Egypt. J. Bot. **Vol. 59**, No. 3. pp. 845-855 (2019)

Egyptian Journal of Botany
http://ejbo.journals.ekb.eg/

Efficacy of Ozonized Water for Fungal Decontamination of Fresh Fruit Pieces Decorating Dessert Cakes

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SEVEN different fresh-cut fruit samples prepared for decorating cakes were screened for fungal contamination. The contamination of the fruits ranged from 8 to 242CFU/g and are arranged in descending order as follow: Strawberry> banana> raspberries> pineapples> mangoes> papaya> kiwi. The most common fungi recovered from the fruits were (CFU/g); *Rhizopus oryzae* (125), *Mucor mucedo* (63), *Botrytis cinerea* (60), *Alternaria alternata* (54), and *Aspergillus flavus* (47). The Efficacy of ozonized water to eliminate fungal contamination associated with fresh fruits was evaluated compared with hypochlorite and acetic acid as commercial disinfectants. Application of 1ppm ozonized water for 2min was the most efficient method and induced a complete reduction of fungi by 100% in case of all investigated fruits except strawberries that revealed 98.2%. Strawberries were the most mycotoxin-contaminated fruit showing aflatoxins, alternariol and patulin recording 2.6, 531.0 and 83.2µg/kg, respectively. Treatment with ozonized water even when applied at 1ppm for one minute was sufficient and effective in complete elimination of mycotoxins in fruits. In SEM image, the ozonized water-treated spores appeared irregular and deformed, with obvious necrotic lesions and disruptions in the walls. As well, there was an increase in activity of hepatic enzymes in serum namely; AST, ALT and ALP that detected in guinea pigs using water mixed with strawberries extract from contaminated fruit compared to those consuming tap water. Guinea pigs consuming tap water mixed with extract from ozonized-treated strawberries did not significantly induce marked change in the most hematological values compared to the control.

Keywords: Ozonized water, Flesh fruits, Fungal contamination, Mycotoxins, SEM, Guinea pigs.

Introduction

Fresh-cut fruits are no longer considered low risk in terms of food safety (Bhagwat, 2006). Many fresh produce items have been linked to outbreaks of microbial disease throughout the world. Mangoes and papayas are regularly served sliced in food as fresh pieces or as juice. In general, these products do not undergo any earlier process to eliminate pathogenic microorganisms before consumption, and a long shelf life could theoretically provide time for these microorganisms to multiply without affecting the organoleptic qualities of the fruit, thereby increasing the risks of food-borne illness. Berries, such as raspberries (*Rubus idaeus*), blueberries (*Vaccinium myrtillus*), blackberries (*Rubus fruticosus*), redcurrant (*Ribes rubrum*), and lingonberries (*Vaccinium vitis-idaea*), represent an important type of fresh produce in several countries, in terms of production volume and economic value; however, little information is available about their contaminated microbiological risks. Berries were responsible for foodborne outbreaks due to virus contamination, including as frozen fruits. Frozen raspberries have been reported in previous studies as contaminated produce responsible for Norovirus outbreaks in Europe (Einoder et al., 2016)

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Received 2/11/2019; Accepted 19/11/2019
DOI: 10.21608/ejbo.2019.19020.1377
Edited by: Prof. Dr. Wael S. El-Sayed, Faculty of Science, Ain Shams University, Cairo, Egypt.

Consumption of fruit products is commonly viewed as a potential risk factor for infection with enteropathogens such as *Salmonella typhimurium* and *Escherichia coli* O157, with recent outbreaks linked to fresh fruits. Routes of contamination are varied and include application of organic wastes to agricultural land as fertilizer, direct contamination by livestock, wild animals and birds and postharvest issues such as worker hygiene (Heaton & Jones, 2007). Contamination of waters used for irrigation with faecal material can also be a source of contamination (Abd El-Salam et al., 2017). The most common sources of water for irrigation include wells, rivers, reservoirs and lakes, all of which are susceptible to contamination by human pathogens. The presence of pathogenic microorganisms in irrigation water and their transfer to vegetables has been reported. (Chigor et al*.,* 2010). Moreover, vegetable cultivation in open areas allows the access of animals (birds, insects, rodents, domestic and wild animals), which can defecate in the fields and, therefore, be a source of contamination. Other studies showed that pathogens such as *S. Typhimurium* and *E. coli* O157:H7 can survive for a long period in soil (>150 days) and in vegetables (>60days) grown after experimental contamination using contaminated fertilizer (manure) or irrigation water (Ijabadeniyi et al*.*, 2011).

These outbreaks show that the quality of the water used for washing is critical factor (CDC, 2009). The mechanical damages caused by the cutting treatment of fresh-cut fruits lead to a series of biological and chemical changes, such as browning, off-flavor, aging, softening and deterioration, which tend to shorten the shelf life (Luo et al., 2015). Therefore, improving the quality and prolonging the shelf life are the key technologies to the processing of fresh-cut fruits. Ankita et al. (2014) indicated that gastroenteritis, giardiasis, hepatitis A, hepatitis E, shigellosis (bacillary dysentery), typhoid fever, *vibrio parahaemolyticus* infections and cholera are very evident examples of fecal-oral route transmitted diseases. Microbes can cause food poisoning in two different ways. Some infect the intestines, causing inflammation and difficulty absorbing nutrients and water, leading to diarrhea. Other produce chemicals in foods (toxins) those are poisonous to the human digestive system. When eaten, these chemicals can lead to nausea and vomiting, kidney failure, and even death.

Food borne fungi, i.e. yeasts and moulds, cause serious spoilage of stored food leading to enormous health problems. Molds can also produce mycotoxins that are associated with several acute and chronic diseases in humans (Miescher et. al., 2011). Mycotoxins are secondary metabolites of filamentous fungi and therefore occur naturally in food. They represent a very large group of different substances produced by different mycotoxigenic species. Although the contamination of some fruits by fungi is not necessarily associated with the formation of mycotoxins, however some are reported as mycotoxin producers.

The mycotoxins commonly found in fruits and their processed products include aflatoxins, ochratoxin A, patulin and *Alternaria* toxins (Moss, 2008; Ouf et al., 2015). Mycotoxins are known for their toxicological properties and several food organizations set maximum allowed levels of mycotoxin to protect animal and public health (Barkai-Golan & Paster, 2008). Kabak & Dobson (2009) reported that the contamination of fruits with mycotoxins has not only caused health hazards but also resulted in economic losses, especially for exporting countries.

The aim of this paper is to identify the different fungi contaminating the fruit decorating the dessert cakes and the possible toxins they produce. As well the efficacy of ozonized water as decontaminating agent will be evaluated.

Materials and Methods

Sample collection

Seven fruit samples were collected randomly between January 2016 and December 2016 from different dessert cake stores in Cairo Egypt. The samples were collected in sterile polythene bags and immediately transferred to the laboratory where the fungal contamination was analyzed immediately.

Isolation of fungi

The fruit surface mycoflora were identified using the dilution plate method (Johnson & Curl, 1972). Different media were employed for isolation of mycoflora including potato dextrose agar (PDA) containing chloramphenicol (30mg/L). The inoculated plates were incubated at 30^{o}C for four days. Fungal colonies were enumerated on the fourth day and were expressed

as the number of colony forming units (CFU) per gram. The developing colonies were identified to the species level based on cultural, morphological and microscopic characteristics according to Raper & Thom (1949), Raper & Fennel (1965), Ramirez (1982), Brandenburg (1985) and Samson et al. (2000). Measurement for each collected sample was carried out in triplicate, the distribution frequency (%) was calculated according to Mirchink (1988).

Treatment

Different disinfectants were employed for the treatment of the fruit pieces. Preliminary experiments showed that 1ppm ozonized water was efficient as inhibitory agent against fungal growth. Therefore, the treatment includes ozonized water at a concentration of 1ppm applied for one and two minutes. The ozonized water was prepared by delivering ozone gas (produced from ozone generator) in sterile water and the concentration of ozone was measured by ozone monitor (Model Z-1200). The effect of ozone was compared with the effect of 0.05% hypochlorite for two minutes, 2% acetic acid for 10min and water as negative control.

Mycotoxins assay

The collected fruits employed in this study had been tested for Aflatoxin (FB2) and Ochratoxin (OTA) production. FB2 was extracted from fruits based on the method reported by D'Arco et al. (2008). In brief, the toxin was extracted from the crushed fruits with 20ml of a mixture of acetonitrile–water (85:15, v/v) using an Ultra Ika T18 basic Ultraturrax (Staufen, Germany) homogenizer for 3min. The extract was centrifuged at 4500 ×*g* for 5 min and then the supernatant evaporated to dryness with a Büchi Rotavapor R-200 (Postfach, Switzerland) and then redissolved in 2ml of the same extraction solvent. This final solution was filtered through a 25mm/0.45µm nylon filter (Análisis, Vinicos, Tomelloso, Spain) and FB2 was determined in the filtrate. OTA extraction from the collected fruits was performed following the method described by Medina et al. (2004). Five replicates, each of three fruit discs were investigated for each treatment. OTA was extracted with 15ml ethyl acetate after acidifying with 10µL concentrated HCl. After shaking for 30min, the supernatants were combined and evaporated to dryness in a rotary evaporator. The residue was redissolved in 250µL of water–acetonitrile–acetic acid (55:44:1, v/v/v). OTA and FB2 were measured quantitatively using enzyme-linked immunosorbent assay (ELISA) test strips, which were examined using the rapid one-step assay (ROSA) system (Charm Biosciences Inc., Lawrence, MA, USA). This system provides results equivalent to those of commercial HPLC methods (Baines et al., 2011). The strip is a quantitative lateral flow immunoassay with a sensitivity range of 0–150µg/kg and a limit of detection of 1µg/kg. One hundred µL of the extracted mycotoxin was diluted with 1ml of mycotoxin dilution buffer, and 300µL of this solution was pipetted onto the strip and incubated for 10min before removing the strip. The strip was then read on a ROSA-M reader within 2min. Each sample was analyzed in triplicate.

The test strip is a one-step procedure. The liquid sample to be analyzed is placed on the sample pad. The membrane pads are usually nitrocellulose based on Shim et al. (2009). The reagent membrane contains the immobilized specific antibodies and the labelled antibodies. Upon addition of the sample, the reacting molecules are solubilized. When solubilized, they combine with the toxin in the sample, and capillary action draws the fluid mixture toward the reaction membrane. Colloidal gold was used as a reagent for visualizing the antigen/antibody interaction in the test strips

Scanning electron microscope (SEM)

The effect of 2ppm of ozonized water on spores of *A. alternata* as a representative example of the isolated fungi was studied using SEM. The spores were detached from agar discs (1cm diameter) of inoculated potato dextrose agar (PDA) medium that had been incubated for 7 days at 25°C. The spores were suspended in demineralized sterile water. The suspension was centrifuged for 15min at 7000*xg*, and the pellet was resuspended in 25ml sterile demineralized water. This operation was performed twice. Then, the pellet was resuspended and centrifuged twice at 5000*xg* and twice at 4000*xg* for 15min each. After the last wash, the purified suspension was collected in 8 Eppendorf tubes each containing 1ml. Four tubes, each was treated with 1ml of 1ppm ozonated water and the other four tubes, each was treated with 1ml of demineralized sterile water and acts as control. Twenty five microlitre aliquots of each treatment were sprayed on 1.0cm diameter polystyrene sheets and dried for 24hr at

room temperature in a laminar flow hood. The sheets were then pasted on a metallic support and gold-plated before observation by SEM at 20.0kV with an FEI/ Philips XL30 microscope.

Hematological and histopathological studies

Twenty male guinea pigs, weighing between 250-300 g were purchased and housed in smooth bottomed plastic cages with steel netting in the animal house of the Faculty of Science, Cairo University, Egypt. The acclimatization period was 15 days. The animals were randomly distributed into two groups each of 10 animals. Beside the daily regular feed supply, both animal groups were supplied with fresh water provided in drip bottle to prevent contamination. The first group was supplied with sterile tap water (control) and the second group of animals was provided with tap water mixed with fruit extract of untreated strawberries (1:1 ratio) and third group was supplied with sterile tap water mixed with ozonized-treated strawberries fruit extract (1:1 ratio). The guinea pigs were anesthetized with isofluorane and blood samples were withdrawn at the beginning of the experiment and after 6 months of drinking water. The sample was allowed to clot and centrifuged at 1000 rpm for 5mins using Uniscope laboratory centrifuge (model SM902B, England) and serum separated for analysis. Analysis for hemoglobin, RBCs, leucocytes, were determined according to Quesenberry & Rosenthal (2004).

Estimation of AST activities and ALT activities were done using Reitman-Frankel method (Reitman & Frank, 1957). Estimation of ALP activities using King and King method (King & King, 1954). Levels of urea and creatinine in serum were determined by kinetic UV methods (Labtest Diagnostica SA, Lagoa Santa, Brazil). The limits of detection for urea and creatinine were 0.056 mg/dl and 0.01mg/ dl, respectively. The method uses an enzymatic system by UV photometry and two-point kinetics (Bartels & Böhmer, 1973). Animal housing and treatment conditions were approved by the ethics committee that determines the ethical and animal welfare practices (approval no. CU/I/S/37/16).

Statistical analysis

Analysis of variance and F-tests were used to identify significant differences among the treatment means at $P \leq 0.05$. Multiple comparisons were made by the least significant difference.

Results

Fungal isolation

Figure 1. shows the fungal contamination of seven different fresh-cut fruit samples ornamenting different dessert cakes. The highest load of fungal contamination was recorded in strawberry (242CFU/g). The contamination of raspberries, pineapples and mango ranged from 390-490 CFU/g. The lowest fungal count was noticed in papaya (10 CFU/g), kiwi (8CFU/g) and banana (51CFU/g).

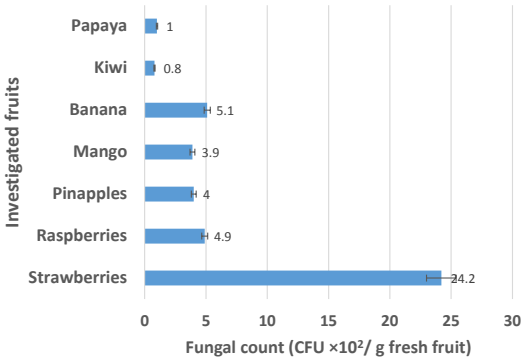

Fig. 1. Total fungal count of different fruit pieces prepared for decoration of dessert cakes.

Quantitively, *Rhizopus oryzae* was the most detected fungus in the investigated samples being isolated in 125CFU/g followed by *Mucor mucedo*, *Botrytis cinerea* ,*Alternaria alternata* and *Aspergillus flavus* counting 63, 60, 54 and 47CFU/g, respectively. *Alternaria alternata*, *Fusarium culmorum*, and *Rhizopus sexualis*, each was isolated in a count around 3CFU/g. The other identified species, each was isolated in count ranging from 11 to 23CFU/g (Table 1).

The data reveal that strawberries was the most contaminated fruit where the total fungal count reached 242CFU/g representing 41.58% from the total count. More than 8 different fungal species were recovered from strawberries. The contamination of the other fruit pieces ranged from 8 to 49CFU/g recorded in the case of kiwi and raspberries, respectively.

Regardless of the diversity of fungal species, *Alternaria alternate* and *F. culmorun*, each was detected in 3 different fruit cut samples while *A. flavus*, *A. niger* and *Mucor mucedo*, each was isolated from two different fruit samples. The other fungi, each looks specific for a definite fruit and isolated from one fruit type (Table 1).

TABLE 1. Fungal species (CFU× 10/g fresh fruit) isolated from fruit pieces prepared for decoration of dessert cakes.

Fungal species	CFU× 10/g fresh cut fruit								
	Banana	Kiwi	Mango	Papaya	Pine-apples	Rasp-berries	Straw-berries	Total	Cases of isolation out of 7
Acremonium strictum						1.7		1.7	1
Alternaria alternata	3.1			0.5			1.8	5.4	3
Aspergillus flavus						1.7	3.0	4.7	2
A. fumigatus			1.1					1.1	1
A. niger			1.1		1.2			2.3	2
Botrytis cinerea							6.0	6.0	1
Colletotrichum musae	1.0							1.0	1
Monilinia fructicola						1.2		1.2	1
Mucor mucedo			1.1				5.2	6.3	2
Penicillium italicum							3.0	3.0	1
Penicillium expansium					1.2			1.2	1
Fusarium culmorum	1.0	0.8			1.2			3.0	3
Rhizopus oryzae								12.5	1
Rhizopus sexualis							3.0	3.0	1
Trichoderma viride				0.5				0.5	1
Ulocladium chartarum							1.8	1.8	1
Others			0.6		0.4	0.3	0.4	3.5	4
Total count	5.1	0.8	3.9	1.0	4	4.9	24.2	58.2	10

Mycotoxin detection

Seven mycotoxins were analyzed in the samples after 5 days of fruit incubation at 5°C using ELISA test strips. Three fruits did not show any of the seven examined mycotoxins namely; kiwi, mango, and raspberries although fungal growth was significantly observed in these fruits (Table 2). On the other hand, strawberries were the most mycotoxin-contaminated fruit showed positive result for aflatoxins, alternariol and patulin recording 2.6, 531.0 and 83.2µg/kg, respectively. Zearalenone was detected in fresh cut pieces of banana and pineapples estimating 377.2 and 112.1µg/kg, respectively.

Effect of different disinfectants on fungal count

The efficacy of 1ppm ozonized water to eliminate fungal contamination associated with fresh fruits was evaluated compared with two commercial disinfectants. The test disinfectants were 0.05% hypochlorite applied for two minutes, and 2% acetic acid for 2min. The percentage of reduction in CFU/g fresh fruit was calculated compared to control (running tap water).

TABLE 2. Mycotoxins (µg/kg) detected in fruit pieces prepared for dessert cakes decoration after incubation for 4 days at 5°C.

Fresh fruit	Mycotoxins						
	Aflatoxins	Alternariol	Citrinin	Fumonisins	Patulin	Satratoxins	Zearalenone
Banana	ND	377.2±22.4	ND	ND	ND	ND	377.2±17.0
Kiwi	ND	ND	ND	ND	ND	ND	ND
Mango	ND	ND	ND	ND	ND	ND	ND
Papaya	ND	256.7	ND	ND	ND	ND	ND
Pineapples	ND	ND	ND	ND	ND	ND	112.1±6.9
Raspberries	ND	ND	ND	ND	ND	ND	ND
Strawberries	2.6±0.9	531.0±26.7	ND	ND	83.2±8.1	ND	ND

Fruit treatment with 1ppm ozonized water for two minutes was the most efficient method and induced a complete reduction of fungi by 100% in case of all investigated fruits except strawberries that revealed 98.2% fungal decontamination. Complete fungal elimination in the case of strawberries was achieved by extending the exposure time to 3min at this dose (unpublished data). Washing with running water was least effective disinfectant as it reduces fungal count in ratios ranging from 67.5% in the case of banana to 18.8% for kiwi (Fig. 2). Application of hypochlorite and acetic as disinfectants looked better than washing water but was not as efficient as ozonized water. The reduction in fungal count ranged from 75.4 to 100% for hypochlorite and from 54.2 to 80.0% in the case of acetic acid.

Effect of different disinfectants on mycotoxin production

Treatment with ozonized water even when applied at 1ppm for one minute was sufficient and effective disinfectant in complete inhibition of mycotoxin production in case of strawberries and raspberries, although both fruits were showing fungal growth. Washing with running water was the least efficient disinfectant as it inhibited the mycotoxin production except in case of banana, pineapples and strawberries, although all the investigated fruits in this case yet showed fungal contamination (Table 3).

Scanning electron microscope (SEM)

The SEM images revealed variation in *A. alternata* spores treated with ozonized water compared to treated tap water (Fig. 3). The ozonized water treated spores appeared irregular and deformed, with obvious necrotic lesions and disruptions in the walls. The spores looked flaccid, plasmolyzed and morphologically collapsed.

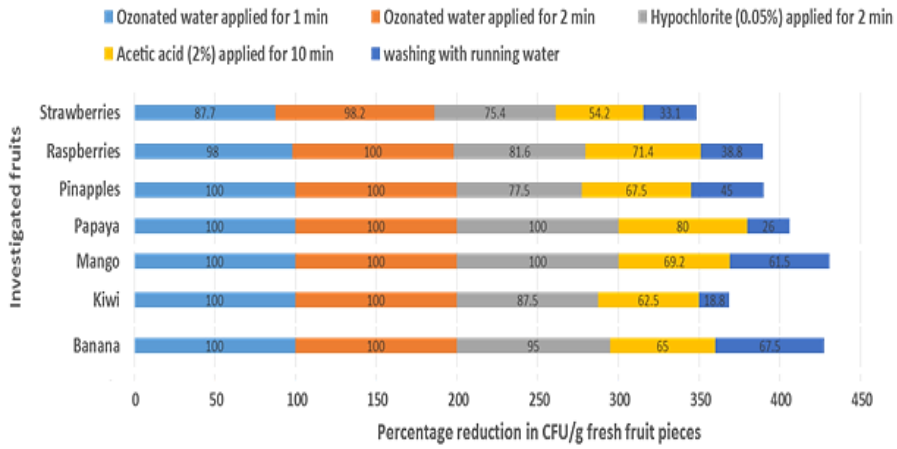

Fig. 2. Percentage reduction in colony-forming units (CFU) of the fungal species contaminating fruit pieces prepared for decoration of dessert cakes using 1ppm ozonized water compared with other disinfectants.

TABLE 3. Detection of mycotoxins in ozonized water-treated decoration fruit pieces compared with two disinfectants and tap water.

Fruit	Treatment				
	Ozonated water applied for 1min	Ozonated water applied for 2min	Hypochlorite (0.05%) applied for 2min	Acetic acid solution (2%) applied for 2min	Washing tap water for 2min
Banana	ND	ND	ND	ND	+
Kiwi	ND	ND	ND	ND	ND
Mango	ND	ND	ND	ND	ND
Papaya	ND	ND	ND	ND	+
Pineapples	ND	ND	ND	ND	+
Raspberries	ND	ND	ND	ND	ND
Strawberries	ND	ND	+	+	+

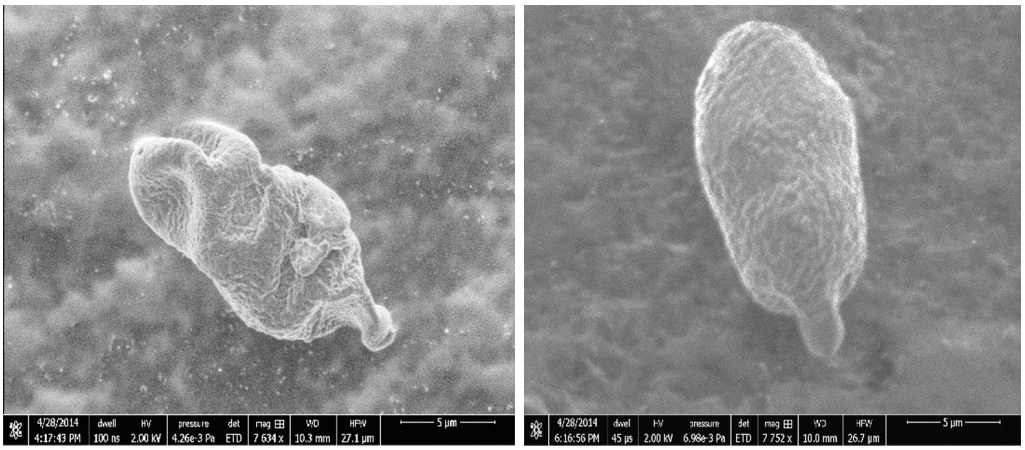

Fig. 3. SEM images of *A. alternata* spores treated with 1ppm ozonized water for 2min (left) as compared with spores treated with washing tap water for the same period (right).

Hematological study

The hematology parameters showed significant decrease in the counts of hemoglobin, RBCs, platelets and lymphocytes and a significant rise in lymphocytes in guinea pigs consuming water mixed with strawberries extract from fungal contaminated fruits compared to those consuming tap water as standard reference (Table 4). As well, there was an increase in activity of serum AST, ALT and ALP detected with guinea pigs using water mixed with extract from contaminated fruit compared to those drinking tap water. These values indicate the efficiency of liver function. The data show a steady increase in blood creatinine and significant increase in urea level in guinea pigs drinking water mixed with extract from contaminated strawberries compared to the control. The latter two parameters are of special significance to evaluate renal function. Urea and creatinine were markedly increased to 642mg/L and 6.7mg/L in guinea pigs using water with contaminated fruit extract compared to 346.2mg/L and 4.9mg/L in the case of water as reference, respectively. Guinea pigs drinking tap water mixed with extract from ozonized-treated strawberries did not significantly induce change in the most hematological values compared to the control.

TABLE 4. Effect fruit extract mixed with drinking water on different hematological values of blood pictures of guinea pigs including liver and kidney markers after 6 months of daily water dinking*.

Item aspect	Drinking tap water mixed with extract from untreated and contaminated strawberries fruit	Drinking tap water mixed with extract from ozonized water- treated strawberries fruit	Drinking tap water (control)
Hemoglobin (g/L)	103±11	133±14	135±13
RBCS ($\times 10^6$/µL)	3.57±0.92	4.81±0.82	4.70±1.5
Leucocytes ($\times 10^3$/µL)	16.2±2.3	12.4±1.6	12.9±1.8
Platelets ($\times 10^3$/µL)	358±34	674±122	622±116
Lymphocytes ($\times 10^3$/µL)	1.66±0.45	1.83±0.7	1.94±0.7
Aspartate aminotransferase (AST) (IU/L)	33.2±4.9	18.0±3.8	17.1±3.8
Alanine aminotransferase (ALT) (IU/L)	27.7±4.3	19.4±4.7	11.8±3.4
Alkaline phosphatase (ALP)(IU/L)	77.5±7.9	52.3±4.8	47.8±5.1
Urea (mg/L)	642±46	337±42	346±42
Creatinine (mg/L)	6.7±1.1	4.5±0.9	4.9±1.1

*Five blood samples were used in each group of guinea pigs
*P value < 0.05

Discussion

Contamination of fresh produce during handling process is considered a common problem and it is usually ignored the use appropriate techniques of decontamination (Kabak & Dobson, 2009). If consumed, contaminated fruits and vegetables can lead to food poisoning, because of the presence of intestinal infectious microbes on the outer surfaces. Drug resistant microbes loaded on fruit surface from air, soil, polluted water or the surrounding environment is probably the cause of fruit spoilage and reduction in their shelf time (Mathur et al., 2014). Such changes can directly affect food poisoning patterns, such as frequency of incidents, number of patients involved, and mortalities. The majority of diseases associated with fresh fruits and vegetables are primarily those transmitted by the fecal-oral route, and therefore, are a result of contamination at some point in the process (DeRoever, 1998). Gastroenteritis, Giardiasis, Hepatitis A, Hepatitis E, Shigellosis (bacillary dysentery), Typhoid fever, Vibrio parahaemolyticus infections and cholera are very evident examples of fecal-oral route transmitted diseases. Microbes can cause food poisoning in two different ways. Some infect the intestines, causing inflammation and difficulty absorbing nutrients and water, leading to diarrhea. Other produce poisonous toxins inducing disturbance and disfunction of the human digestive system. that can lead to nausea and vomiting, kidney failure, and even death (Titarmare et al., 2009).

In this research, strawberries showed the highest fungal contaminated fruit followed by raspberries, pineapples and mango. The high contamination of strawberries by fungi (242CFU/g) may be due to the high concentration of various sugars, minerals, vitamins, amino acids, high water content and low pH that support and enhance the successful growth and survival of several varieties of fungi (Droby, 2006). The filamentous fungi are reportedly involved in the efficient degradation of plant cell wall as they utilize the complex plant cell wall polysaccharides as a sole source of carbon by producing a wide range of hydrolases such as pectinases, cellulases, hemicellulases and amylases. Most fungi isolated from strawberries and other investigated fruits have been proved to be pectolytic and/or cellulolytic producer including *Botrytis cinerae* (Lionetti, 2015), *Rhizopus oryzae* (Chowdhury et al., 2017), *Mucor* spp. (Alves et al., 2002), *P. italicum* (Alaña et al., 1989).

The detection of mycotoxins in the investigated fruits depends of the diversity of isolated fungi and their mycotoxigenic potential as well as the physical and chemical composition of the fruit and ecological factors that play an important role in mycotoxin formation. Three types of mycotoxins were detected in strawberries; aflatoxins probably due to *Aspergillus* spp., alternariol and patulin mostly due to *A. alternata*. The presence of these fungi provides an indication of mycotoxin contamination although its occurrence is not necessarily assure the formation of mycotoxins. The contamination of fruits with mycotoxins has not only caused health hazards but also resulted in economic losses, especially to exporting countries (Salunkhe et al., 1987). The contamination with banana with alternariol and zearalenone is consistent with the being contaminated with *A. alternata* (Tournas & Stack, 2001) and *F. culmorum* (Waśkiewicz et al., 2008), respectively.

Ozone is a strong oxidant and has different food applications including fruits and vegetables to ensure food safety. Ozone treatment is considered an eco-friendly An environmentally friendly approach, cost-effective food processing technique and has spontaneous decomposition without forming hazardous residues. In this research, the application of ozone at 1ppm for one or two minutes achieved complete elimination of fungal contamination as well degradation of the associated mycotoxins. Most of the preliminary studies reviewed by Freitas-Silva & Venâncio (2010) have indicated that an application of ozone for a short period of exposure is capable of controlling the proliferation of filamentous fungi and of degrading many mycotoxins. Haas & Kaymak (2003) reported that the antimicrobial effect of ozone depends on varied factors, such as ozone concentration and quantity of microoganism, exposure period and variables in the microbial permeability that verified the occurrence of diverse effects on each microorganism.

One of the mechanism route of ozone application is the malformation of the microbial cell. The ozone- treated *A. alternata* as an example in this research showed significant deformation and shrunken patterns that may result in its inability to germinate. This may result in a loss of membrane integrity, leakage of intracellular components, and, ultimately, focal dissolution of the cell surface with longer exposure times (Ouf et al., 2016). Moisan et al. (2001) indicated that,

in microorganisms subjected to intense electron or ion bombardments, spore coatings or cell wall materials are eroded and occasionally rupture, resulting in fatal outcomes. This investigation substantiates supports the proposed mechanism of the microbial inactivation by ozone that caused cell membrane destruction and finally lysis reaction.

The risk profile on the microbiological contamination of raw fresh fruits and vegetables has reported from patients attending different clinics and hospitals throughout the world including united states (Harris et al., 2003). Surveys of raw fruits and vegetables demonstrate that there is potential for a wide range of these products to become contaminated with enteric pathogenic microorganisms and may consequently pose health risks. Outbreaks of gastrointestinal illness have been reported for intact products, cut/sliced/skinned/shredded or minimally processed products, sprouted seeds and unpasteurized fruit and vegetable juices (Tauxe et al., 1997). The consumption of guinea pigs with contaminated fruit extract induces hepatic and renal dysfunction and disturbance in the other hematological aspects within 6 months mainly due to mycotoxin existence. Application ozone to the fruit cut pieces was shown to eradicate the microbial contamination of fruits and did not cause disturbance of aspect items related to liver, kidney or blood features of guinea pigs when consume these fresh produces.

Conclusion

In conclusion, the most efficient way to improve safety of fruits and vegetables is to rely on a positive system reducing risk factors during production and handling. Apart from washing, the other methods of decontamination seem to have a significant impact on protection and safety even though it may leave unpleasant smell. Moreover, care should be considered to avoid consumption of raw fruits and minimize the possible risk of infection of pathogens.

References

Abd El-Salam, S.S., Amer, M.M., Nasr-Eldin, M.A., Radwan, A.M., Abo taleb, S.H. (2017) Physical-chemical and bacteriological evaluation of river Nile water and drinking water in Benha city, Egypt. *Egypt. J. Bot.* 57(3), 495-506.

Alaña, A., Gabilondo, A.., Hernando, F., Moragues, M.D., Dominguez, J.B., Llama, M.J., Serra, J.L. (1989) Pectin lyase production by a *Penicillium italicum* strain. *Appl. Environ. Microbiol.* **55**, 1612-1616.

Alves, M.H., Campos-Takaki, G.M., Porto, A.L.F., Milanez, A.I. (2002) Screening of *Mucor* spp. for the production of amylase, lipase, polygalacturonase and protease. *Brazil. J. Microbiol.* **33**, 325-330.

Ankita, M., Akshay, J., Dharmesh, H. (2014) Microbial contamination of raw fruits and vegetables. *Internet Journal of Food Safety*, **16**, 26-28.

Baines, D., Erb, S., Lowe, R., Turkington, K., Kuldau, G. (2011) Moldy feed, mycotoxins and shiga toxin-producing *Escherichia coli* colonization associated with jejunal hemorrhage syndrome in beef cattle. *BMC Vet. Res.* **7**, 24.

Barkai-Golan, R., Paster, N. (2008) "*Mycotoxins in Fruits and Vegetables*". Academic Press.

Bartels, H., Böhmer, M. (1973) A kinetic method for measurement of creatinine in serum. *Med. Lab. (Stuttg),* **26**, 209-215.

Bhagwat, A. A. (2006) Microbiological safety of fresh-cut produce: Where are we now? In: "*Microbiology of Fresh Produce*", Matthews, K.R. (Ed.), pp. 121–165. Chapter 5. ASM Press, Washington, DC.

Brandenburg, W. (1985) "*Parasitische Pilze an Gefasspflanzen in Europa*", Fischer, Stuttgart, Germany.

Centers for Disease Control and Prevention (CDC) (2009) Centers for Disease Control and Prevention Investigation update: Outbreak of *Salmonella Typhimurium* infections, Nov. 2008- March 2009.

Chigor, V., Umoh, V., Smith, S. (2010) Occurrence of *Escherichia coli* O157 in a river used for fresh produce irrigation in Nigeria. *Afr. J. Biotechnol.* **9**, 178-182.

Chowdhury, T.I., Jubayer, F., Uddin, B., Aziz, G. (2017) Production and characterization of pectinase enzyme from *Rhizopus oryzae*. *Slovak J. Food Sci.* **11**(1), 641-51.

DeRoever, C. (1998) Microbiological safety evaluations and recommendations on fresh produce. *Food Control,* **9**, 321-347.

D'Arco, G., Fernández-Franzón, M., Font, G., Damiani, P., Mañes, J. (2008) Analysis of fumonisins B1, B2 and B3 in corn-based baby food by pressurized liquid extraction and liquid chromatography/ tandem mass spectrometry. *J. Chromatogr A,* **1209**, 188-194.

Droby, S. (2006) Improving quality and safety of fresh fruits and vegetables after harvest by the use of biocontrol agents and natural materials. *Acta Hortic.* **709**, 45-51.

Einoder-Moreno, M., Lange, H., Grepp, M., Osborg, E., Vainio, K., Vold, L. (2016) Non-heat-treated frozen raspberries the most likely vehicle of a norovirus outbreak in Oslo, Norway, November 2013. *Epidemiol. Infect.* **144**, 2765-2772.

Freitas-Silva, O., Venâncio, A. (2010) Ozone applications to prevent and degrade mycotoxins: A review. *Drug Metabolism Reviews*, **42**(4), 612-620.

Haas, C., Kaymak, B. (2003) Effect of initial microbial density on inactivation of *Giardia muris* by ozone. *Water Res.* **37**, 2980-8.

Harris, L.J., Farber, J.N., Beuchat, L.R. (2003) Outbreaks associated with fresh produce: Incidence, growth and survival of pathogens in fresh and fresh-cut produce. *Compr. Rev. Food Sci. Food Saf.* **2**, 78-141.

Heaton, J.C., Jones, K. (2007) Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: A review. *J. Appl. Microbiol*. **104**(3), 613-26.

Ijabadeniyi, O., Debusho, L., Vanderlinde, M., Buys, E. (2011) Irrigation water as a potential preharvest source of bacterial contamination of vegetables. *J. Food. Saf.* **31**, 452-461.

Johnson, L., Curl, E. (1972) "*Method for the Research on Ecology of Soil Borne Plant Pathogens*", Burgess, Minneapolis.

Kabak, B., Dobson, A. (2009) Biological strategies to counteract the effects of mycotoxins. *Journal Food Prot.* **72**(9), 2006-16.

King, E.J., King P.R.N. (1954) Estimation of plasma phosphatase by determination of hydrolyzed phenol with amino-antipyrene. *J. Chem. Path.* **7**, 322-326.

Lionetti, V. (2015) Pectoplate: The simultaneous phenotyping of pectin methylesterases, pectinases, and oligogalacturonides in plants during biotic stresses. *Front. Plant Sci.* **6**, 331.

Luo, Z., Wang, Y., Jiang, L., Xu, X. (2015) Effect of nano-$CaCO_3$-LDPE packaging on quality and browning of fresh-cut yam. *LWT-Food Science and Technology*, **60**, 1155-1161.

Medina, A., González, G., Sáez, J.M., Mateo, R., Jiménez, M. (2004) Bee pollen, a substrate that stimulates ochratoxin A production by *Aspergillus ochraceus* Wilh. *Syst. Appl. Microbiol.* **27**, 261-267.

Mathur, A., Joshi, A., Harwani, D. (2014) Microbial contamination of raw fruits and vegetables. *Internet Journal of Food Safety*, **16**, 26-28.

Miescher, S., Meile, L., Lacroix, C. (2011) Antifungal lactic acid bacteria and propionibacteria for food biopreservation, Woodhead Publishing Series in Food Science, Technology and Nutrition, 2011, 27-62.

Mirchink, T. (1988) "*Soil Mycology*", Moscow State University, Moscow.

Moisan, M., Barbeau, J., Moreau, S., Pelletier, J., Tabrizian, M., Yahia, L.H. (2001) Low-temperature sterilization using gas plasmas: A review of the experiments and an analysis of the inactivation mechanisms. *Int. J. Pharm*. 226 (1–2), 1-21

Moss, M. (2008) Fungi, quality and safety issues in fresh fruits and vegetables. *J. Appl. Microbiol.* **104**(5), 1239-43.

Ouf, S. A., Basher, A. H., Mohamed, A-A. H. (2015) Inhibitory efect of double atmospheric pressure argon cold plasma on spores and mycotoxin production of *Aspergillus niger* contaminating date palm fruits. *Journal of the Science of Food and Agriculture*, **95**(15), 3204-3210.

Ouf, S.A., Mohamed, A-A.H., El-Sayed, W.S. (2016) Fungal decontamination of fleshy fruit water washes by double atmospheric pressure cold plasma. *Clean, Soil, Air, Water,* **44**(2), 134-142.

Quesenberry, K.E., Rosenthal, K.L. (2004) Endocrine diseases. In: "*Ferrets, Rabbits and Rodents Clinical Medicine and Surgery*", Quesenberry, K.E., Carpenter, J.W. (Eds.), pp.79-90, 2nd ed.

Philadelphia, W.B. Saunders.

Ramirez, C. (1982) "*Manual and Atlas of the Penicillia*", Elsevier Biomedical Press, New York.

Raper, K., Thom, C.A. (1949) "*Manual of the Penicillia*", Williams & Wilkins, Baltimore.

Raper, K., Fennell, D. (1965) "*The Genus Aspergillus*", Williams & Wilkins, Baltimore.

Reitman, S., Frank, S. (1957) Transaminases. *Am. J. Clin. Pathol.* **28**, 56.

Salunkhe, D.K., Adsule, R.N., Padule, D.N. (1987) Aflatoxins in foods and feeds. *J. Food Sci. Technol.* **33**, 95-107.

Samson, R., Hoekstra, E., Frisvad, J., Filtenborg, O. (2000) "*Introduction to Food and Airborne Fungi*", 6th ed., Centraalbureau voor Schimmelcultures, Utrecht 2000.

Shim, W., Dzantiev, B., Eremin, S.A., Hung, D. (2009) One-step simultaneous immunochromatographic strip test for multi-analysis of ochratoxin A and zearalenone. *J. Microbiol. Biotechnol.* **19**, 83-92.

Tauxe, R., Kruse, H., Hedberg, C., Potter, M., Madden, J., Wachsmuth, K. (1997) Microbiological hazards and emerging issues associated with produce. A preliminary report to the National Advisory Committee on Microbiologic Criteria for Foods. *J. Food Prot.* **60**, 1400-1408.

Titarmare, A., Dabholkar, P., Godbole, S. (2009) Bacteriological analysis of street vended fresh fruit and vegetable juices in Nagpur City, India. *Internet J. Food Safety*, **11**, 1-3.

Tournas, V.H., Stack, M.E. (2001) Production of alternariol and alternariol methyl ether by *Alternaria alternata* grown on fruits at various temperatures. *J. Food Prot*. 4, 528-532.

Waśkiewicz, A., Gromadzka, K., Wiśniewska, H., Goliński, P. (2008) Accumulation of zearalenone in genotypes of spring wheat after inoculation with *Fusarium culmorum*. *Cereal Res. Commun.* 36, 401-404.

فعالية الماء المعالج بالأوزون لإزالة التلوث الفطري من قطع الفاكهة الطازجة التي تزين كعك الحلوى

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تم فى هذا البحث فحص التلوث بالفطريات لسبع عينات فواكه طازجة مختلفة أعدت لتزيين كعك الحلوى. وتراوح التلوث بالفطريات من 8-242 وحدة مكونة للمستعمرة ويمكن ترتيب التلوث بالفطريات للعينات المستهدفة تنازليا كالأتى: الفراولة > الموز> توت > أناناس> مانجو> بابايا> فاكهة الكيوي. وكانت الفطريات الأكثر شيوعًا المستخلصة من الفواكه (وحدة مكونة للمستعمرة/جرام) هى فطر الريزوبس اوريزا (125) والميكور ميوسيدا (63) وبوتريتس سينارياً (60) والترناريا الترناتا (54) واسبرجلس فلافس (47). وتمت مقارنة فعالية الماء المعالج بالأوزون للقضاء على التلوث الفطري المرتبط بالفواكه الطازجة مع الهيبوكلوريت وحمض الخليك كمطهرات تجارية. وعند استخدام 1 جزء من المليون من الماء المعالج بالأوزون لمدة دقيقتين أمكن تطهير جميع العينات من التلوث بنسبة 100% باستثناء الفراولة حيث بلغت نسبة ازالة التلوث الفطرى 98.2%. وكانت الفراولة أكثر الفواكه الملوثة بالسموم الفطرية التي تظهر الأفلاتوكسينات والاترناريول والباتولين، حيث سجلت 2.6 و531.0 و83.2 ميكروجرام/ كيلوجرام على التوالي. وكان العلاج بالماء المعالج المؤوزن حتى عند استخدامه في جزء واحد في المليون لمدة دقيقة كافيا وفعال في التخلص التام من السموم الفطرية في الفاكهة.وتظهر صورة الميكرسكوب الألكترونى لجراثيم فطر الألترناريا الترناتا المعاملة بالماء المؤوزن ان الجراثيم غير منتظمة ومشوهة، مع اماكن نخرية واضحة في الجدران. وكذلك هناك زيادة في نشاط الإنزيمات الكبدية ALT، AST وALP في فئران التجارب التى تغذت على الماء المخلوط مع مستخلص الفراولة الملوثة بالفطريات مقارنة بالمستهلكات لمياه الصنبور. ولم تظهر حيوانات التجارب التى استخدمت المياه المستخلصة من الفراولة المعالجة بالأوزون أى تغيير ملحوظ في صورة الدم مقارنة بالعينة الحاكمة.