing only anthrone reagent was used as blank. The test was performed in triplicates.

4.10.4 Estimation of moisture content

The moisture content of plasma treated and untreated mung bean samples were determined as per the standard method of AOAC 2010. Briefly describing, two grams of plasma treated and untreated mung bean grains were placed in a pre-weighed dried crucible (W_1) and its weight was recorded as W_2. Subsequently, crucibles containing wheat grains were incubated at 130˚C for 1 hour and then allowed to cool inside a desiccator. The crucibles were then again weighed and weight was designated as W_3 (AOAC, 2010). The % age moisture content of the sample was calculated by using following formula

% Moisture content = $(W_2- W_3 / W_2- W_1) X 100$

Where, W_1= weight of empty dried crucible, W_2= weight of the crucible containing wheat grains, W_3 = Final weight of the crucible containing wheat grains after incubation.

4.10.5. Ash content

The ash content of plasma treated and untreated mung bean was done as per the methodsof AOAC, 2010. To pre-weighed dried crucible (W_1), 2g of plasma treated and untreated mung beans were placed and weight was again noted as W_2. Further, these crucibles were placed in muffle furnace at 600ºC for 6 hours. After 6 hours of incubation, crucibles were taken out and allowed to cool in the desiccator. The final weight of the crucibles were noted and designated as W_3. The % age ash content of the sample was calculated by using following formula

% Ash content = $(W_3 - W_1 / S) X 100$

Where W_1 = weight of empty dried crucible, W_3 = Final weight of the crucible containing mung bean after Muffle furnace treatment.

4.11 Effect of plasma treatment on protein profiling:

4.11.1 Extraction of crude protein and estimation

For Protein extraction, 5g of plasma treated and untreated mung bean grains were crushed in a mortar and pestle using 50 mM Tris-HCl buffer, pH=7.5 over a ice bath and the homogenized sample in buffer was further transferred into 15 ml centrifuge tubes. Centrifugation was done at 12,000 rpm for 15 minutes at 4ºC to separate the pellet and crude extract (Ranjan and Matcha 2012). The pellet was discarded and supernatant (crude extract) was further subjected to Bradford's Assay to estimate the protein content (Bradford 1976).

Concentration	**BSA stock solution (1mg/ml) (µl)**	**Double Distilled water(µl)**
20ppm	20	980
40ppm	40	960
60ppm	60	940
80ppm	80	920
100ppm	100	900

Table 2: Different dilutions of Bovine serum albumin (BSA)

A standard curve of BSA was prepared against which the values of the unknown protein samples were plotted. Different concentrations of BSA (Stock- 1mg/ml) ranging from 20 ppm to 100 ppm were prepared (Table 2) and protein content was estimated using Bradford's method in a 96 well titer plate. To each well, 10µl of each BSA concentration and crude protein extract of plasma treated and untreated samples were dispensed followed by addition of 90µl of Bradford's Reagent (Sigma-Aldrich). The titer plate was incubated at 37˚C for 15 min and absorbance was recorded at 595 nm using Biotek Powerwave 340 Microplate Reader. All the tests were carried out in triplicates and values were expressed as Mean ± SD.

4.11.2 SDS-PAGE

For SDS-PAGE, the glass plates were assembled on the casting apparatus and water was poured into the glass plates to ensure that the assembly is not leaking. Further, 10% resolving and 5% stacking gel was prepared according to the composition as described in Table 2. After pouring the resolving gel, the top of the gel was covered with butanol. After polymerization of the separating gel, butanol was drained off and stacking gel was poured above the solidified separating gel followed by insertion of 10 well comb into it.

Simultaneously, the sample was prepared by adding 20µl of crude extract of plasma treated and untreated samples with 10µl of 5X loading dye and incubated at boiling water bath for 3 min. After solidification of gel, 30µl of sample was loaded on to per well. A molecular weight marker of 29KDa to 205KDa was also loaded as standard marker. Gel was allowed to run at 80V for 3 hours in 1X electrophoresis buffer until the tracking dye reaches at the bottom. After the gel electro-phoresis was done, the gel was stained with staining solution (Methanol - 20%, Glacial Acetic Acid -10 %, Coomassie Blue R-250 - 0.15 %) for 2-3 h and further subjected to de-staining solution ((Methanol - 20%, Glacial Acetic Acid -10 %) for the visualization of protein bands (Turi et al. 2010) .

Ingredients	Separating gel (10%)	Stacking gel (5%)
Autoclaved Double distilled water	2.20 ml	2.50 ml
30% Acrylamide: bis-acrylamide	2.20 ml	980 µl
1.5M Tris HCl (pH 8.8)	1.25 ml	----
0.5M Tris HCl (pH 6.8)	----	1.25 ml
10% SDS	50 µl	70 µl
10% Ammonium persulfate	50 µl	70 µl
TEMED	06 µl	07 µl

Table 3: Composition of Separating and stacking gel of SDS-PAGE

4.12 Tetrazolium Staining

The plasma treated and untreated mung beans were prepared by soaking them overnight in distilled water. After overnight incubation, the water was discarded and the seeds coat was removed after moistening. Further, cut the seeds in two parts from the centre using the sterile scalpel without damaging the embryo of the seeds. Then the seeds were dipped into 1.0% TTC (tetrazolium solution, pH- 7) at 37°C for 30 min. Further, the seeds were rinsed with distilled water for 2-3 times. The staining pattern on the embryo of seeds was evaluated. During evaluation, 2-3 drops of lactophenol clearing agent (Lactic acid: Phenol: Glycerin: water in ratio of 20:20:40:20) was added visualization of embryo of seeds (Deminicis et al. 2014).

Chapter 5

Results & Discussion

5. RESULTS

5.1. Collection of Sample and Culture

Mung bean (*Vigna radiata L.)* variety SML-668 collected from the PAU, Ludhiana was kept in sterile zip pouches (Figure 5a). The test pathogen *Aspergillus flavus* (Figure 5b) was identified by observing various features listed in Table 4.

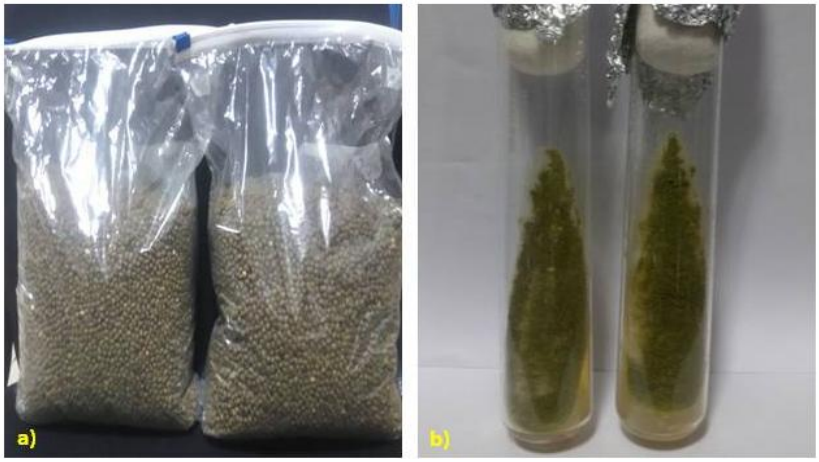

Figure 5: (a) Collected mung bean (*Vigna radiata*) sample (b) PDA-glycerol slants of *Aspergillus flavus*

Characteristics	**Observation on Czepak Dox Agar plates after 7 days**
Growth rate	Fast, covering 90 mm in 7 days
Colony appearance	Granular
Colony color	Yellow then turning to yellow-green from front and green in color from back
Colony margin	Smooth
Colony surface	Flat with radial grooves

Table 4: Observed colony characteristics of *Aspergillus flavus*

5.2 Plasma decontamination of Mung bean

Plasma treatment is an emerging technology to combat post harvest losses of grains by inhibiting growth of pathogenic fungi during storage. In addition to this, plasma technology is being used in range of industrial applications processes such as

anticorrosion coating, waste treatment of toxic materials, microelectronic technology etc. In the present piece of work, *Aspergillus flavus,* the common contaminant of mung bean, was used to infect the mung beans prior to low pressure SF_6 plasma treatment. After plasma treatment, surface modification of mung bean sample was observed in comparison to untreated sample. The plasma treated grains exhibited shiny and smooth surface (Figure 6).

Figure 6: (a) Untreated Mung bean (b) Plasma treated mung bean (*Vigna radiata*)

5.3 Disinfection of *Aspergillus flavus* on the mung bean

The decontamination of *Aspergillus flavus* from the mung bean sample was performed by using SF_6 gas and exposing then for varying durations ranging from 2 - 10min (Figure 7). The total number of fungal spores before plasma treatment was 4.23 X 10^6 CFU/g. However, after plasma treatment of 5 min, the total number of fungal spores was reduced to 1 X 10^4 CFU/g (Table 5).

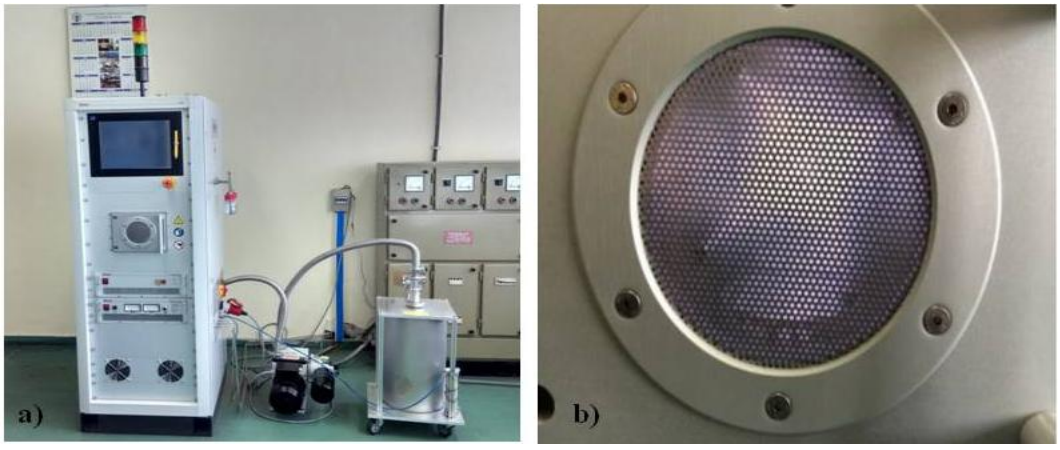

Figure 7: (a) Low Pressure Plasma reactor PICO (b) Plasma chamber

Duration	CFU/g	Log_{10} CFU/g
0 minute	4.23×10^6	6.626
2 minute	1.8×10^5	5.255
5 minute	1.0×10^4	4.964
10 minute	0	0

Table 5: CFU/g and Log_{10} CFU/g of fungal spores on plasma treated mung bean for different durations

There is 1- log growth inhibition of fungal spores was observed after 2 min of plasma treatment which was escalated to 2-log inhibition after 5 min of plasma treatment. Further, infected mung bean samples were subjected for plasma treatment for 10 min duration and get fully inactivated but on further extending the exposure time, the reduction effect remains stationary (Figure 8). The optimal duration of SF_6 plasma treatment was found to be 10 min with complete inhibition of microbial population.

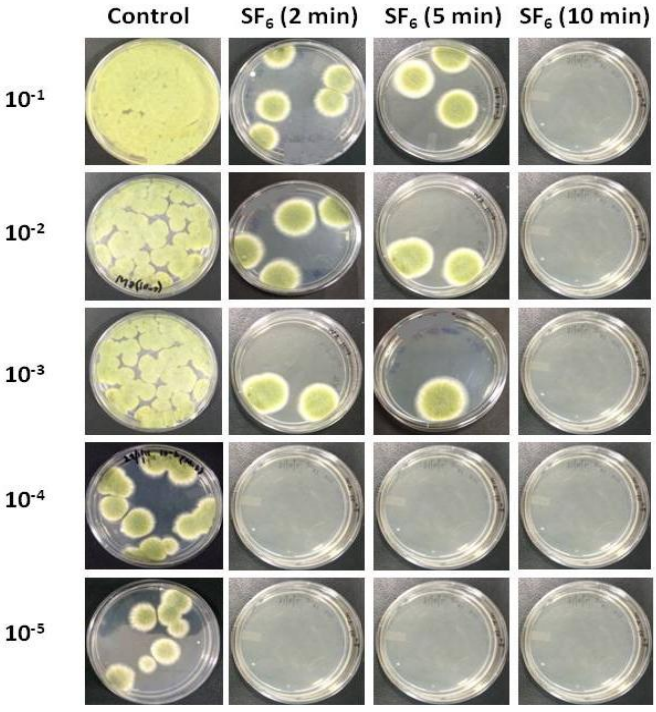

Figure 8: Colonies of different dilutions of untreated and plasma treated sample for different durations.

5.4 Effect on cooking properties after plasma treatment

5.4.1 Cooking Time

The cooking time of plasma treated sample was reduced to 30 min in comparison to untreated sample with cooking time of 35 min. The probable reason of reduced cooking time can be easy penetration of water into the plasma treated wheat surface.

5.4.2 Water uptake

Water uptake capacity of plasma treated sample was increased to 2.045 in comparison to 1.98 of untreated sample (Table 6). As plasma treatment is defined as surface phenomena, modification in the surface of plasma treated mung beans can be correlated with decreased water uptake ratio.

Sample	Initial weight (A)	Final weight (B)	Water uptake (B/A)
Plasma treated sample	2.01	3.98	1.98
Untreated sample	2.00	4.09	2.045

Table 6**:** Water uptake capacity of plasma treated and untreated wheat sample

5.5 Estimation of Antioxidant activity

5.5.1 Extraction of Mung bean sample

The plasma treated and untreated mung bean sample exhibited a little variation in the color of the crude extract. The extract of plasma treated mung bean was slightly darker than untreated sample extract (Figure 9a).

5.5.2 Ferric reducing antioxidant power (FRAP) assay

The antioxidant activity of plasma treated sample was found to be increased (+ 0.20 mg AA/g) in comparison to untreated sample. The Ascorbic acid equivalent of plasma treated sample and untreated sample was found to be 0.92 mg/g and 0.71 mg/g respectively as calculated from standard curve of ascorbic acid (Figure 10). However, a little variation in

the intensity of green color formed in plasma treated and untreated sample also supports the increase reducing activity (Figure 9b)

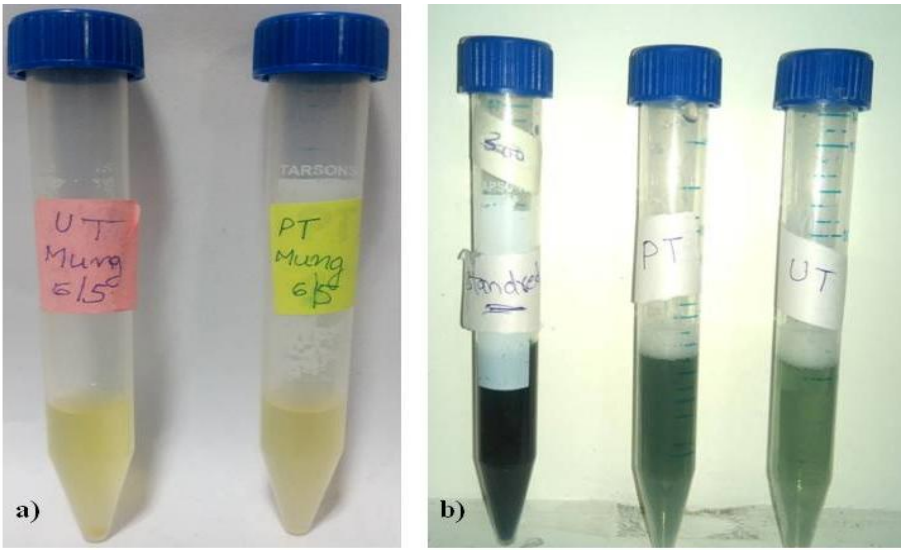

Figure 9: (a) Mung bean extact of untreated sample (left) and plasma treated (right) (b) FRAP assay of standard, plasma treated and untreated mung bean extract

Mung bean extract	Absorbance	FRAP	FRAP(mg AA/g)
Untreated sample	0.7522	71.53	0.71
Plasma treated sample	0.8929	92.12	0.92

Table 7: FRAP value and Ascorbic acid equivalents of plasma treated and untreated mung bean extracts

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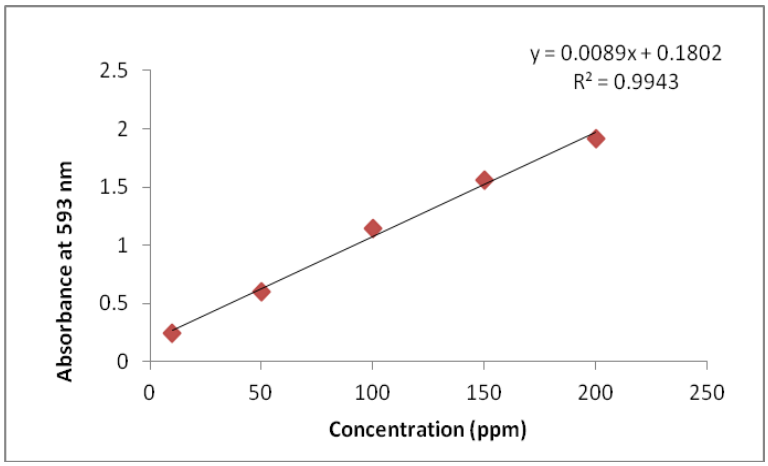
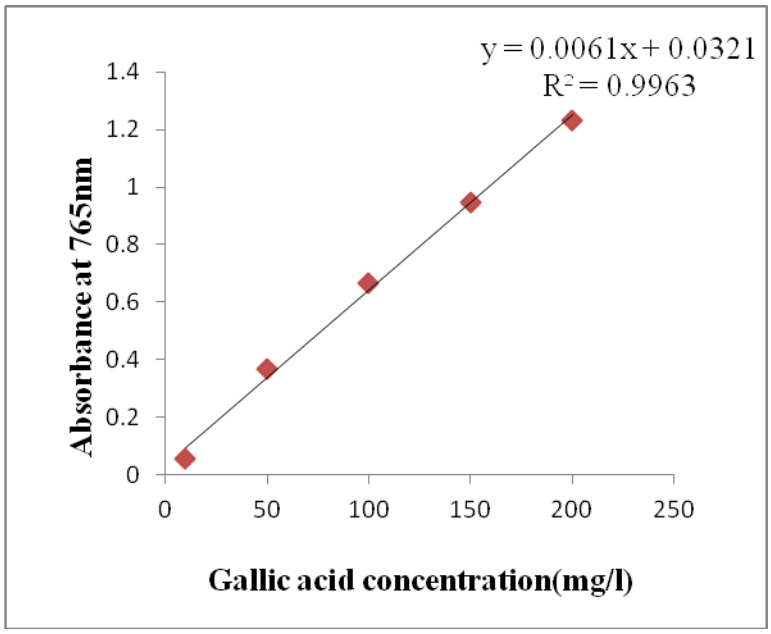


Figure 10: Standard curve of Ascorbic acid

5.5.3 Total Phenolic content

Total phenolic content estimation using Folin–Ciocalteu method is based upon formation of blue colored complex formation due to phenolic groups. The total phenolic content (TPC) of plasma treated and untreated sample was calculated by preparing a standard curve of gallic acid (Figure 11). The TPC was calculated by putting absorbance values in equation, y = 0.0061x+0.0321. The TPC of untreated sample was 1.93 mg GAE/g and was found to be increased (+0.26 mg GAE/g) to 2.19 mg GAE/g (table 8). Similar increase in phenolic content was observed in plasma treated naringin samples (Kim et al 2015).



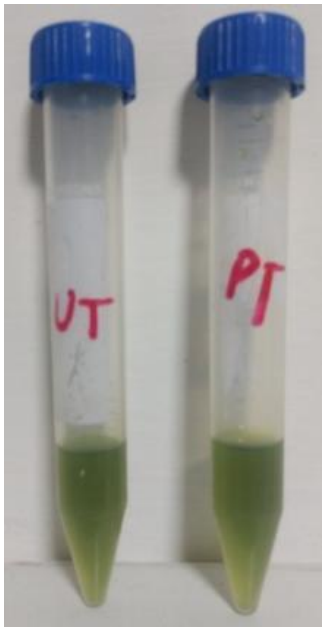


Figure 11: (a) Standard curve of gallic acid (b) TPC estimation of untreated (UT) and plasma treated (PT) mung bean extract

Mung bean extract	Absorbance	TPC (µg/mg)	Gallic Acid equivalents
Untreated sample	1.1935	193.42	1.93 mg GAE/g
Plasma treated sample	1.3277	217.39	2.19 mg GAE/g

Table 8: Total phenolic content of plasma treated and untreated mung bean extract

5.5.4 Total Flavonoid content estimation

The flavonoid content of plasma treated and untreated mung bean sample was calculated by putting the absorbance values in equation, y = 0.0033x + 0.0148, of catechin standard curve (Figure 12a). The total flavonoid content of the mung bean sample was found to be increased from 0.9444 to 1.030 mg CAE/g after 10 minutes of plasma treatment (Table 9). The change in color was observed due to increase flavonoid content (Figure 12b).

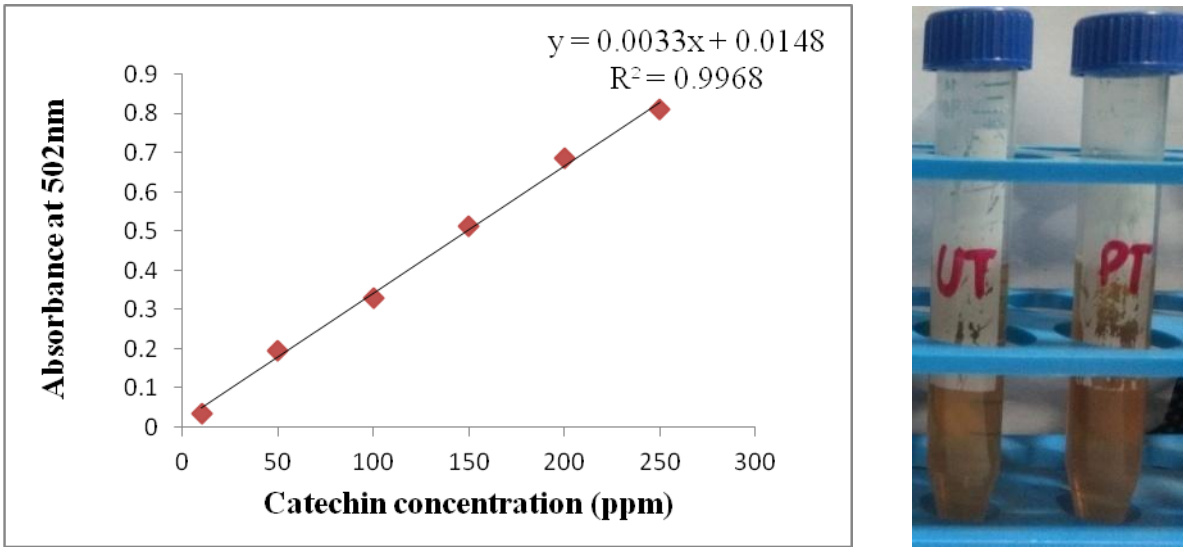


Figure 12: (a) Standard curve of catechin (b) TFC estimation of untreated (UT) and plasma treated mung bean extract

Mung bean extract	Absorbance	TFC (µg/mg)	Catechin equivalents
Untreated sample	0.2973	94.44	0.944 mg CAE/g
Plasma treated sample	0.3232	103.07	1.030 mg CAE/g

Table 9: Total flavonoid content of the untreated and plasma treated sample

5.5.5 DPPH free radical scavenging activity

DPPH free radical scavenging assay is based upon abstraction of protons from antioxidant moieties thereby forming the reduced DPPH. The scavenging potential is correlated with discoloration of DPPH reagent from purple to yellow which is further quantified by decrease in absorbance recorded at 517 nm. Free Radical scavenging

potential of plasma treated mung bean sample was found to increase by 4% .in comparison to untreated samples (Table 10). The degree of discoloration indicates the free radical scavenging potentials of the sample/antioxidant by their hydrogen donating ability (Figure 13).

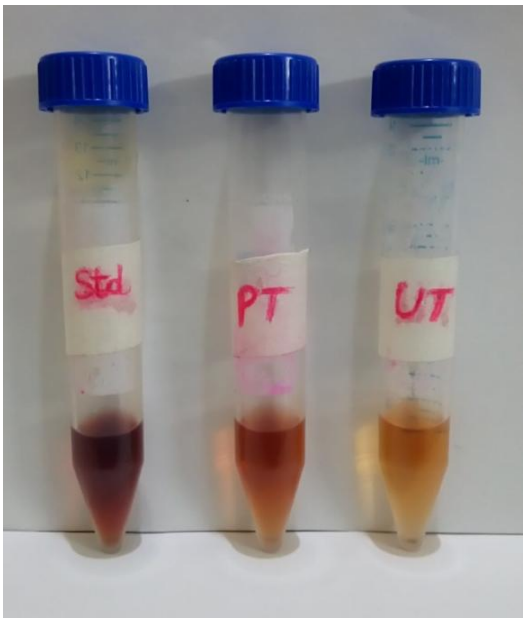


Figure 13: DPPH free radical assay of Plasma treated and untreated mung bean sample

Mung bean extract	Absorbance	DPPH scavenging activity (%)
Untreated sample	0.6453	59.65%
Plasma treated sample	0.5766	63.95%

Table 10: DPPH free radical scavenging activity of the untreated and plasma treated mung bean extract

5.6 Effect of plasma treatment on the proximate analysis of mung bean

5.6.1 Protein estimation

The Protein content of plasma treated sample exhibited slight increase in protein content in comparison to untreated sample. The protein content of plasma treated sample was increased by 3% i.e. 30.90% in comparison to 27.12% of untreated sample (table 11). Increase in the Protein content might be due to breakage of the proteins present on the

surface and other proteineceous matter because of the application oxygen which plays a major role in degradation reaction (Deng et al.,2007).

Sample	Initial volume	Final volume	Nitrogen %	Protein %
Untreated sample	50.9ml	60.2ml	4.34%	27.12%
Plasma treated sample	21.5ml	32.1ml	4.94%	30.90%

Table 11: Percentage protein content of plasma treated and untreated sample

5.6.2 Fat content estimation

The initial fat content of untreated sample was found to be 1.30%. However, fat content was found to be increased (+ 0.5%) in plasma treated sample as compared to untreated sample (Table 12). Variation in the fat content value in treated and untreated sample may be due to the reduction in moisture content of sample or oxidation of the free radicals present. These difference varies depending upon the parameters decided for plasma treatment application (Lii et al. 2015)

Sample	Initial Weight W1	Final Weight W2	Fat %
Untreated sample	79.376 g	79.389 g	1.30%
Plasma treated sample	82.979 g	82.997 g	1.80%

Table 12: Fat content of plasma treated and untreated mung bean samples

5.6.3 Estimation of the Moisture content:

Moisture content is highly responsible for the contamination of the grains and fungi growth. It is well known that plasma treatment is an effective method to reduce the moisture content without heating. Initially, the moisture content of the sample was 8.22% and further got decreased to 7.53% after plasma treatment.

Figure 14: Mung plasma treated and untreated samples in dessicator

Sample	W1 (g)	W2 (g)	W3 (g)	Moisture content %
Untreated sample	19.5081	2.0015	21.3021	8.22%
Plasma treated sample	19.3020	2.0211	21.1602	7.53%

Table 13: Percentage moisture content of plasma treated and untreated sample

5.6.5 Estimation of the Ash content:

The mineral present inside the food is determined by the estimation of ash content. Ash content of the untreated sample was 3.5% and then after treatment it was increased to 3.8% (Table 14).

Sample	W1 (g)	W2 (g)	W3 (g)	Ash content %
Untreated sample	19.5141	2.0498	19.5864	3.5%
Plasma treated Sample	21.6007	2.0105	21.6780	3.8%

Table 14: Percentage ash content of plasma treated and untreated samples

5.6.6 Carbohydrate estimation

Carbohydrates are the major component of our diet. At the time of storage, there is degradation of carbohydrate content due to mycotoxins synthesis by storage fungi. The initial carbohydrate content of untreated mung bean was 11.86 µg/ml which was calculated from standard curve of Glucose (Figure 15). Further, plasma treated samples showed slight increase (+ 7.49 µg/ml) in carbohydrate content viz. 19.35 µg/ml.

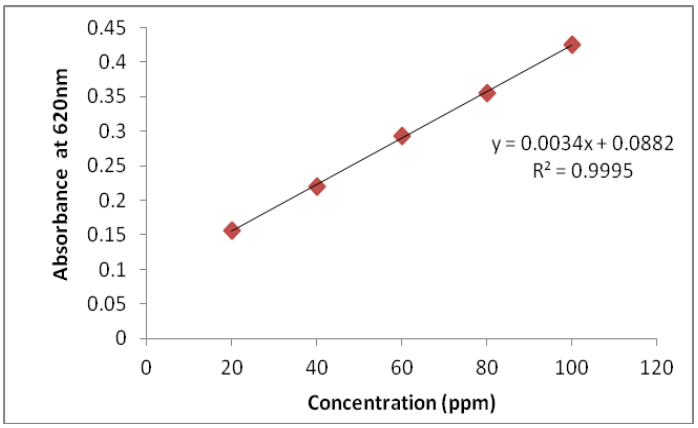


Figure 15: Standard curve of glucose

5.7 Fourier transform infrared spectroscopy (FTIR)

Chemical composition of the different samples can be predicted by observing the graph. FTIR result for untreated and plasma treated sample and can be identified by black and red color respectively (Figure 16). It can be noticed from figure 5.15 that there is no change in the peaks of the untreated and plasma treated sample which signifies that plasma treatment has no negative effect on the mung bean sample.

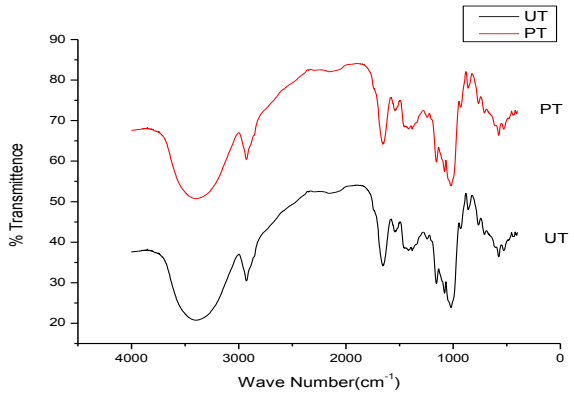


Figure 16: FTIR spectrum of the untreated and plasma treated sample

5.8 Effect on protein profiling

There was slight difference in the protein concentration of plasma treated and untreated mung bean sample. The protein concentration of plasma treated sample was found to be 0.936 µg/ml and untreated sample was 0.738 µg/ml as calculated by plotting the absorbance values in the equation, y = 0.745x - 0.0002 of standard curve of BSA (Figure 17).

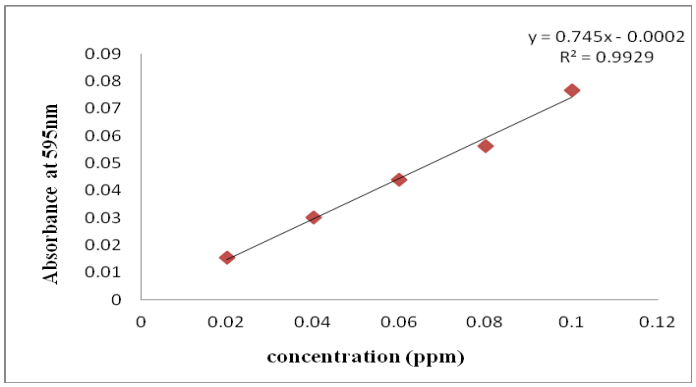


Figure 17: Standard curve of BSA by Bradford assay

5.8.1 SDS-PAGE

The variability of seed storage protein profiling was evaluated by running SDS-PAGE (Figure 18). The bands of protein observed in plasma treated (lane 2 and 3) were same as that of untreated sample protein (lane 4 and 5). There was no difference in molecular weight of protein bands in treated as well as untreated samples thereby confirming that plasma treatment with SF_6 gas did not exert any negative effect on the nutritive value of mung bean.

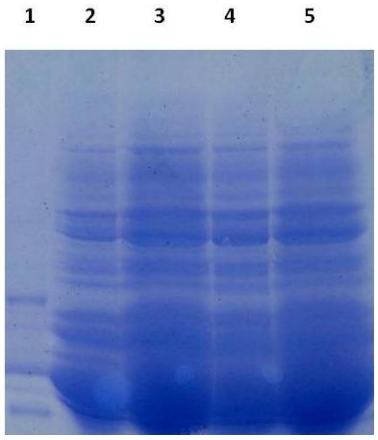


Figure 18: SDS-PAGE gel of plasma treated (lane 2, 3) and untreated sample (lane 4,5)

5.9 Tetrazolium staining

Tetrazolium staining test was used for determining the viability of the mung bean seeds and it was observed after staining that the seeds are 98% viable after plasma treatment (Figure 19).

Figure 19: Viability of the untreated (right) and plasma treated (left) mung bean.

Chapter 6

Conclusion

6. CONCLUSION

The main objective of our project was to inactivate *Aspergillus flavus* from the surface of mung bean using the low pressure cold plasma. It was successfully carried out by using the SF_6 gas and complete inactivation of the fungal spores was achieved within 10 minutes of plasma treatment.

Tetrazolium staining was performed for checking the viability of the seeds and it was observed that 98% of the mung bean seeds were viable after plasma treatment. Protein profiling and FTIR peaks of treated and untreated samples did not impart any variation.

Total phenolic and flavonoid content of treated samples was found to be increased from 1.93 to 2.19 mg GAE/g and 0.9444 to 1.030mg CAE/g respectively after plasma treatment. Radical scavenging activity of the mung bean also increased from 59.65% to 63.95% by DPPH assay.

Protein, fat, carbohydrate and ash content of mung bean was investigated which was found to be increased from 27.12-30.90%, 1.30-1.80%, 11.86-19.35µg/ml and 3.5%-3.8% respectively. Cooking properties of the seeds were also studied and it was observed that cooking time of the mung bean got reduced after plasma treatment due to increase in the water uptake ratio. Plasma treatment proved to be an effective method as no damage to the grains was observed after the treatment.

Chapter 7

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