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Prevention and Control Methods of Mycofloa Spoilage Contaminated some Fresh Juices

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ABSTRACT

Prevention and control of mycoflora spoilage contaminated some fresh juices resulted that, all hot water used i. e. 45, 50 and 55^{0}c were found to reduce significantly the number of spores' viability with all tested fungi i. e. *Aspergillus flavus, A. parasiticus, Pencillium digitatum, P. expansum, P. italicum* and *Rhizopus stolonifer* compared with un-treated (control). Enhanced effect was recorded when treated these spores with hot water treatment at 55^{0}c for 5min. The numbers of spore viability were decreased significantly with increasing the temperature degree used. Both pasteurization (at 80°C) and sterilization (at 105°C) treatments have a great effect on spore viability of the tested fungi compared with un-treated (control) which were highly significant and gave completely inhibition of spore viability of the tested fungi with hundred percent of reduction. All tested alternative fungicides used i. e. Benzoic acid, Citric acid and Sodium carbonate were found to reduce significantly spore viability of all the tested fungi i. e. *Aspergillus flavus, Pencillium digitatum, P. expansum, P. italicum* and *Rhizopus stolonifer* compared with un-treated (control). Benzoic acid substance was enhanced than others. Tested of ozone exposure on spore viability of *A. flavus* and *P. expansum* with different exposure times i.e. 15, 30 and 45 minutes at ozone concentration in the gas supply was varied (1-4.8% w/w of oxygen) were found to reduce significantly the total numbers of spore' viability for these fungi compared with untreated control. The numbers of spore viability were continuing decreased significantly with increasing the times period of ozone exposure used.

Key words: Fresh juices, Fungi, Heating, Pasteurization, Sterilization, Ozonation

Introduction

Mycoflora contaminants: Fruit juices contain a micro-flora which is normally present on the surface of fruits during harvest and postharvest processing which include transport, storage, and processing (Tournas *et al.,* 2006 and Kamal *et al.,* 2014). Fresh fruits, fresh vegetables are susceptible to rot by different types of molds from genera *Penicillium, Aspergillus, Alternaria, Botrytis, Rhizopus*, and others. According to the changes in appearance, the mold spoilages are designated as black rot, gray rot, blue rot, soft rot, brown rot, and others (Neeraj and Sharma, 2007). In addition the common postharvest and storage fungi of fruits are *Alternaria* spp., *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp. (Ammar and El-Naggar, 2014). *Aspergillus, Penicillium, Fusarium,* and *Alternaria* are the principal genera concerning mycotoxins and widespread occurrence (Ester *et al.,* 2014). Water used for juice preparation can be a major source of microbial contaminants. Fruit juices are available in essentially the same form almost anywhere in the world. From polar bases to the tropics and from the largest developed countries, fruit juices are available in bottles, cans, laminated paper packs, pouches, cups and almost every other form of packaging known. Therefore, maintaining the quality of processed fruit juices is an important concern (Tasnim *et al.,* 2010). In general, microbial growth in fruit juice is restricted by using preservatives and also through other environmental factors during production. Preservation of fruit juice by pasteurization, refrigeration and sterilization are popular methods used to attain microbiological stability by destroying pathogenic microorganisms and to preserve the color, aroma and taste of fresh juice (Rahman *et al.,* 2011). The major ingredients of the juice such as water, sugar, color, natural fruit pulp and other additives ...etc may also carry some microbial contaminants. The presence of microbial contaminants in water, sugar and pulp may cause spoilage of the drinks or gastrointestinal diseases to the consumers. Fruit juices contain various concentrations of sucrose, which constitutes a very important component of the medium for the growth of fungi. Microbial spoilage is a serious problem for the food industry as fungal contamination can occur during processing as well as handling of the end products (Koc *et al.,* 2007).

Prevention and Control of Mycoflora Spoilage: Many thermal and non-thermal technologies have been developed to control microorganisms on fresh-cut produce. Types of thermal processing used to treat fresh-cut produce include hot water, hot steam, and hot sanitizing solution. Thermal processing is a relatively new technology to the fresh-cut produce industry. Bio-steam R Technologies trademarked Thermo-Safe system is an example of thermal technology developed for fresh-cut processing. The process time (from seconds to minutes)

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and temperature (from 60 to 100°C) are dependent upon the commodities being treated (Farber *et al.* 2003 ; Sapers *et al.,* 2005). Common methods for preservation and processing of fruit juices include pasteurization, freezing, evaporation, canning and spray drying (Fasoyiro *et al.,* 2005). Non-thermal processing technologies can be classified as either physical or chemical (Farber *et al.,* 2003; Sapers *et al.,* 2005). Chemical technologies can be divided into gas-phase sanitation and liquid-phase sanitation based on the physical state of the chemical used. Examples of gas-phase sanitation include ozone can be used to help eliminate mycotoxin contamination and chlorine dioxide (Sengun *et al.,* 2008). Organic acids have widespread application for preventing food spoilage in fermented and acidified foods (Brul and Coote, 1999). The mode of action of organic acids is attributed to depression of intracellular pH by ionization of the un-dissociated acid molecule or disruption of substrate transport, by alteration of cell membrane permeability (Naidiu, 2000). Types of organic acids added to foods include salts of benzoic acid and sorbic acid, as well as acetic, lactic, and propionic acids. A given acid concentration and pH may independently affect the growth and death of bacterial cells.Organic acids have been used for years to control fungal spoilage of foods. They find wide use because of solubility, taste, and low toxicity (Oranusi *et al.,* 2012).

Ozonation treatment: Ozone (O_3) is a strong antimicrobial agent with numerous applications in the food industry. It has been used for decades in many countries. Ozone in the aqueous or gaseous phase is active against a wide range of bacteria, molds, and yeasts. Most applications are targeted to decontamination of fruit and vegetable surfaces by washing in ozonated water. A second application is fruit and vegetable storage Xu, (1999) and Barth *et al.* (1995) assessed ozone exposure on storage of blackberries stored at 28^0C in air with 0.3 ppm ozone. Fungal development was suppressed while 20% of the control fruits showed decay. The effectiveness of ozone is influenced by the intrinsic factors of a food. It also oxidizes food surfaces when used at high levels. Further research may reduce some of these concerns so; ozone can be used in broader food applications. Rapid decomposition of ozone to oxygen and lack of toxic residues make it a favorable environment friendly sanitizer (Kim, *et al.,* 1999; Achen and Yousef, 2001).

This work aimed to prevention and control of mycoflora spoilage contaminated some fresh juices by some physical and chemical technologies. 1-Physical methods include a-heating, b-pasteurization and c-sterilization. 2-Chemical technologies include; a- some organic acids, salts, and b-ozonation.

Materials and Methods

Reduction of fungi and their mycotoxins of some isolated fungi i. e. *Aspergillus flavus, A. parasiticus, Pencillium digitatum, P. expansum, P. italicum* and *Rhizopus stolonifer* were studied in laboratory of Plant Pathology, National Research Centre (NRC), Cairo, Egypt by using somephysical and chemical treatments as follow:-

I-Physical treatment:-

I.1-Hot water treatment:

Aspergillus flavus, A. parasiticus, P. expansum, P. digitatum, P. italicum and *R. stolonifer* isolates which isolated from some fresh juices as Apple, Grape-fruit and Navel-orange (Embaby *et al.,* 2015) were surface harvested after 7 days old culture from incubation. Spores were harvested by adding 9 ml of water sterile from the plate surface with a sterile glass rod, and passing through two layers of sterilized cheesecloth to removing the mycelial growth. The suspension was diluted with sterilized water to an optical density (OD). Spore suspension (0.2 ml) of a concentrated each fungus was added to sterilize distilled water in the sterile glass tubes, to achieve a final concentration of 2 x 10^2 spore's /ml (Zamani *et al.,* 2009; Embaby *et al.,* 2013 II&III). All sterile glass tubes were placed in water bath at 45, 50, and 55°C, and allowed to equilibrate for 5 min. After 5min., tubes were removed from the water bath and placed immediately on ice. Aliquots (2 ml) of the spore suspensions were transferred to Potato Dextrose Agar (PDA) kept in Petri dishes padded with treated spore suspension, and incubated for three days at 22±2ºC in darkness. The numbers of colony-forming of viable spores were counted for each treatment. These treatments included: hot water (45, 50 and 55ºC) for 5 min, comparing with un-treated control treatment were recorded according to Plaza *et al.* (2004); Zamani *et al.* (2009); Fatemi and Hassan, (2011) and Embaby and Hagag (2014).

I.2-Pasteurization treatment:

Other sterile glass tubes were processed. And treated by High temperature short time (HTST) at 80°C for 30 s according to Moyer and Aitken (1980) and Rupasinghe, and Juan, (2012), and then transferred to Potato Dextrose Agar (PDA). All Petri dishes were incubated for three days at 22±2ºC in darkness. The numbers of colony-forming of viable spores were counted for each treatment, comparing with un-treated control treatment were recorded according to De Donno *et al.* (1998); Sant'Ana *et al.* (2009) and Embaby and Hagag (2014).

I.3- High temperature (autoclaving or sterilization treatment):

Another sterile glass tubes were autoclaving at 105 °C for 5 minutes as previously described (Sant'Ana *et al.,* 2008 & 2009), then transferred to Potato Dextrose Agar (PDA). All Petri dishes were incubated for three days at 22±2ºC. in darkness. The numbers of colony-forming of viable spores were counted for each treatment, comparing with un-treated control treatment were recorded according to Embaby, and Hagag (2014).

II- Chemical treatment:-

II.1-Alternative fungicides:

Effect of some alternative fungicides in vitro: All tested fungi were surface harvested after 7 days old culture from incubation. Spores were harvested by adding 9 ml of water sterile from the plate surface with a sterile glass rod, and passing through two layers of sterilized cheesecloth to removing the mycelial growth. The suspension was diluted with sterilized water to an optical density (OD). Spore suspension (0.2 ml) of a concentrated each fungus was added to sterilize distilled water in the sterile glass tubes, to achieve a final concentration of 2 x 10^2 spore's /ml (Zamani *et al.,* 2009 and Embaby *et al.,* 2013 II&III). The efficacies of the tested compounds i. e. Benzoic acid, Citric acid and Sodium carbonate of tested fungi were evaluated *in vitro*. The tested compounds were added to sterilized PDA medium, before solidifying to obtain the proposed concentrations Benzoic acid, Citric acid (6, 8, 10 g/l) and Sodium carbonate (1, 2 & 3 g/l) then, gentle rotation was done for five minutes to ensure the equal distribution of the added compound (s). Aliquots (2 ml) of the spore suspensions were transferred into sterilized Petri dishes. After that, the concentrations were dispensed in sterilized Petri plates (9-cm-diameter). PDA medium free of these compounds was used for the check treatment (Control), and incubated for three days at 22±2ºC in darkness. The numbers of colony-forming of viable spores were counted for each treatment (Zamani *et al.,* 2009 and Fatemi and Hassan, 2011).

II.2-Ozonation treatment:

Ozone gas was produced from ozone generator in Food Toxicology and Contaminants Dep., National Rerearch Center, Giza, Egypt. Model OZO 6 VTTL OZO Max Ltd, Shefford, Quebec Canada. Disinfestations' with ozone: 100 ml of Apple juice (contain 10^{-2} spore suspensions of *Aspergillus flavus* and *P. expansum*) were transferred in to 500 ml flask, and the flask was plugged with a silicone stopper with 2 holes in it. One hole was for the ozone line and the other was for tubing connected to the ozone destruct unit. Apple juice was treated at selected conditions of 30% generator power of oxygen gas, the ozone gas concentrations produced were about 4.8 mg. L^{-1} (1-4.8% w/w of oxygen) which reported by Wang *et al.* (2010) and Torres *et al.* (2011) and agitated for 15, 30 and 45 min using a magnetic stirrer at room temperature. Aliquots (2 ml) of the spore suspensions were transferred to Potato Dextrose Agar (PDA) kept in Petri dishes padded with treated spore suspension, and incubated for three days at 22±2ºC in darkness. The numbers of colony-forming of viable spores were counted for each treatment comparing with un-treated (control) treatment.

Statistical analysis:

Obtained data were subjected to analysis of variance as a simple experiment in a complete randomizes block design. LSD method was used to difference means according to Snedecor and Cochran, (1989).

Results

Reduction of fungi and their mycotoxins of all the isolated fungi i. e. *Aspergillus flavus, A. parasiticus, Pencillium digitatum, P. expansum, P. italicum* and *Rhizopus stolonifer* were studied in laboratory of Plant Pathology, National Research Centre (NRC), Cairo, Egypt by using some physical and chemical treatments as follow:-

I-Physical treatment

I.1-Aspergillus flavus

I.1.a-Heat treatment:

Data show in Table (1) that, all hot water treatments were found to reduce significantly the number of spores' viability of *Aspergillus flavus* fungus. The numbers of spore viability were decreased with increasing the temperature degree used. Spores viability of *A. flavus* was found to be decreased from $51x10^{-2}$ to 24 when treated with hot water treatment at 45^0c for 5min and loosed 27 equal 47.06% of spores viability and 52.94 % reduction and reduced from $51x10^{-2}$ with un-treated (control) to 19 spores when treated with hot water treatment at 50^0c for 5min and loosed 38 spores equal 25.49% of spores viability and 74.51% reduction. Also, enhanced effect was recorded when treated the spores of A. *flavus* with hot water treatment at 55^0c for the same time which decreased from $51x10^{-2}$ to 8 and loosed 43 spores equal 15.69% of spore viability with 84.31 % reduction.

I.1.b-Pasteurization and Sterilization treatments:

Also, data in Table (1) presented that, both pasteurization and sterilization treatments have a great effect on spore viability of *A. flavus* fungus. Both pasteurization and sterilization were highly significant and gave completely inhibition of spore viability for *A. flavus* with hundred percent of reduction which reduced from ($51x10^{-2}$ spores/ml) to zero with 100% reduction.

Table 1: Effect of heat treatments on reducing spore viability of *A. flavus*

Parameters	Treatments °C					LSD 5%
	H. w.			P.	St.	
	45°C	50°C	55°C	80°C	105°C	
No. Treated colony	24	13	8	0	0	3.36^{s}
No. Loss of colony	27	38	43	51	51	11.21^{s}
% Spore viability	47.06	25.49	15.69	00	00	8.74^{s}
% Reduction	52.94	74.51	84.31	100	100	16.14^{s}
No. of un-treated colony	51 x10-2					

H. w. = Hot water P. =Pasteurization St. =Sterilization ^{S}=significant

I.2-*Aspergillus parasiticus* fungus:-

I.1.a- Heat treatment:

Data in Table (2) shows that, all hot water treatments were found to reduce significantly the total number of spores' viability of the tested *Aspergillus parasiticus* fungus. The numbers of spore viability were decreased significantly with increasing the temperature degree used. Spores viability of *A. parasiticus* was found to be decreased from $64x10^{-2}$ with un-treated (control) to 40 spores when treated with hot water treatment at 45^{0}c for 5min and loosed 24 equal 62.5% of spores viability and 37.5 % reduction and reduced from $64x10^{-2}$ with un-treated (control) to 19 spores when treated with hot water treatment at 50^{0}c for 5min and loosed 45 spores equal 29.69% of spores viability and 70.31% reduction. Also, enhanced effect was recorded when treated the spores of *A. parasiticus* with hot water treatment at 55^{0}c for the same time which decreased from $64x10^{-2}$ to 10 and loosed 54 spores equal 15.63% of spore viability with 84.38 % reduction.

I.2.b-Effect of pasteurization and sterilization treatments:

Data in Table (2) indicated that, both pasteurization and sterilization treatments have a great effect on spore viability of *A. parasiticus* fungus. Data also show that either pasteurization or sterilization were highly significant and gave completely inhibition of spore viability with hundred percent of reduction. In case of *A. parasiticus*, pasteurization and sterilization effected on spore viability which are reduced from ($64x10^{-2}$ spores/ml) to zero with 100% reduction.

Table 2: Effect of heat treatmentson reducing spore viability of *A. parasiticus*

Parameters	Treatments °C					LSD 5%
	H. w.			P.	St.	
	45°C	50°C	55°C	80°C	105°C	
No. Treated colony	40	19	10	0	0	0.15^{s}
No. Loss of colony	24	45	54	64	64	16.34^{s}
% Spore viability	62.5	29.69	15.63	0	0	11.14^{s}
% Reduction	37.5	70.31	84.38	100	100	28.22^{s}
No. of un-treated colony	$64 x10^{-2}$					

H. w. = Hot water P. = Pasteurization St. = Sterilization ^{S}=significant

I.3-*Penicillium digitatum* fungus:-

I.3.a- Heat treatment:

Effect of hot water treatment on reducing spore viability of *P. digitatum* was tabulated in Table (3). Data presented that, hot water used was found to be reduced significantly spore viability of *P. digitatum* from $59 x10^{-2}$ to $39x10^{-2}$ when treated by 45^{0}C for 5minutes which loosed $20x10^{-2}$ spores and gave 66.10% of spore viability with 33.90 reduction percent. Increasing the reduction percent with increasing the degree of temperature used. Hot water treatment at 50^{0}C was found to decrease significantly spore viability of *P. digitatum* from $59 x10^{-2}$ to only one $x10^{-2}$ spore viability and loosed 58 spores with only 1.69% spore viability and 98.31% reduction. Enhanced temperature used was 55^{o}C at the same time which record zero percent of spore viability with *P. digitatum* equal hundred percent of reduction.

I.3.b-Effect of pasteurization and sterilization treatments:

Effect of pasteurization and sterilization treatments on spore viability of *P. digitatum* was studied. Data in Table (3) indicated that, both pasteurization and sterilization treatments have a great effect on spore viability of *P. digitatum* fungus. Data also show that either pasteurization or sterilization were highly significant and gave

completely inhibition of spore viability with hundred percent of reduction. In case of *P. digitatum*, pasteurization and sterilization effected on spore viability which are completely reduced significant from ($59x10^{-2}$ spores/ml) to zero with 100% reduction.

Table 3: Effect of heat treatment on reducing spore viability of *P. digitatum*

Parameters	Treatments °C			P.	St.	LSD
	45°C	50°C	55°C	80°C	105°C	5%
No. Treated colony	39	1	00	00	00	3.16^{s}
No. Loss of colony	20	58	59	59	59	14.17^{s}
% Spore viability	66.10	1.69	0	0	0	1.33^{s}
% Reduction	33.90	98.31	100	100	100	24.37^{s}
No. of un-treated colony	$59 x10^{-2}$					

H. w. = Hot water P. = Pasteurization St. = Sterilization ^{S}=significant

I.4- *P. expansum* fungus:-

I.4.a- Heat treatment:

Effect of hot water treatment on reducing spore viability of *P. expansum* was tabulated in Table (4). Data presented that, hot water used was found to be reduced significantly spore viability of *P. expansum* from $73 x10^{-2}$ to $6x10^{-2}$ when treated by 45^{0}C for 5minutes which loosed $67x10^{-2}$ spores and gave 8.22% of spore viability with 91.78 reduction percent. Also, increasing the reduction percent with increasing the degree of temperature used. Hot water treatments at 50 and 55^{0}C at the same time which record zero percent of spore viability with *P. expansum* equal hundred percent of reduction.

I.4.b-Effect of pasteurization and sterilization treatments:

Effect of pasteurization and sterilization treatments on spore viability of *P. expansum* was studied. Data in Table (4) indicated that, both pasteurization and sterilization treatments have a great effect on spore viability of *P. expansum* fungus. Data also show that either pasteurization or sterilization were highly significant and gave completely inhibition of spore viability with hundred percent of reduction. In case of *P. expansum*, pasteurization and sterilization effected on spore viability which are reduced significantly from ($73x10^{-2}$ spores/ml) to zero with 100% reduction.

Table 4: Effect of heat treatments on reducing spore viability of *P. expansum*

Parameters	Hot water treatments °C			P.	St.	LSD
	45°C	50°C	55°C	80°C	105°C	5%
No. Treated colony	6	00	00	00	00	-
No. Loss of colony	67	73	73	73	73	4.34^{s}
% Spore viability	8.22	00	00	00	00	-
% Reduction	91.78	100	100	100	100	6.24^{s}
No. of un-treated colony	$73 x10^{-2}$					

H. w. = Hot water P. = Pasteurization St. = Sterilization ^{s}=significant - =highly significant

I.5-On *P. italicum* fungus:-

I.5.a- Heat treatment:

Effect of hot water treatment on reducing spore viability of *P. italicum* was tabulated in Table (5). Data presented that, hot water used was found to be reduced significantly spore viability of *P. italicum* from $66 x10^{-2}$ to $40x10^{-2}$ when treated by 45^{0}C for 5minutes and loosed 22 spores then gave 60.61% of spore viability with 39.39 reduction percent. Also, increasing significantly the reduction percent with increasing the degree of temperature used. Hot water treatments at 50 and 55^{0}C at the same time which record zero percent of spore viability with *P. italicum* equal hundred percent of reduction.

Table 5: Effect of heat treatments on reducing spore viability of *P. italicum*

Parameters	Treatments °C			P.	St.	LSD
	45°C	50°C	55°C	80°C	105°C	5%
No. Treated colony	40	00	00	00	00	-
No. Loss of colony	22	66	66	66	66	14.37^{s}
% Spore viability	60.61	00	00	00	00	-
% Reduction	39.39	100	100	100	100	18.21^{s}
No. of un-treated colony	$66 x10^{-2}$					

H. w. = Hot water P. = Pasteurization St. = Sterilization ^{S}=significant - =highly significant

I.5.b-Effect of pasteurization and sterilization treatments:

Effect of pasteurization and sterilization treatments on spore viability of *P. italicum* was studied. Data in Table (5) indicated that, both pasteurization and sterilization treatments have a great effect on spore viability of *P. italicum* fungus. Data also show that either pasteurization or sterilization were highly significant and gave

completely inhibition of spore viability with hundred percent of reduction. In case of *P. italicum*, pasteurization and sterilization effected on spore viability which are completely reduced from ($66x10^{-2}$ spores/ml) to zero with 100% reduction.

I.6-*Rhizopus stolonifier* fungus:-

I.6.a- Heat treatment:

Effect of different hot water treatments on spore viability of *Rhizopus stolonifier in vitro* was recorded in Table (6). Data show that, all hot water treatments were found to reduce significantly the total number of spores' viability of *Rhizopus stolonifier* fungus. The results of obtained data indicated that, the numbers of spore viability were continuing decreased with increasing the temperature degree used. Spores viability of *R. stolonifier* was found to be decreased significantly from 75 $x10^{-2}$ with un-treated (control) to 55 spores when treated with hot water treatment at 45^0c for 5minutes and loosed 20 spores and gave 73.33% of spores viability with 52.94 %reduction, while reduced from 75 $x10^{-2}$ with un-treated (control) to 30 spores when treated with hot water treatment at 50^0c for 5minutes and loosed 45 spores equal 40.0% of spores viability and 60.0% reduction. Enhanced effect was recorded when treated the spores of *R. stolonifier* with hot water treatment at 55^0c for the same time which decreased from 75 $x10^{-2}$ to 9 spores and loosed 66 spores equal 12.0% of spore viability with 88.0 % reduction.

I.6.b-Effect of pasteurization and sterilization treatments:

Pasteurization and Sterilization treatments: Also, effect of pasteurization and sterilization treatments on spore viability of *R. stolonifier* was studied**.** Data in the same table presented that, both pasteurization and sterilization treatments have a great effect on spore viability of *R. stolonifier*. Data also show that either pasteurization or sterilization gave completely inhibition of spore viability with hundred percent of reduction. In case of *R. stolonifier*, pasteurization and sterilization effected on spore viability which are completely reduced significantly from ($75x10^{-2}$ spores/ml) to zero with 100% reduction.

Table 6: Effect of heat treatments on reducing spore viability of *Rhizopus stolonifier*

Parameters	Treatments °C			P.	St.	LSD
	45°C	50°C	55°C	80°C	105°C	5%
No. Treated colony	55	30	9	00	00	12.13 ^{s}
No. Loss of colony	20	45	66	75	75	14.36 ^{s}
% Spore viability	73.33	40	12	00	00	18.14 ^{s}
% Reduction	26.67	60	88	100	100	26.04 ^{s}
No. of un-treated colony	75 $x10^{-2}$					

H. w. = Hot water P. = Pasteurization St. = Sterilization ^{S}=significant

II-Chemical treatment

II.a-Effect of some alternative fungicides in reducing spore viability

II.a.1-On Aspergillus flavus

Data were recorded in Table (7). Data indicated that, all tested alternative fungicides were found to reduce significantly spore viability of *A. flavus* fungus**.** Data also presented that; increased reduction percent with increasing the concentrate of these substance. Benzoic acid substance was the enhanced than the others which record zero percent of reduce spore viability and gave hundred percent of reduction with all the three tested concentrates i. e. 2, 5 and 10%, followed by Sodium carbonate which reduced significantly spore viability of *A. flavus* from $95x10^{-2}$ with un-treated (control) to 74 and loosed 21 spores equal 77.89 percent of spore viability and gave 22.11 percent of reduction at 2%. Sodium carbonate used at 5% was found to be decreased spore viability of *A. flavus* from $95x10^{-2}$ to 32 and loosed 63 spores equal 33.68% of spore viability and gave 66.31% of reduction. Sodium carbonate at 10% used was enhanced which record zero percent of reduce spore viability and gave hundred percent of reduction. Citric acid substance was less affected. Citric acid used at 2% was found to be reduced spore viability of *A. flavus* from $95x10^{-2}$ to 84 of spore viability and loosed 11spores equal 88.42% of spore viability and record 11.58% of reduction.

Table 7: Effect of chemical treatments in reducing spore viability of *A. flavus*

Parameters	% Concentration of Substrate									LSD
Substrates	Benzoic acid			Citric acid			Sodium carbonate			5%
Concentrates	2	5	10	2	5	10	2	5	10	
No. Treated colony	0	0	0	84	69	40	74	32	00	12.36 ^{s}
No. Loss of colony	95	95	95	11	26	55	21	63	95	14.18 ^{s}
% Spore viability	00	000	00	88.42	72.63	42.11	77.89	33.68	00	18.25 ^{s}
% Reduction	100	100	100	11.58	27.37	57.89	22.11	66.31	100	11.07 ^{s}
No. of un-treated colony	95 $x10^{-2}$									

^{S}=Significant

Citric acid used at 5% reduced spore viability of *A. flavus* from 95x10^{-2} to 69 of spore viability and loosed 26 spores equal 72.63% of spore viability and gave 27.37% of reduction while, Citric acid used at 10% was found to be reduced spore viability of *A. flavus* from 95x10^{-2} to 40 of spore viability and loosed 55 spores equal 42.11% of spore viability and record 57.89% of reduction.

II.a.2-On Penicillium digitatum:

Data indicated that, all tested alternative fungicides (i. e. Benzoic acid, Citric acid and Sodium carbonate) were found to reduce significantly spore viability of *P. digitatum* fungus as shown in Table (8). Data also presented that, increased reduction percent with increasing the concentrate of these substances. Benzoic acid substance was the enhanced than the others which record zero percent of reduce significantly spore viability and gave hundred percent of reduction with all the three tested concentrates i. e. 2, 5 and10%, followed by Sodium carbonate which reduced spore viability of *P. digitatum* from 80x10^{-2} with un-treated (control) to 55 and loosed 25 spores equal 68.75% of spore viability and gave 31.25% of reduction. Sodium carbonate at 5 and 10% used were enhanced which recorded zero percent of reduce spore viability and hundred percent of reduction. Citric acid substance was less affected. Citric acid used at 2% was found to be reduced significantly spore viability of *P. digitatum* from 80x10^{-2} to 70 of spore viability and loosed 10 spores equal 87.5% of spore viability and record 12.5% of reduction. Citric acid used at 5% reduced spore viability of *P. digitatum* from 80x10^{-2} to 55 of spore viability and loosed 25 spores equal 68.75% of spore viability and gave 31.25% of reduction while, Citric acid used at 10% was found to be reduced spore viability of *P. digitatum* from 80x10^{-2} to 30 of spore viability and loosed 50 spores equal 37.5% of spore viability and record 62.5% of reduction.

Table 8: Effect of chemical treatments on reducing spore viability of *P. digitatum*

Parameters	% Concentration of Substrate									LSD 5%
Substrates	Benzoic acid			Citric acid			Sodium carbonate			
Concentrates	2	5	10	2	5	10	2	5	10	
No. Treated colony	0	0	0	70	55	30	55	00	00	16.40 ^{s}
No. Loss of colony	80	80	80	10	25	50	25	80	80	19.32 ^{s}
% Spore viability	00	00	00	87.5	68.75	37.5	68.75	00	00	23.14 ^{s}
% Reduction	100	100	100	12.5	31.25	62.5	31.25	100	100	13.22 ^{s}
No. of un-treated colony	80 x10^{-2}									

^{S}=*Significant*

II.a.3-On Penicillium expansum:

Data in Table (9) indicated that, all tested alternative fungicides were found to reduce significantly spore viability of *P. expansum* fungus**.** Data also presented that; increased reduction percent with increasing the concentrate of these substance. Benzoic acid substance was the enhanced than the others which record zero percent of reduce spore viability and gave hundred percent of reduction with all the three tested concentrates i. e. 2, 5 and 10%, followed by Sodium carbonate which reduced significantly spore viability of *P. expansum* from 100x10^{-2} with un-treated (control) to 58 and loosed 42 spores equal 58 percent of spore viability and gave 42 percent of reduction at 2%. Sodium carbonate used at 5% was found to be decreased spore viability of *P. expansum* from 100x10^{-2} to 15 and loosed 85 spores equal 15% of spore viability and gave 85% of reduction. Sodium carbonate at 10% used was enhanced which record zero percent of reduce significantly spore viability and gave hundred percent of reduction. Citric acid substance was less affected. Citric acid used at 2% was found to be reduced significantly spore viability of *P. expansum* from 100x10^{-2} to 75 of spore viability and loosed 25 spores equal 75% of spore viability and record 25% of reduction. Citric acid used at 5% reduced spore viability of *P. expansum* from 100x10^{-2} to 50 of spore viability and loosed 50 spores equal 50% of spore viability and gave 50% of reduction while, Citric acid used at 10% was found to be reduced spore viability of *P. expansum* from 100x10^{-2} to 23 of spore viability and loosed 77 spores equal 23% of spore viability and record 77% of reduction.

Table 9: Effect of chemical treatments on reducing spore viability of *Penicillium expansum*

Parameters	% Concentration of Substrate									LSD 5%
Substrates	Benzoic acid			Citric acid			Sodium carbonate			
Concentrates	2	5	10	2	5	10	2	5	10	
No. Treated colony	00	00	00	75	50	23	58	15	00	11.26 ^{s}
No. Loss of colony	100	100	100	25	50	77	42	85	100	14.50 ^{s}
% Spore viability	00	00	00	75	50	23	58	15	00	11.26 ^{s}
% Reduction	100	100	100	25	50	77	42	85	100	14.50 ^{s}
No. of un-treated colony	100 x10^{-2}									

^{S}=*Significant*

II.a.4-On Penicillium italicum:

Data in Table (10) indicated that, all tested alternative fungicides were found to reduce significantly spore viability of *P. italicum* fungus**.** Data also presented that; increased reduction percent with increasing the concentrate of these substance. Benzoic acid substance was the enhanced than the others which record zero percent of reduce significantly spore viability and gave hundred percent of reduction with all the three tested concentrates i. e. 2, 5 and 10%, followed by Sodium carbonate which reduced spore viability of *P. italicum* from $90x10^{-2}$ with un-treated (control) to 85 and loosed 5 spores equal 94.44 percent of spore viability and gave 5.56 percent of reduction at 2%. Sodium carbonate used at 5% was found to be decreased spore viability of *P. italicum* from $90x10^{-2}$ to 15 and loosed 75 spores equal 16.67% of spore viability and gave 83.33% of reduction. Sodium carbonate at 10% used was enhanced which record zero percent of reduce significantly spore viability and gave hundred percent of reduction. Citric acid substance was less affected. Citric acid used at 2% was found to be reduced spore viability of *P. italicum* from $90x10^{-2}$ to 75 of spore viability and loosed 15 spores equal 83.33% of spore viability and record 16.67% of reduction. Citric acid used at 5% reduced spore viability of *P. italicum* from $90x10^{-2}$ to 50 of spore viability and loosed 40 spores equal 55.56% of spore viability and gave 44.44% of reduction while, Citric acid used at 10% was found to be reduced spore viability of *P. italicum* from $90x10^{-2}$ to 23 of spore viability and loosed 67 spores equal 25.56% of spore viability and record 74.44% of reduction.

Table 10: Effect of chemical treatments on reducing spore viability of *Penicillium italicum*

Parameters	% Concentration of Substrate									LSD 5%
Substrates	Benzoic acid			Citric acid			Sodium carbonate			
Concentrates	2	5	10	2	5	10	2	5	10	
No. Treated colony	00	00	00	75	50	23	85	15	00	11.26 ^{s}
No. Loss of colony	90	90	90	15	40	67	5	75	90	19.02 ^{s}
% Spore viability	00	00	00	83.33	55.56	25.56	94.44	16.67	00	26.13 ^{s}
% Reduction	100	100	100	16.67	44.44	74.44	5.56	83.33	100	17.40 ^{s}
No. of un-treated colony	$90 x10^{-2}$									

^{S}=*Significant*

II.a.5-On Rhizopus stolonifier:

Data indicated that, all tested alternative fungicides (i. e. Benzoic acid, Citric acid and Sodium carbonate) were found to reduce significantly spore viability of *R. stolonifier* fungus as shown in Table (11). Data also presented that; increased reduction percent with increasing the concentrate of these substance. Benzoic acid substance was the enhanced than the others which record zero percent of reduce spore viability and gave hundred percent of reduction with all the three tested concentrates i. e. 2, 5 and10%, followed by Sodium carbonate which reduced significantly spore viability of *R. stolonifier* from $78x10^{-2}$ with un-treated (control) to 55 and loosed 23 spores equal 70.51% of spore viability and gave 29.49% of reduction. Sodium carbonate at 5 and 10% used were enhanced which recorded zero percent of reduce spore viability and hundred percent of reduction. Citric acid substance was less affected. Citric acid used at 2% was found to be reduced spore viability of *R. stolonifier* from $78x10^{-2}$ to 62 of spore viability and loosed 16 spores equal 79.49% of spore viability and record 20.51% of reduction. Citric acid used at 5% reduced significantly spore viability of *R. stolonifier* from $78x10^{-2}$ to 42 of spore viability and loosed 36 spores equal 53.85% of spore viability and gave 46.15% of reduction while, Citric acid used at 10% was found to be reduced spore viability of *R. stolonifier* from $78x10^{-2}$ to 20 of spore viability and loosed 58 spores equal 25.64% of spore viability and record 74.36% of reduction.

Table 11: Effect of chemical treatments on reducing spore viability of *Rhizopus stolonifier*

Parameters	% Concentration of Substrate									LSD 5%
Substrates	Benzoic acid			Citric acid			Sodium carbonate			
Concentrates	2	5	10	2	5	10	2	5	10	
No. Treated colony	00	00	00	62	42	20	55	00	00	16.19 ^{s}
No. Loss of colony	78	78	78	16	36	58	23	78	78	23.52 ^{s}
% Spore viability	00	00	00	79.49	53.85	25.64	70.51	00	00	18.90 ^{s}
% Reduction	100	100	100	20.51	46.15	74.36	29.49	100	100	14.27 ^{s}
No. of un-treated colony	$78 x10^{-2}$									

^{s}=*significant*

II.b- Ozonation

II.b.1-Effect of ozone exposure on spore viability of Aspergillus flavus:

Effect of different times of ozone exposure on spore viability of *A. flavus in vitro* was recorded in Table (12). Data show that, all different times of ozone exposure were found to reduce significantly the total number of spores' viability of *A. flavus* fungus. The results of obtained data indicated that, the numbers of spore viability were continuing decreased with increasing the times period of ozone exposure used. Spores viability of *A. flavus*

was found to be decreased significantly from 325.5x10^{-2} with un-treated (control) to 225 spores when treated with ozone treatment at 4.8% w/w of oxygen for 15 minutes period and loosed 70.5 spores to gave 78.34% of spores viability with 21.66 %reduction, while reduced significantly from 325.5 with un-treated (control) to 235.5 spores when treated with ozone exposure for 30 min period and loosed 90 spores equal 72.35% of spores viability and 27.65% reduction. Enhanced effect was recorded when treated the spores of *A. flavus* with ozone exposure for 45 min which decreased from 325.5 to 205 spores and loosed 120.5 spores equal 62.98% of spore viability with 37.02% reduction.

Table 12: Effect of ozone exposure on spore viability of *A. flavus*

Parameters	Time periods of ozone exposure/ minutes			LSD 5%
	15	30	45	
No. Treated colony	255	235.5	205	18.14 ^{s}
No. Loss of colony	70.5	90	120.5	12.08 ^{s}
% Spore viability	78.34	72.35	62.98	7.37 ^{s}
% Reduction	21.66	27.65	37.02	4.12 ^{s}
No. of un-treated colony	325.5 x10^{-2}			

^{s}=*Significant*

II.b.2-Effect of ozone exposure on spore viability of Pencillium expansum:

Effect of different times of ozone exposure on spore viability of *Pencillium expansum in vitro* was recorded in Table (13). Data show that, all different times of ozone exposure were found to reduce significantly the total number of spores' viability of *P. expansum* fungus. The results of obtained data indicated that, the numbers of spore viability were continuing decreased with increasing the times period of ozone exposure used. Spores viability of *P. expansum* was found to be decreased significantly from 308 with un-treated (control) to 292 spores when treated with ozone treatment at 4.8% w/w of oxygen for 15 minutes period and loosed only 16 spores to give 94.81% of spores viability with 5.19 %reduction, while reduced significantly from 308 with un-treated (control) to 220.5 spores when treated with ozone exposure for 30 min period and loosed 87.5 spores equal 71.59% of spores viability and 28.41% reduction. Enhanced effect was recorded when treated the spores of *P. expansum* with ozone exposure for 45min which decreased from 308 to 175 spores and loosed 133 spores equal 56.82% of spore viability with 43.18% reduction.

Table 13: Effect of ozone exposure on spore viability of *Pencillium expansum*

Parameters	Times period of ozone exposure/ minutes			LSD 5%
	15	30	45	
No. Treated colony	292	220.5	175	35.18 ^{s}
No. Loss of colony	16	87.5	133	12.34 ^{s}
% Spore viability	94.81	71.59	56.82	8.29 ^{s}
% Reduction	5.19	28.41	43.18	4.38 ^{s}
No. Un-treated colony	308 x10^{-2}			

^{s}=*significant*

Discussion

All hot water used i. e. 45, 50 and 55^{0}c were found to reduce the number of spores' viability of all tested fungi i. e. *A. flavus*, *A. parasiticus Pencillium digitatum, P. expansum, P. italicum* and *Rhizopus stolonifer* compared with un-treated (control).Enhanced effect was recorded when treated the spores of all the tested fungi with hot water treatment at 55^{0}c for 5min. The numbers of spore viability were decreased with increasing the temperature degree used. Both pasteurization (at 80°C) and sterilization (at 105°C) treatments have a great effect on spore viability of the tested fungi compared with un-treated juices which gave completely inhibition of spore viability of the tested fungi with hundred percent of reduction.

Lemessa *et al.* (2004) and Irtwange, (2006) reported that, Fruits were passed through hot dips for a few minutes at 49 ºC to kill mold spores on citrus fruit. Also, Zamani *et al.* (2009) reported that, pre–storage hot water dips of fruit at temperatures above 40ºC have been shown to be effective in controlling storage decay, not only by reducing the pathogen but also by enhancing the resistance of fruit tissue, influencing host metabolism and ripening. Postharvest dips are applied for a few minutes at high temperatures, because fungal spores and latent infections of the pathogen are either on the surface or in the first few cell layers under the peel of the fruit. (Porat *et al.,* 2000; Fallik, 2004 and Irtwange, 2006) found that, pre-storage dipping of ' Fortune ' mandarins in water at 50, 52 or 54ºC for 3 min reduced decay and simulated shelf-life at 20ºC without causing adverse effect to the rind surface. Fruits are dipped in water at 50-55ºC for 15 min before storage for control of fungus. Nafussi *et al.* (2001) found that, hot water dip for 2 min at 52–53°C inhibited the development of decay in lemons inoculated with *P. digitatum*. Green mould incidence caused by *P. digitatum* was reduced by hot water (HW)

from 97.9 and 98% on untreated Eureka lemons (*Citrus limon*) and Valencia oranges (*Citrus sinensis*) to 14.5 and 9.4%, respectively. While, short-term heating, where the fruit or vegetable is dipped in hot water at temperatures above 40°C (generally 44-55°C) for a short time (from a few minutes to 1 h). Fruits and vegetables commonly tolerate such temperatures for 5-10 min, and that even shorter exposure to these temperatures is sufficient to control many of the post-harvest pathogens.

Smilanick *et al.,* (2008) reported that, pre-storage application of hot water treatment for a short time (about a few minutes), is only effective on pathogenic agents found on external layers of fruit skin. The results showed that floating of fruits in hot water 55°C for 2 to 3 min caused the control of green mould and improved the post-harvest quality in tangerine. Also Embaby and Hagag, (2014) found that, Immersion of fruits in 55°C hot water for 2 and 3 min and 50°C for 3 min had the best effect in controlling and delaying activity of the tangerine green mold activity. Hot water treatment increased the protective effect of fruits and increased significantly the shelf life of Navel orange fruits. Pre–storage hot water dips of fruit at temperatures above 40ºC have been shown to be effective in controlling storage decay, not only by reducing the pathogen but also by enhancing the resistance of fruit tissue, influencing host metabolism and ripening. Moyer & Aitken (1980) and Rupasinghe and Juan, (2012) stated that, High temperature short time (HTST) treatment could minimize those undesirable quality changes made by batch heating due to the much less duration of heat treatment. Currently, HTST pasteurization is the most commonly used method for heat treatment of fruit juice. For example, orange juice is processed by HTST at 90 to 95°C for 15 to 30 s and apple juice is treated by HTST at 77 to 88°C for 25 to 30 s.

All tested alternative fungicides i. e. Benzoic acid, Citric acid and Sodium carbonate were found to reduce spore viability of all the tested fungi i. e. *Aspergillus flavus, Pencillium digitatum, P. expansum, P. italicum* and *Rhizopus stolonifer* compared with un-treated (control). Benzoic acid substance was enhanced than the others.

Embaby *et al.,* (2013 II &III) and Embaby *et al.,* (2015) found that, all tested alternative fungicides i. e. Ascorbic acid, Benzoic acid, Citric acid and Potassium sorbate were found to be reduced significantly the growth rate of *P. digitatum* and *P. italicum* compared with untreated (control). Data also presented that; Benzoic acid was the most effective than the others followed by potassium sorbate and Ascorbic acid while; citric acid was less. 1500 ppm concentration was more effective on the fungal growth rate than other concentrates. Also, both 1000 and/or 1500 ppm was better conc. for reducing the entire fungal growth rate compared with 500 ppm. *P. italicum* was the most affecting growth rate followed by *P. digitatum* comparing with non-treated control. Data also show that, under room temperature data show that, all alternative fungicides were found to decrease significantly Alternaria rot decay (*Alternaria citri*) compared with untreated control (500 and 1000 ppm). Benzoic acid substance had higher affective than others while, citric acid was less effective. Alternative products such as Sodium Bicarbonate, Sodium Carbonate, Boric Acid, Potassium Sorbate, Sodium Metabisulfite and essential oil of *Cymbopogon citratus* were promising in the control of *Penicillium digitatum* over bitter orange in postharvest.

Tested of ozone exposure on spore viability of *A. flavus* and *P. expansum* show that, all different exposure times i.e. 15, 30 and 45 minutes at ozone concentration in the gas supply was varied (1-4.8% w/w of oxygen) were found to reduce the total numbers of spore' viability for *A. flavus* as well as *P. expansum* compared with untreated control. The numbers of spore viability were continuing decreased with increasing the times period of ozone exposure used.

Raila *et al.,* (2006) reported that, Micromycetes of mold fungi are less resistant than spores to ozone exposure. Activity of mold fungi *A. flavus* can be inhibited by grain exposure to 5 ppm ozone concentration. Ozone was used also for detoxification of food products infected by mycotoxins. Mson *et al.,* (2009) found that, radial groth of *A. flavus* and *F. moniliforme* in agar media was inhibited for the first 2 days. Hyphal growth and sporulation were both completely inhibited by an ozone environment. Aflatoxin production by *A. flavus* was also reduced by more than 97%in ozone exposed cultures. Wang *et al.,* (2010) and Torres *et al.,* (2011) stated that, ozone, or tri-atomic oxygen (o_3), is a powerful disinfectant and oxidizing agent. It has been considered as a generally recognized as safe (GRAS) substance and used in a number of applications in the food industry for destruction or detoxification of chemicals or microorganisms. Ozone gas also could effectively degrade mycotoxins in solution or in vitro (present as pure standards), such as aflatoxins, cyclopiazonic acid (CPA), fumonisin B_1, ochratoxin A (OTA), patulin, secalonic acid D (SAD) and zeralenone (ZEN) and degradation products are generally harmless. As a powerful oxidant, ozone has been successfully used for controlling stored product fungi and pests.

Conclusion:

Contaminated food and juices are the source of various food borne conditions due to gastroenteritis in human. The consumption of food and juices could have both positive and negative effect on the part of consumers. Today the emphasis is on total quality of food and juices which means that not only food should be nutritionally balanced but should be microbiologically safe too. The various sources through which microorganisms gain entry into the foods are micro-flora present in soil, water, air, present on plant and plant products, present on food utensils and equipments, present in animal feeds, present on animal hides, present in

intestinal tracts of humans and animals and food handlers. Microbiological problems may occur at all stages in the production. Prevention and control of mycoflora spoilage contaminated some fresh juices by some physical and chemical technologies are more strategies. Physical methods include heating, pasteurization and sterilization which more safe and chemical technologies include organic acids and salts which widespread application and safe too for preventing spoilage of fresh juices include i. e. Benzoic acid, Citric acid and Sodium carbonate in addition ozone which is lack of toxic residues makes it a favorable environment friendly sanitizer.

References

Achen, M. and A.E. Yousef, 2001. Efficacy of Ozone against *E. coli* O157:H7 on apples. Food microbiology and safety vol: 66; No 9.

Ammar, M. I. and M. A. El-Naggar, 2014. Screening and Characterization of Fungi and their associated Mycotoxins in some Fruit Crops. International Journal of Advanced Research, Volume 2, Issue 4, 1216-1227.

Barth, M. M., C. Zhou, J. Mercier, and F. A. Payne, 1995. Ozone storage effects on anthocyanin content and fungal growth blackberries. J. Food Sci 60:1286–1288.

Brul, S. and P. Coote, 1999. Preservative agents in foods. Mode of action and microbial resistance mechanisms. International Journal of Food Microbiology, 50, 1–17.

De Donno, A., M.T. Montagna, R. Erroi, D. Liaci, S. Sanapo, and G. Caggiano, 1998. Food products and fungal contamination. Note II. Study on moulds presence in pasteurized and fresh fruit juices. Journal of Preventative Medicine and Hygiene; 39: 71-73.

Embaby, E. M. and L. F. Hagag, 2014. Citrus fruit decay and its control. Journal of Agricultural Technology, Vol. 10(5):1273-1288.

Embaby, E. M., M. Hazaa, L. F. Hagag, T. I. El-Sayed and F. S. Abd El-Azem, 2013, II: Decay of Some Citrus Fruit Quality Caused by Fungi and their Control: II- Control Alternaria rot or core rots decay by using some alternative fungicides. Journal of Applied Sciences Research 9(11):5671-5678.

Embaby, E.M., M. Hazaa, L. F. Hagag, T. I. El-Sayed and F. S. Abd el-Azem, 2013, III: Decay of Some Citrus Fruit Quality Caused by Fungi and their Control: III- Control Blue and Green mould decay by using some alternative fungicides. Journal of Applied Sciences Research 9(8):5086-5096.7. 8. 2.

Embaby, E. M., Nadia M. Awni, Mona M. Abdel-Galil and Heba I. El-Gendy, 2015. Mycoflora and Mycotoxin Contaminated some Juices. Journal of Agricultural Technology 11(3): 693-712.

Ester, P., V. M. Liliana, G. C. Jesús and L. Joan Maria, 2014. Toxicology Unit, GRET-CERETOX, INSA-UB, Food and Nutrition Torribera Campus, University of Barcelona, Spain; 2Biothani Europe. S.L: Can Lleganya, SantFeliu de Buixalleu, Spain.

Fallik, E., 2004. Hot water treatments for control of fungal decay on fresh produce. Microbiology of Fruits and Vegetables. (Sapers, G.M., Gorny, J.R. and Yousef, A.E. Ed). CRC Press, Inc., Boca Raton, Florida.

Farber, J. N., L. J. Harris, M. E. Parish, L. R. Beuchat, T. V. Suslow and J. R. Gorney, 2003. Microbiological safety of controlled and modified atmosphere packaging of fresh and fresh-cut produce. In Comprehensive reviews in food science and food safety (Vol. 2, pp. 142–160.

Fasoyiro, S. B., A. O. Ashaye, A. Adeola and F. O. Samuel, 2005. Chemical and storability of fruits flavoured (*Hibiscus Sabdariffa*) drinks. World J. Agric Sci. 1: 165-168.

Fatemi, S. and B. Hassan, 2011. The effect of physical treatments on control of *Penicillium digitatum* decay orange cv. Valencia during storage period. African Journal of Agricultural Research, 6 (26): 5757-5760.

Irtwange, S. V., 2006. Hot water treatment: A non-chemical alternative in keeping quality during postharvest handling of citrus fruits. Agricultural engineering international: the CIGR Ejournal. Invited Overview, pp. 5.

Kamal R. A., R. Dhiman, N. K. Aggarwal, V. Kumar and M. Kaur, 2014. Microbes Associated with Freshly Prepared Juices of Citrus and Carrots. International Journal of Food Science Volume, Article ID 408085, 7 pages.

Kim, J. G., A. E. Yousef and G. W. Chism, 1999. Use of ozone to inactivate microorganisms on lettuce. J Food Safety19:17-34.

Koc, A. N., S. Silici, M. S. Fatma and S. Osman, 2007. Antifungal Activity of Propolis in Four Different Fruit Juices. Food Technol. Biotechnol. 45 (1) 57–61.

Lemessa, F., W. Shunli, J. Agravante, C. Amuoh, and F. Kiyimba, 2004. Effect of hot water treatment on the quality of citrus fruit. Unpublished Group Project Report. International Research and Development Course in Postharvest Biology and Technology, the Volcani Center, Israel. Postharvest Biol. Technol, 18:235-244.

Mason, L. J., C. P. Woloshuk, F. Mendoza, D. E. Maier and S. A. Kells, 2009. Ozone: A new control strategy for stored grain. 9th International Work Conference on Stored Product Protection, 904-907.

Moyer, J. C. and H. C. Aitken, 1980. Apple juice. In P. E. Nelson & D. K. Tressler (Eds.), Fruit and vegetable juice processing technology (pp. 212-267). Westport: AVI.

Nafussi B., S. Ben-Yehoshua, V. Rodov, J. Peretz, B.K. Ozer, G. D'hallewin, 2001. Mode of action of hot-water dip in and reducing decay of lemon fruit. J. Agric. & Food Chem, 49(1):107-113.

Naidiu, A. S., 2000. Natural food antimicrobial systems overview. In: Naidiu AS ed. Natural Food Antimicrobial Systems. Boca Raton: CRC Press. pp 1–14.

Neeraj, D. and S. Sharma, 2007. Food and industrial microbiology; Food spoilage, food infections and intoxications caused by microorganisms and methods for their detection. Products. 17th Edition. American Public Health Association, Pp. 42

Oranusi, U. S., W. Braide, and H. O. Nezianya, 2012. Microbiological and chemical quality assessment of some commercially packed fruit juices sold in Nigeria. Greener Journal of Biological Sciences; 2(1): 001-006.

Plaza P., J. Usall, R. Torres, M. Abadias, J. L. Smilanick, I. Viñas, 2004. The use of sodium carbonate to improve curing treatments against green and blue moulds on citrus fruits. Pest Manag Sci. 60: 815–821.

Porat, R., A. Daus, B. Weiss, L. Cohen, E. Fallik, and S. Droby, 2000. Reduction of post-harvest decay in organic citrus fruit by a short hot water brushing treatment. Post-harvest Biology and Technology 18:151–157.

Rahman, T., S. Hasan, and R. Noor, 2011. An Assessment of Microbiological Quality of Some Commercially Packed and Fresh Fruit Juice Available in Dhaka City: A Comparative Study. Stamford Journal of Microbiology, Vol. 1, Issue 1, 13-18.

Raila, A., S. Dainius, R. Marija, S. Ausra, and Z. Egidijus, 2006. Investigation of physical prevention means to reduce mycological contamination of grain surface. Ekologija. Nr. 3. P. 88-95.

Rupasinghe, V. H. and Y. L. Juan, 2012. Emerging Preservation Methods for Fruit Juices and Beverages, Food Additive, Yehia El-Samragy (Ed.), ISBN: 978-953-51-0067-6, InTech, Available from: http://www.intechopen.com /books/food-additive/emerging preservation-methods-3-for-fruit-juices-and beverages.

Sant'Ana, A. S., A. Rosenthal, and P. R. de Massaguer, 2008. The fate of patulin in apple juice processing: A review. Food Research International, Vol.41, No.5, pp.441-453, 0963-9969.

Sant'Ana, A.S., A. Rosenthal and P. R. Massaguer, 2009. Heat resistance and effects of continuous pasteurization on the inactivation of *Byssochlamys fulva* ascospores in clarified apple juice. Journal of Applied Microbiology 107, 197–209.

Sapers, G. M., J. R. Gorney and A. E. Yousef, 2005. Microbiology of fruits and vegetables. Boca Raton, Fl: CRC Press.

Sengun I. Y., D. B. Yaman and S. A. Gonul, 2008. Mycotoxins and mould contamination in cheese: a review. World Mycotoxin Journal, August; 1(3): 291-298.

Smilanick, J. L., M. Mansour, F. M. Gabler and D. Sorenson, 2008. Control of citrus postharvest green mold and sour rot by potassium sorbate combined with heat and fungicides. Postharvest Biol. Technol. 47, 226–238.

Snedecor, G.W. and W. G. Cochran, 1989. Statistical methods.7th Ed. Lowa state Univ. Press. Ames Iowa, USA.

Tasnim, F., A. M. Hossain, S. Nusrath, K. M. Hossain, D. Lopa, and F. K. M. Haque, 2010. Quality Assessment of Industrially Processed Fruit Juices Available in Dhaka City, Bangladesh. Mal J. Nutr. 16 (3): 431-438

Torres, B., B. K. Tiwari, A. Patras, H. W. Hilde, N. Brunton, P. J. Gullen, and C. P. Donnell, 2011. Effect of ozone processing on the color, rheological properties and phenolic content of apple juice. Food chemistry, 124, 721-726.

Tournas, V. H., J. Heeres, and L. Burgess, 2006. Moulds and yeasts in fruit salads and fruit juices. Food Microbiology, vol. 23, no. 7, pp. 684–688.

Wang, S., H. Liu, J. Lin, and Y. Cao, 2010. Can ozone fumigation effectively reduce aflatoxin B_1 and other mycotoxins contamination on stored grain? 10th International working conference on stored product protection. Julius-Kuhn-Archiv, 425, 582-589.

Xu, L., 1999. Use of ozone to improve the safety of fresh fruits and vegetables. Food Technol 53:58–62.

Zamani, M., T. A. Sharifi, M. Ahmadzadeh, V. Housseininaveh, and Y. Mostofy, 2009. Control of *Penicillium digitatum* on orange fruit combining Pantoea Agglomerans with hot sodium bicarbonate dipping. Australisian Plant Pathology 37:605-608.