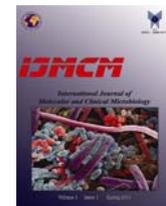International Journal of
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Plasma jet impacts on Citrinin production in isolates belonging to *Penicillium* Spp.

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ABSTRACT

Mycotoxins such as Citrinin are small toxic molecules produced by a great variety of microorganism, which encompass several classes of secondary metabolites with no common chemical structure or mode of action. Citrinin produces by different species of *Penicillium.* Today, the cold atmospheric pressure plasma (CAPP) method has a potential for mycotoxin detoxification. In this study, a new and promising approach involving the use of cold atmospheric pressure plasma is considered, which may overcome multiple weaknesses associated with the classical methods. In this study, samples included wheat, corn; rice and Barley were obtained from northern parts of Iran (Lahijan). Initially, the output of the power supply was set to 50 kV, 100 watts, and the electron frequency of 30 kHz (optimal conditions to effective plasma). Then, the effect of cold atmospheric jet plasma (argon) on gas was investigated in 30, 60 and 360 seconds (gas flow rate of 6 liters per minute). The results showed us that the cold atmospheric pressure plasma method effectively production pure mycotoxins. The rate of various mycotoxins destruction varies according to their structure. It was also shown in the study that the combination of mycotoxins in foods would reduce the plasma's effect, but not prevent them from degrading. Finally, in this study, descendants in all samples were performed successfully. The atmospheric pressure plasma method has a natural biodegradation effect and has the least negative effect on food products in comparison with conventional methods of disinfection of food products in addition to the detoxification characteristics.

1. Introduction

Penicillium is one of the most commonly found fungi in nature. *Penicillium* has many benefits, including the ability to produce various enzymes, various antibiotics, anti-tumor and antifungal drugs and the production of effective compounds against insects. The purpose of this study was to investigate the effect of atmospheric cold plasma on the reduction of mycotoxins produced by *Penicillium* isolated from five different food sources (wheat, barley, corn, rice, and flour). Today, plasma science is a new way of overcoming the problems faced by

food biotechnology researchers. The unique properties of plasma make it widely available in industry and medicine. Classification in terms of pressure divides plasma into two general classes of low-pressure plasma and atmospheric pressure plasma. Currently, the atmospheric pressure plasma is more considered due to its simplicity and low cost compared to low pressure plasma. Atmospheric discharges have been extensively investigated in recent years due to the advantages such as cost-effectiveness of the test, the operation without vacuum and the ability to access and process the sample without limitations in size and high reactivity. Therefore, the use of atmospheric plasma jet is a new technology that has been considered in recent years due to its high potential for destruction of microorganisms and its low cost as a non-thermal method. Hayashi et al. (2013) carried out sterilization with plasma jet. They investigated the combining of oxygen-helium and argon-oxygen gas and found that the combination of argon and oxygen gas in 40 seconds could destroy *Bacillus thuringiensis* bacteria, if the helium and oxygen gas in 180 seconds could reduce the number of bacteria (Hayashi et al. 2013). Mortazavi et al. (2016) studied the diffusion of electrons, ions, and free radicals of oxygen in the oxygen plasma and its microbial effect on *E. coli* at the level of the Polytetrafluoroethylene (PTFE). In addition, they assess the number of destroyed proteins and fatty peroxides produced in the bacterial suspension and DNA state. They concluded that after sterilization to inactivate the bacteria and mechanism analysis, the results showed that the concentration of electrons and ions decreased significantly with increasing distance from the center of the inductive coil, while the concentration of oxygen radicals decreased much more slowly (Mortazavi et al., 2016).

Song et al. (2011) aimed at measuring the ability of plasma (a plasma capable of operating in atmospheric pressure) to clean pieces of cheese and pork infected to three different species of *Listeria monocytogenes*. The logarithmic decrease of the microbial population increased with increasing input power and increasing the duration of treatment. After 120 seconds, the plasma treatment decreased at 75, 100 and 125 watts of live *L. monocytogenes* as much as 1.7, 2.78 and 5.8 logarithmic cycles in cheese pieces, respectively. More than eight logarithmic cycles of microbial load could be observed at 150 watts with 120-second treatment *(*Song et al., 2011*)*.

In a study by Park et al. (2013) on AFB1 and Citrinin toxins, they observed that plasma jet has fine deterrence effects on toxins; they have confirmed it by using HPLC method. They have also noticed that AFB1 toxin compared to Citrinin will be destroyed more slowly (Park et al., 2013).

Siciliano et al. (2016) aimed at measuring the ability of plasma (a plasma capable of operating in atmospheric pressure) to clean corn infected to Citrinin. They reported that decrease of the microbial population increased with increasing input power and increasing the duration of treatment. In this study, researchers found that after 60 seconds the concentration of Citrinin decreased from 25.9 ppm to 13.66ppm (Siciliano et al., 2016).

2. Materials and Methods
2.1. Sampling

The way of sampling depends on the aim of the microbiological study, and must be done at the selected site with sterile equipment. Sampling should be performed aerobically with sterile sampling equipment. In this survey, simple randomized or infinite random sampling was used. Simple random sampling is the basic selection process of sampling and is easiest to understand.

In this research, the samples were taken from the northern regions of Iran included wheat, corn, oats, flour and rice. These samples were collected by Dr. Arash Chaychi Nosrati and included in the study (Table 1). Samples were transported to the laboratory immediately after collection, and stored for determination of the amount of Citrinin as well as plasma detoxification at 4 °C in the refrigerator.

2.2. Culture of samples in solid media

In order to isolate the mycotoxin derivative Citrinin, the samples were cultured in a medium of Oatmeal agar in a needle culture (at 3 dots, 2cm apart and at the edge of the plate). Then, each plate was transferred to the incubator for 7 days at 25°C. Plates were examined for incubation (every 3 days from the 7-day incubation period). Identification of isolated

fungi was done according to the morphological and macroscopic characteristics of fungal colonies such as surface color and behind the colonies, panoramic view of the colonies in terms of pleat, radial lines or concentric circles, smooth or folded colony levels, and level of colonies such as powder, cotton, velvet, and etc. given the identification keys of Barnett and Hunter, Klich and Dugan and (Barnett and Hunter 1998) and (Klich 2002).

2.3. Cultivation in a liquid medium

In this step, two cultures of Sabouraud Dextrose Broth + Malt Extract (SB+ME) as well as Sabouraud Dextrose Broth + Yeast Extract (SB+YE) were used to increase mycotoxin production, which were used in 10mililiter falcon tubes containing 5ml from liquid medium and sterile glass stubs (used in this study to accelerate the cellular and lysed cell failure process for the extraction of mycotoxins from glass). In order to inoculate the samples from solid to liquid medium, each pure specimen was removed by sterile loop and transferred to the liquid medium at 25°C, 200rpm for 7 days in a shaker incubator. Phosphate buffered saline solution (PBS) was added to each of the tube to prevent the drying of liquid media during incubation (On the third day) (Devi et al., 2017).

2.4. Preparation of the sample slides from selected samples

The Oatmeal medium agar was used to prepare slide culture in this research. Using sterilized scalpel, a few cubes of square 1cm × 1cm were cut and sterilized by placing them on a slide (Figure 1). After placing the agar completely in the center of the lam, it is placed horizontally on a U- tube that is placed inside a large glass plate (Figure 2) (containing 10ml distilled sterile water). Then, using sterile anise, the pure samples were inoculated on four slices of the medium on the lam. Then, the sterilized lamel was placed on an inoculated agar and kept in the incubator for 7 days at 25°C (James 1986). During incubation (every 3 days from the 7-day incubation period), the plates were examined and distilled water was used to prevent the plates from drying. After 7 days of incubation, cover slip is removed. Then two drops of lactophenol are poured onto another clean slide and the cover slip with a mold (purified sample) is placed on the lam. Then microscopic and macroscopic characteristics of microorganisms were investigated (James, 1986).

Table 1. Location and type of samples collected

| Sample Number	Sample type	Sampling location	Sampling Time
V_1	Rice	Lahijan, Cereal and grain storage	From May 2015 to September 2016
V_2	Rice	Lahijan, Cereal and grain storage	From May 2015 to September 2016
V_3	Corn	Lahijan, Corn storage place	From May 2015 to September 2016
V_4	Wheat	Lahijan, Wheat storage location	From May 2015 to September 2016
V_5	Barley	Lahijan, Cereal and grain storage	From May 2015 to September 2016
STD_1	*Penicillium digitatum*	Herbarium Ministerii Iranici Agriculturae "IRAN"	-
STD_2	*Penicillium italicum*	Herbarium Ministerii Iranici Agriculturae "IRAN"	-
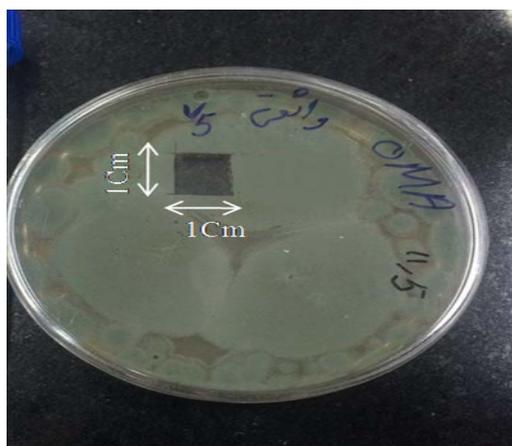
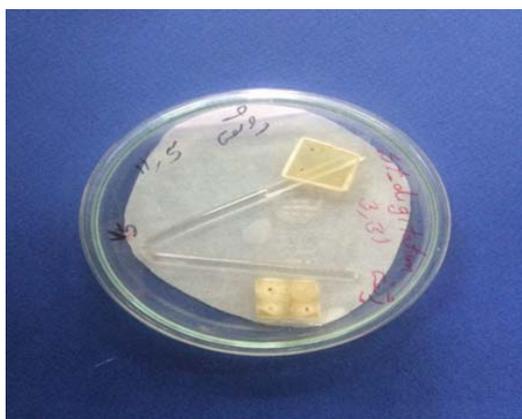**Figure 1.** slide culture from selected strains

Figure 2. Preparation of culture slide on U-shaped loop

2.5. Extraction of mycotoxins from liquid

Extraction of mycotoxins from liquid media (Sabouraud Dextrose Broth with malt extract and yeast) after incubation (seventh day) requires pure cell-free media to extract and purify the mycootoxins produced by strains. For this purpose, the Falcon tubes containing liquid medium were placed in a freezer for 15 min with a 35°C vortex and at -70°C. In the next step, the Falcon tubes are removed from the freezer and de-frozen at the laboratory temperature. Then, 2.5 ml of extraction solvent was added to the Falcon tubes and they were vortexed at three times in three 15-minute periods at 30-35°C (twice to increase the extraction rate). A total of 5ml of extraction solvent was used for each of the falcons. In the final stage, the extraction of mycotoxins (after two times during the vortex process and adding extra solvent) was separated by a Whatman filter (0.5 micron) and then sterilized in sterile microtiops (2 ml) to carry out an ELISA test and kept at 4°C (Rahmani et al., 2009).

2.6. Extraction of mycotoxin from the solid medium (Oatmeal Agar)

After incubation (seventh day) a free cell media was required to extract and purification of mycotoxin produced by pure strains. For this purpose, the Oatmeal Agar medium was cut using a sterile scalp of 1×3 cm and inoculated with sterile falcon tubes (10 ml) containing sterile glasses and 5 ml soluble phosphate buffered saline (PBS). It was placed in a freezer for 15 min with a 35-degree vortex angle and at -70°C. (Rahmani et al., 2009).

2.7. Citrinin Analysis by ELISA method

The ELISA Enzyme Kit and standard of Citrinin was prepared from r-biopharm Germany. The r-biopharm Citrinin kit (R6302)

is a competitive immune enzyme type for determining the amounts of these toxins in foods. Methods of extraction and testing of mycotoxins in the sample were carried out according to the manufacturer's instructions (Leszczynska et al., 2001). Fifty µl of Citrinin standard solutions (0, 15, 45, 135 and 405 ppb or micrograms per kilogram) and 50 µl of samples were prepared for the test, each in two replicates separately in microtitre plates (the number of the applied kit wells is 48). Then, 50 µl of anti-Citrinin antibody solution (black Door vial) was added to each well, then slowly shaken the microplate and incubated at 20-25°C. The wells were then washed or deionized with distilled water three times (250 microliters each). After that, 100 µl of conjugate enzyme (red Door vial) was added to each well and incubated for 10 minutes at 20-25°C. The wells were then washed or deionized with distilled water three times (250 microliters each). In the next step, 100 µl of the substrate/chromogen solution (brown door vial) was added to each well. Then, it slowly shook the microplate and it was incubated at 20-25°C (in dark space). In the final step, 100 µl of the stopper solution (yellow door vial) was added to each well, microplate was slowly shaken, and the absorbance at 450 nm was measured in an ELISA reader (ELISA reader) (The well was adsorbed 10 minutes after pouring the stopper solution). Data on the absorption (OD) of each cavity was recorded separately. The absorption percentage was obtained with the deduction of absorption of samples and standards by the zero standard absorbance rates. The calibration curve standard was plotted based on the absorption rate of the standard samples and the amount of citrinin in the samples. Subsequently, the Citrinin of each sample was obtained in a µg/kg scale based on the absorption percentage of each sample and the correlation with the calibration curve.

$$\text{Adsorption (\%)} = 100 \times \frac{\text{sample absorption}}{\text{zero standard absorption}}$$

2.8. Mycotoxin purification with atmospheric cold jet plasma

In this study, a device was used to purify mycotoxins that the produced jet plasma had the greatest impact on mycotoxins (In this research, the jet plasma device invented by Dr. Hamed

Nikmaram from Islamic Azad University, Science and Research Branch of Tehran was used). In this device, the container in which the plasma is formed is made up of a helix to make the jet plasma most in contact with the surface of the passing mycotoxin and acts as a plasma reactor. The length of the glass tube used in the construction of Helix is 12 cm, its internal diameter is 2 mm, and its outer diameter is 3mm. The spiral glass made with a core diameter of 3cm and a step spacing of 1.5 cm. The two-headed Helix is transformed into three ways to allow the simultaneous entry of gas and mycotoxin at the entrance, and can be separated from each other at the exit. During the experiments, argon gas was selected as the main gas used to make the plasma.

2.9. The testing and treatment step

In order to conduct the tests, initially, the output of the power supply was set to 50 kV, 100 watts and the electron frequency of 30 kHz (optimal conditions for stable and effective plasma) and their exact values were measured with high voltage probes and oscilloscope.

Then, the effect of cold atmospheric jet plasma (argon-air) was investigated for 30, 60 and 360 seconds by setting the gas flow (gas speed of 6 liters per minute) and creating a high potential difference between the two connected electrodes. The treatments were performed for mycotoxins (supernatant) with three replications.

2.10. Statistical analysis

One-way ANOVA analysis ($p < 0.05$) was performed on data to determine significant for comparing the amount of Citrinin at different times (30, 60 and 360 seconds. Statistical analysis was performed using SPSS (Version 185) software.

3. Results
3.1. Results of preliminary experiments for the selection of mycotoxin producing microorganisms

In this research, the samples were collected from northern regions of Iran including wheat, corn, oats, flour, and rice. Five different types of sources (wheat, corn, oats, flour and rice) were

isolated and identified from five species of *Penicillium* genus (encoded by Strains V_1, V_2, V_3, V_4 and V_5) (Table2).

In this study, the standard strains of *Penicillium digitatum* and *Penicillum italicum* were used in the microbial bank of the country's plant health research institute to compare the results and produced of mycotoxins with the collected samples.

Results of the ELISA test before and after the cold jet plasma radiation of atmospheric pressure on the produced mycotoxin (Citrinin) of isolated fungi

The amount of turbidity (maximum absorption) of mycotoxins was measured by mycotoxins before and after treatment with cold atmospheric argon-air jet plasma in the 450 nm wavelength spectrophotometer and the concentration of the produced mycotoxins was determined according to the standard calibration curve (before and after treatment). The results of this study showed that the concentration of citrinin in more than 90% of samples of wheat, maize, oatmeal, flour and rice was higher than the limit specified in the national standard of Iran. The permitted level of styrene concentration according to the national standard of Iran is "Human Feed - Maximum Tolerability of Mycotoxins" with the number 5925, 5µg/kg.

Analysis of the Citrinin concentration of the isolated samples in two Sabouraud dextrose broth + malt extract (SB+ME) and Sabouraud dextrose broth + Yeast Extract (SB+YE) before and after plasma radiation

In this study, the mycotoxin turbidity of the samples was measured. In the next step, the concentration of the citrinin samples (Figures 6, 7, 8, 9 and 10) before and after Plasma jet treatment was designed as Lin/Log (Linear–logarithmic) charts and Log/Lin (logarithmic-Linear) tables according to the standard curve of citrinin (Figure 3) and standard *Penicillium* samples (STD1/STD2) (Figures 4 and 5).

The results of assessing the concentration of citrinin obtained from analyzing Lin/Log charts in Sabouraud dextrose broth + malt extract (SB+ME)

The initial concentration mean of Citrinin reached 16.38, 16.23, and 29.27 µg/kg from 47.203 µg/kg at 60, 120, and 360 seconds, respectively (Figure 11).

The results of assessing the concentration of citrinin obtained from analyzing Lin/Log charts in subrose dextrose broth+ Yeast Extract (Citrinin / YE + SB)

The initial concentration mean of Citrinin reached 24.75, 30.04, and 34.78µg/kg from 57.14 µg/kg at 60, 120, and 360 seconds, respectively (Figure 11).

The results of assessing the concentration of citrinin obtained from analyzing Log/Lin charts in Sabouraud dextrose broth+ Malt Extract (Citrinin / ME + SB)

The initial concentration mean of Citrinin reached 41.66, 11.22, and 20.24µg/kg from36.05 µg/kg at 60, 120, and 360 seconds, respectively (Table 3).

The results of assessing the concentration of citrinin obtained from analyzing Log/Lin charts in Sabouraud dextrose broth+ Yeast Extract (Citrinin / YE + SB)

The initial concentration mean of Citrinin reached 29.88, 13.49, and 31.61µg/kg from 63.01 µg/kg at 60, 120, and 360 seconds, respectively (Table 4).

3.2. Investigation of Pearson correlation (Lin/Log) on the mycotoxin concentration produced before (citrinin) radiation in two media (ME + SB) and (YE + SB)

In this section, the statistical correlation, the citrinin produced by each isolated strain (V_1 to V_5) was investigated in two (ME + SB), (YE + SB) media.

Table 2. Macroscopic and Microscopic characteristics of isolated strains

Sample	Genus, Species	Macroscopic characterizations	Microscopic characterizations
V₁	*Penicillium variable*	Limited growth Colonial diameter 10-15 mm in 7 days Fluffy and woolly colonies Yellow colonies	Conidiospores are often concentrated in the center conidia are Cone-shaped and soft Conidiophore have a smooth wall Containing 5-7 units in Conidiophore The size of the conidia is routine (2.5-3) × (5.7-12) micrometers
V₂	*Penicillium variable*	Limited growth Colonial diameter 10-15 mm in 7 days Fluffy and woolly colonies Yellow colonies	Conidiospores are often concentrated in the center conidia are Cone-shaped and soft Conidiophore have a smooth wall Containing 5-7 units in Conidiophore The size of the conidia is routine (2.5-3) × (7.5-12) micrometers
V₃	*Penicillium digitatum*	fast growth Colonial diameter 40-60 mm in 7 days Low sporulation Powder colony Colnect (originally yellow to green-brown) Smooth colony level	Conidiospores are Distinct and irregular conjunctivitis with short base and at the end of 3-6 fialides Two-row fisheye around the vesicles conidia color(green olives) The size of the conidia is routine (5.3-8) × (3-4) micrometers
V₄	*Penicillium nalgiovense*	Colonial diameter 25-30 mm in 7 days Colony color green to yellow Fluffy and fluffy colonies The color behind the yellow colony	Conidiophores are single and single but less than *P.italicum* Contains 6-2 units in Conidiophore Conidiophore have a smooth wall conidia with a diameter of 3-4 micrometers Conidiophores are transparent 2- 6 long fialides at the end
V₅	*Penicillium italicum*	Slow growth The colony diameter is 50-60 mm in 14 days Has a fragrant smell Color behind the colorless colony or yellow-brown Colony color (green-gray) Smooth colony level	Conidiophores alone and individual Conidiophores have a smooth wall Conidiophores are transparent 3-6 long fialides The color of the conidia (green) The size of the conidia is typically (5-4) × (5.2-5.3) micrometers
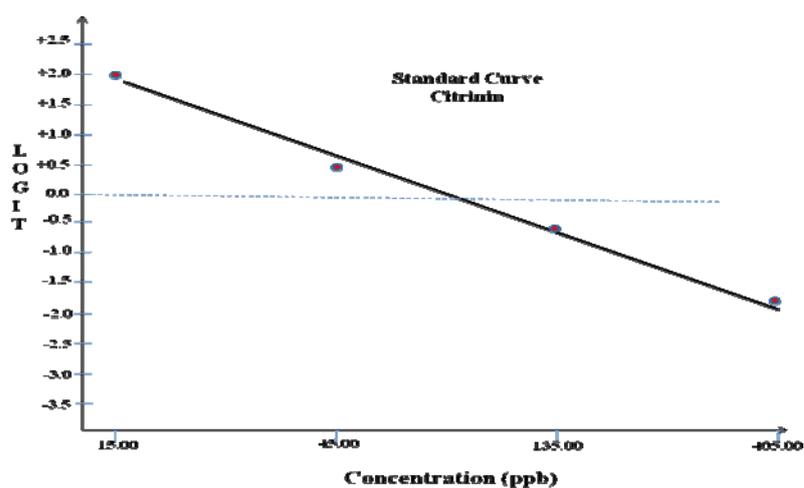
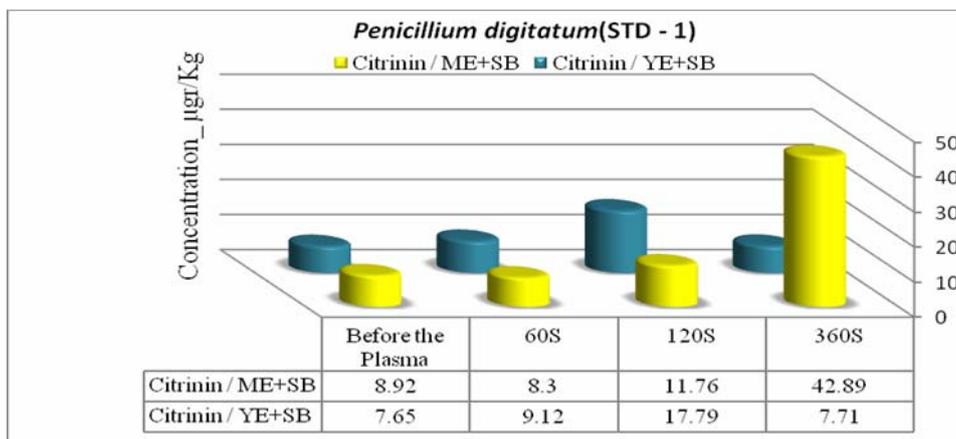
Figure3. Citrinin standard curve

Penicillium digitatum(STD - 1)

Concentration_μgr/Kg

■ Citrinin / ME+SB   ■ Citrinin / YE+SB

	Before the Plasma	60S	120S	360S
Citrinin / ME+SB	8.92	8.3	11.76	42.89
Citrinin / YE+SB	7.65	9.12	17.79	7.71

Figure 4. The concentration of Citrinin before and after plasma radiation in standard 1 (STD1)

Penicillium italicum(STD - 2)

Concentration_μgr/Kg

■ Citrinin / ME+SB   ■ Citrinin / YE+SB

	Before the Plasma	60S	120S	360S
Citrinin / ME+SB	18.18	16.005	12.73	19.99
Citrinin / YE+SB	18.02	9.75	12.7	12.7

Figure 5. The concentration of Citrinin before and after plasma radiation in standard 2 (STD2)

Penicillium variable Strain 1 (V1)

Concentration_μgr/Kg

■ Citrinin / ME+SB   ■ Citrinin / YE+SB

	Before the Plasma	60S	120S	360S
Citrinin / ME+SB	22.52	17.16	17.88	20
Citrinin / YE+SB	17.16	43.65	101.26	23.09

Figure 6. The concentration of Citrinin before and after plasma radiation in sample V1

	Before the Plasma	60S	120S	360S
Citrinin / ME+SB	34.781	18.32	18.61	22.89
Citrinin / YE+SB	20.29	20	50.7	31.02

Figure 7. The concentration of Citrinin before and after plasma radiation in sample V2

	Before the Plasma	60S	120S	360S
Citrinin / ME+SB	202.5	19.27	18.91	26.26
Citrinin / YE+SB	108.96	61.38	14.07	69.96

Figure 8. The concentration of Citrinin before and after plasma radiation in sample V3

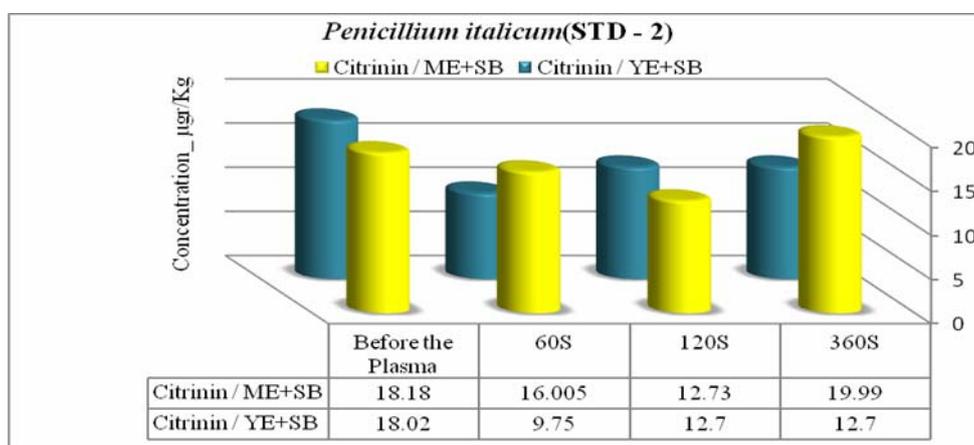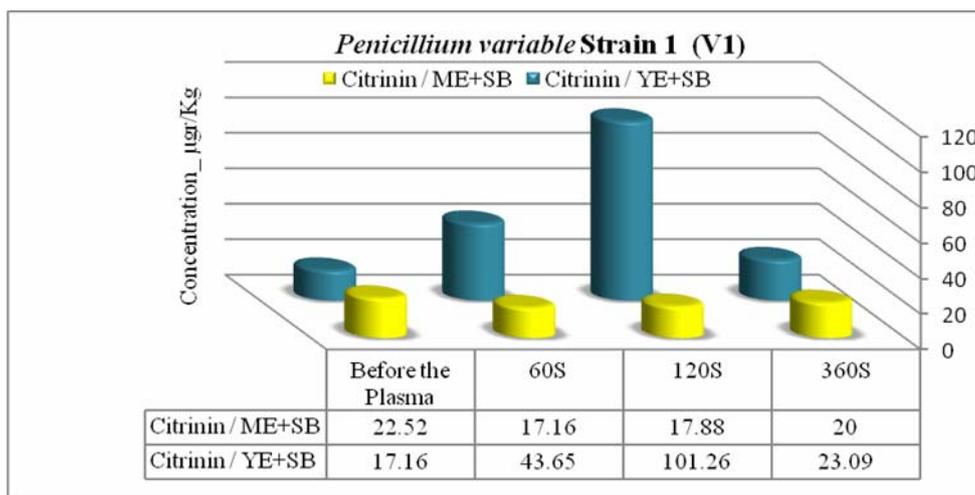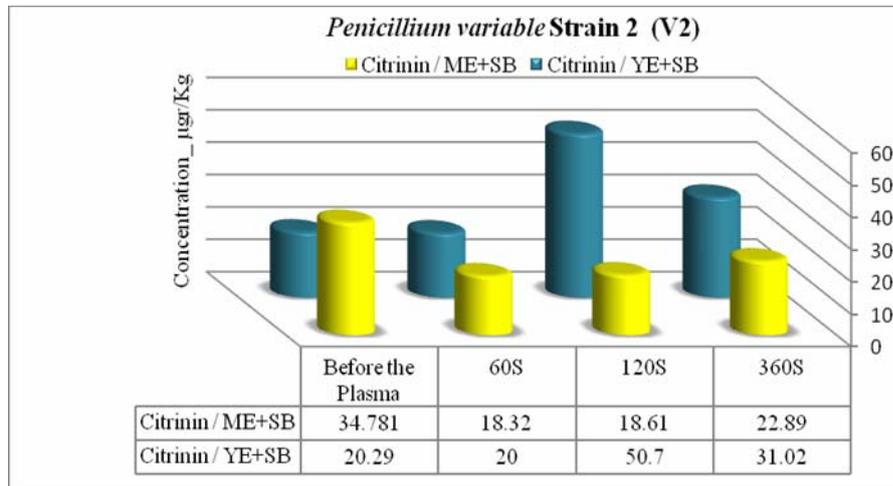|	Before the Plasma	60S	120S	360S
Citrinin / ME+SB	19.68	25.35	18.56	24.09
Citrinin / YE+SB	25.4	16.3	0.283	66.513

Figure 9. The concentration of Citrinin before and after plasma radiation in sample V4

Figure 10. The concentration of Citrinin before and after plasma radiation in sample V5
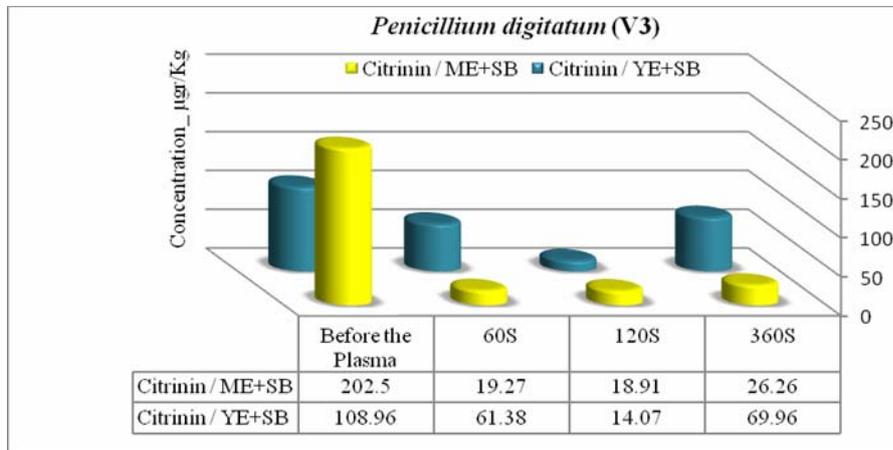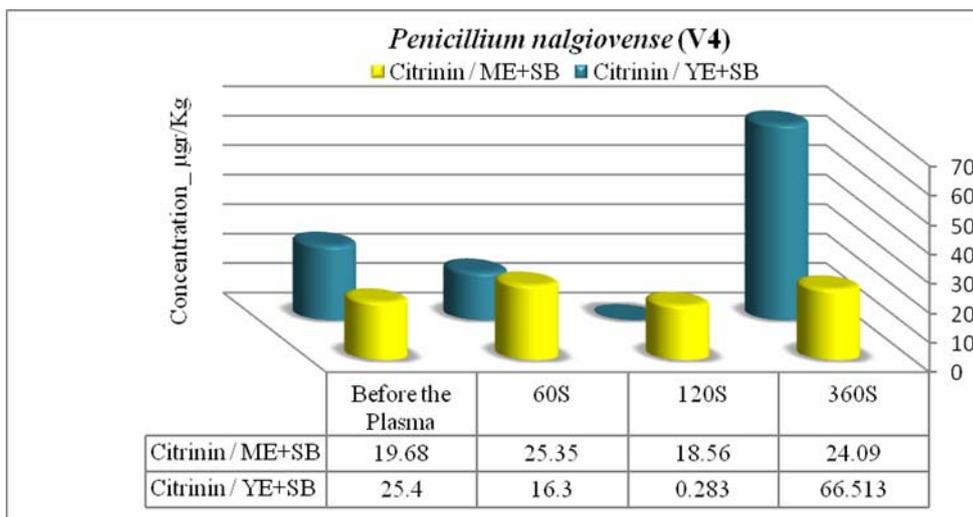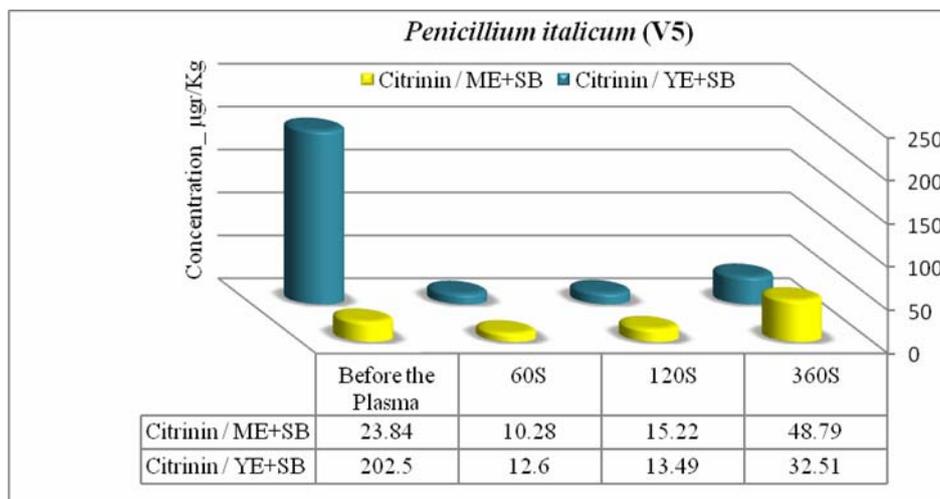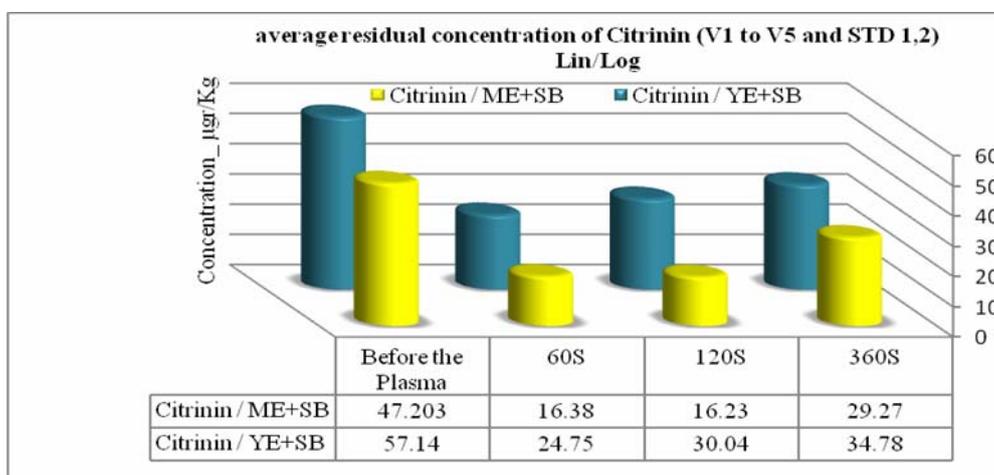
	Before the Plasma	60S	120S	360S
Citrinin / ME+SB	23.84	10.28	15.22	48.79
Citrinin / YE+SB	202.5	12.6	13.49	32.51

Figure 11. The concentration of Citrinin before and after plasma radiation

	Before the Plasma	60S	120S	360S
Citrinin / ME+SB	47.203	16.38	16.23	29.27
Citrinin / YE+SB	57.14	24.75	30.04	34.78

3.3. Statistical analysis of Pearson correlation (Lin/Log) for Citrinin concentration in two (ME + SB), (YE + SB)

In the study of Pearson correlation, the reduction of Citrinin in (ME + SB) medium was more than its reduction in (YE + SB) medium after 60, 120, and 360 seconds of jet plasma treatment. A significant correlation was observed in this regard (Table 5). In the study of Pearson correlation there was a significant correlation between the amounts of Citrinin produced after 60 seconds of Plasma jet treatment according to Table. This significant correlation indicates that the plasma jet effect reduces respectively in two Sabouraud dextrose broth + malt extract and Sabouraud dextrose -

broth + Yeast Extract media as much as 34.7% and 43.33%. In the study of Pearson correlation, according to Table 6, the correlation between the amount of Citrinin toxin produced in the two studied media shows that the studied fungal isolates in two Sabouraud dextrose broth + malt extract and Sabouraud dextrose broth + Yeast Extract media have been able to produce toxin. According to Table 6, the greatest reduction and effect of jet plasma among treatments was observed for Citrinin toxin after 360 seconds of treatment in in two Sabouraud dextrose broth + malt extract and Sabouraud dextrose broth + Yeast Extract media. During this time, the amount of toxin decreased as much as 86.86%.

3.3. Statistical analysis of Pearson correlation (Log/Lin) in the concentration of citrinin in two (ME + SB) and (YE + SB) media

As shown in Table 6:

1. For Citrinin, the highest reduction in Sabouraud dextrose broth + Yeast Extract after 60 seconds of treatment, which decreased as much as 47.4% after 60 seconds.

2. For Citrinin, the highest reduction in Sabouraud dextrose broth + Malt Extract after 120 seconds of treatment, which decreased as much as 31.12% after 120 seconds.

It can be concluded that non-thermal (cold) atmospheric jet plasma can cause detoxification. Due to the presence of charged particles of electrons and ions, ultraviolet radiation, free radicals and reactive chemical species, the plasma can cause changes in the cell wall, morphology, or genetic features of microorganisms and cause them to die. Therefore, the cold plasma as a new technology in various fields of food and agriculture can provide effective solutions in order to promote the goals of the food industry, especially in the areas of mycotoxin detoxification.

3.5. Review and compare the current research with previous research

In Table 7, a comparison of the performance of jet plasma in detoxification in this study and previous studies has been shown.

Table 3. The concentration of Citrinin Sabouraud dextrose broth+ Malt Extract

Log/Lin	sample Name	Citrinin Malt	Citrinin Malt/60s	Citrinin Malt\120s	Citrinin Malt/360s
V1	*Penicillium variable*	44.99	42.36	9.36	22.39
V2	*Penicillium variable*	28.68	47.42	8.89	7.52
V3	*Penicillium digitatum*	40.5	88.28	0.03	0.01
V4	*Penicillium nalgiovense*	38.1	23.39	0.3	20.5
V5	*Penicillium italicum*	48.61	64.27	44.61	42.34
V STD (1)	*Penicillium digitatum*	16.81	10.43	19.82	24.38
V STD (2)	*Penicillium italicum*	34.66	15.51	5.51	24.54
Mean		36.05	41.66	11.22	20.24

Table 4. The concentration of Citrinin Sabouraud dextrose broth+ Yeast Extract

Log/Lin	sample Name	Citrinin Yeast	Citrinin Yeast 60s	Citrinin Yeast 120s	Citrinin Yeast 360s
V1	*Penicillium variable*	32.43	35.04	13.54	72.2
V2	*Penicillium variable*	39.53	22.5	7.91	6.72
V3	*Penicillium digitatum*	226.68	57.8	0.03	0.2
V4	*Penicillium nalgiovense*	52.36	12.38	0.1	66.01
V5	*Penicillium italicum*	40.5	55.73	36.3	44.48
V STD (1)	*Penicillium digitatum*	15.54	12.61	17.08	8.08
V STD (2)	*Penicillium italicum*	34.04	13.11	19.51	23.58
Mean		63.01	29.88	13.49	31.61

Table 5. Citrinin concentration before and after radiation (Lin/Log)

Citrinin concentration	Initial concentration of Citrinin before treatment	Concentration after 60_S Radiation (μgr/Kg)	Concentration after 120_S Radiation (μgr/Kg)	Concentration after 360_S Radiation (μgr/Kg)
Citrinin . ME+SB	47.203	16.38	16.23	29.27
Citrinin . YE+SB	57.14	24.75	30.04	34.78

Table 6. Citrinin concentration before and after radiation (log/Lin)

Citrinin concentration	Initial concentration before treatment	Concentration after 60 seconds of radiation (μg.kg)	Concentration after 120 seconds of radiation (μg.kg)	Concentration after 360 seconds of radiation (μg.kg)
Citrinin . ME+SB	36.5	41.66	11.22	20.24
Citrinin . YE+SB	63.01	29.88	13.49	31.61

Table 7. Comparison of previous research with this research

Condition of the jet plasma	Microorganism / mycotoxin	toxin removal (%)	Comparison of previous research with this research	Reference
Argon gas, voltage 50 kV, 400 watts, electron frequency 30 kHz, 1 minute time	25 (microgram / kg) Citrinin and Ochratoxin A penicillium	95	The concentration of Citrinin from 47.203 to 16.38 µg / kg after 60 seconds, reduced the amount of Citrinin after 60 seconds by 65.29 %	Siciliano et al., (2016)
Argon gas, 60 watts power in 5 minutes	Citrinin and Ochratoxin A penicillium	80	The concentration of Citrinin from 47.203 to 29.27 µg / kg after 360 seconds, reduced the amount of Citrinin after 360 seconds by 37.99 %	Devi, A. and et al., (2017)
Argon gas, voltage 30 KV, 500 W power, inlet gas speed 4 liters per minute, 1 minute	Citrinin and penicillium	80	The concentration of Citrinin from 47.203 to 16.38 µg / kg after 60 seconds, reduced the amount of Citrinin after 60 seconds by 65.29 %	Park, J.C and et al., (2007).
Sulfur hexafluoride gases, voltage 20 kV, power 100 watts, in 2 minutes	Citrinin and penicillium	100	The concentration of Citrinin from 47.203 to 16.23 µg / kg after 120 seconds, reduced the amount of Citrinin after 120 seconds by 65.61 %	Basaran and et al., (2008).

3. Conclusion

In comparison with the methods previously, the cold atmospheric pressure plasma (CAP) mycotoxin decontamination of food overcomes many of the disadvantages and obstacles of physical, chemical and microbial decontamination procedures. As depicted in Table 8, most of the CAP systems used for decontamination of food are environmentally benign, require a low energy input and are economically favourable. Beside this, plasma approaches have proven to have a negligible effect on the quality of many types of treated food. These advantages are based on the reactivity of the plasma species which enable the high decontamination efficiency in a very short time compared to alternative decontamination methods (Niemira, 2012), (Niemira, 2011) and (Misra et al., 2016).

Mycotoxin contaminated food represents a significant and increasing threat to human health and an enormous burden for the global economy. Decontamination methods to tackle this problem are based on physical, chemical and biological principles. In spite of constant improvements, these methods can still suffer from a lack of mycotoxin removal efficiency; they can be environmentally harmful and economically unfavourable. With no doubt, the food industry continuously strives for more effective mycotoxin decontamination approaches. One of the most promising new procedures to deactivate mycotoxins on food is cold atmospheric pressure plasma (CAP) technology. On the laboratory level, it has been convincingly demonstrated that CAP efficiently kills fungi on the surface of food and destroys the mycotoxins that these organisms secrete. In favour over many of the traditional food decontamination methods, plasma-based decontamination methods are generally lower-cost and ecologically benign. Most importantly, plasma-based mycotoxin decontamination of food has been demonstrated significantly more efficient in both the mycotoxin degradation level and speed of decontamination in comparison to conventional decontamination methods.

Table 8. The comparison between mycotoxin decontamination methods

Decontamination method	Highest decontamination Rate obtained	Food product	Process Duration	Energy Consumption	Impact on the food quality	Reference
Thermal treatment	85–100% (FBs, Citrinin, AFs)	Corn	Long	High	Significant	Scott et al., 1993
Gamma irradiation	90% (mixture)	Grains, seeds	Short	Low	Significant	Calado et al., 2014
UV light irradiation	90% (Citrinin, AFB_1)	Peanut oil; apple, juice;	Short	Low	Negligible	(Mao et al., 2016) and (Zhu et al., 2014)
Pulsed light technology	90% (Citrinin, AFB_1)	Rice product	Short	Low	Negligible	Wang et al., 2016
Ammoniation	90-100% (Citrinin, AFB_1)	Rice, corn flour, peanuts	Long	High	Significant	Millan et al., 2003
Ozonation	80% (Citrinin, AFB_1)	Rice, corn flour, peanuts	Long	Low	Negligible	(Luo et al., 2014) and (Diao et al., 2013)
CAP technology	100% (Citrinin, AFB_1)	Seeds, Crops, Cereals	Short	Low	Negligible	(Park et al., 2007) and (Siciliano et al., 2016)

Before industrialization of CAP technology can be realised, the molecular mechanisms and kinetics of plasma-based mycotoxin decontamination should be better characterized in order to become standardized. For this reason, additional experimental work is needed to:
• Draw firm correlations between different plasma operating parameters and the specific reactive chemical species formed.

• Draw correlations between the composition of the plasma and the structure of the mycotoxin degradation products. As toxicities of the mycotoxin degradation products can be experimentally determined, in this way, the mycotoxin decontamination efficiency would be defined as well.

• Examine the effects of different plasma treatments on the quality of food products, for example on their nutritional value and organoleptic qualities.

• Design plasma-forming systems for efficient mycotoxin decontamination of various types and sizes of food products.

• Test if hybrid plasma-conventional systems for mycotoxin decontamination of food products can be even more effective.

Refereces

Barnett, H. and Hunter, B. (1998). Illustrated Genera of Imperfect Fungi. 4th ed. Minneapolis. Burgess.

Basaran, P., and et al., (2008). Elimination of Aspergillus parasiticus from nut surface with low pressure cold plasma (LPCP) treatment. Food Microbiology. 25: 626–632.

Calado, T., Venâncio, A., Abrunhosa, L. (2014). Irradiation for mold and mycotoxin control: A review. Compr. Rev.Food Sci. Food Saf., 13:1049–1061.

Devi, A. and et al. (2017). Influence of cold plasma on fungal growth and aflatoxins production on groundnuts. Food Control. 77: 187e191.

Diao, E., Hou, H., Chen, B., Shan, C., Dong, H. (2013). Ozonolysis efficiency and safety evaluation of aflatoxin B1 in peanuts. Food Chem. Toxicol., 55: 519–525.

Hayashi, N. and et al. (2013). Inactivation characteristics of Bacillus thuringiensis spore in liquid using atmospheric torch plasma using oxygen. Vaccum. 88: 173-176.

James, L. (1986). Modified Method for Fungal Slide Culture. Harris Journal of Clinical Microbiology. 460-461.

Klich, M.A. (2002). Identification of Common Aspergillus Species. United States. Centraalbureau Voor Schimmelcultures.

Leszczynska, O. and et al. (2001). Determination of Aflatoxins in Food Products by the ELISA Method. Czech J. Food Sci. 19(1): 8–12

Luo, X., Wang, R., Wang, L., Li, Y., Wang, Y., Chen, Z. (2014). Detoxification of aflatoxin in corn flour by ozone. J. Sci. Food Agric. 94: 2253–2258.

Mao, J., He, B., Zhang, L.X., Li, P.W., Zhang, Q., Ding, X.X., Zhang, W. (2016). A structure identification and toxicity assessment of the degradation products of aflatoxin B1 in peanut oil under UV irradiation. Toxins. 8: 332.

Millan, T.F., Martinez, Y.A. (2003). Efficacy and stability of ammoniation process as aflatoxin B1 decontamination technology in rice. Archivos Latinoamer. Nutr. 53: 287–292.

Misra, N.N., Schlüter, O., Cullen, P.J. (2016). Chapter 1 - plasma in food and agriculture. In Cold Plasma in Food and Agriculture; Academic Press: San Diego, CA, USA. 1–16.

Mortazavi, S.M., Colagar, A.H. and F. Sohbatzadeh. (2016). The Efficiency of the Cold Argon-oxygen Plasma jet to reduce Escherichia coli and Streptococcus pyogenes from solid and liquid ambien. Iran J Med Microbiol. 10(3): 13-19.

Niemira, B.A. (2012). Cold plasma decontamination of foods. Annu. Rev. Food Sci. Technol. 3: 125–142.

Niemira, B.A. (2011). Gutsol, A. Nonthermal plasma as a novel food processing technology. Nonthermal Process. Technol. Food. 272–288.

Park, J.C. (2007). Degradation of mycotoxins using microwave-induced argon plasma at atmospheric pressure. Surf. Coat. Technol, 201: 5733–5737.

Park, R. and et al. (2013). Plasma Medicine: How can Nonthermal Atmospheric Plasma are Applied to Medicine. Journal of Life Science. 23838.

Rahmani, A., Jinap, S., and Soleimany, F. (2009). Qualitative and Quantitative Analysis of Mycotoxins. Comprehensive Review in Food Science and Food Safety. 8: 13-19.

Scott, P., Lawrence, G. (1993). Stability and problems in recovery of fumonisins added to corn-based foods. J. AOAC Int. 77: 541–545.

Siciliano, I., and et al. (2016). Use of cold atmospheric plasma to detoxify hazelnuts from aflatoxins. Toxins. 8: 125.

Song, F. and et al. (2011). Meta-analysis of plasma amyloid-β levels in Alzheimer's disease. J Alzheimers Dis; 26(2): 365–375.

Wang, B.; Mahoney, N.E.; Pan, Z.L.; Khir, R.;Wu, B.G.; Ma, H.L.; Zhao, L.M. (2016). Effectiveness of pulsed light treatment for degradation and detoxification of aflatoxin B1 and B2 in rough rice and rice bran. Food Control, 59, 461–467.

Zhu, Y., Koutchma, T., Warriner, K., Zhou, T. (2014). Reduction of patulin in apple juice products by UV light of different wavelengths in the UVC range. J. Food Prot. 77: 963–971.