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Mycoflora and Mycotoxin Contaminated some Juices

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Fruit juices are popular drinks as they contain antioxidants, vitamins, and minerals that are essential for human being and play important role in the prevention of heart diseases, cancer, and diabetes. Consumption of fresh juices increased dramatically due to their freshness, high vitamin content, and low caloric consumption. Fruit and vegetable markets are known to contain several species of fungi. A total of 60L juice samples were examined for their microbiological association. Ten fungal species including seven fungal genera were isolated and identified from the three types of fresh juices survied i. e. apple, grape-fruit and navel orange. These are *Alternaria citri, Aspergillus flavus, A. parasiticus, Botryodiplodia theobromae, Colletotrichum gloeosporioides, Fusarium* sp., *Penicillium expansum, P. digitatum, P. italicum* and *Rhizopus stolonifer*. Tested of mycotoxin production indicated that, *A. flavus* (No. 20) was found to be produced only 0.10 ng/ml of aflatoxin AFB_1, *A. parasiticus* (No. 4) produced 0.52 ng/ml of aflatoxin AFB_1, 0.10 ng/ml of aflatoxin AFB_2, 0.32 ng/ml of aflatoxin AFG_1 and 0.10 ng/ml of aflatoxin AFG_2. Decontamination of fungi resulted that, all hot water treatments i. e. 45, 50 and 55^0c were found to reduce the number of spores' viability of the tested fungi (*A. flavus, A. parasiticus* and *P. expansum*) compared with un-treated. The numbers of spore viability were decreased with increasing the temperature degree used. Both pasteurization (at 80°C) and sterilization (at 121°C) treatments have a great effect on spore viability of the three tested fungi compared with un-treatedjuices which gave completely inhibition of spore viability of the three tested fungi with hundred percent of reduction.

Kay word: Fresh juice, Apple, Grapefruit, Navel orange, Fungi, Mycotoxin

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Introduction

Fruit juices are popular drinks as they contain antioxidants, vitamins, and minerals that are essential for human being and play important role in the prevention of heart diseases, cancer, and diabetes. They contain essential nutrients which support the growth of acid tolerant bacteria, yeasts, and moulds**.** Consumption of fresh juices increased dramatically due to their freshness, high vitamin content, and low caloric consumption. Extracted juices from fruits contain most substances which are found in the original ripe and sound fruit from which the juice is made. The high potassium and low sodium characteristic of most juices help in maintaining a healthy blood pressure. Vitamin C is naturally present in juices which are essential for the body to form collagen, cartilage, muscle, and blood vessels. It also helps in the absorption of iron **(Rathnayaka, 2013** and **Kamal, et al., 2014).**

Apple (*Malus domestica*) is a highly nutritious fruit containing essential food elements such as carbohydrates, protein, fat and water. Apart from its energy value, apple is a good source of soluble and insoluble fiber (**Herforth, 2000**). Apples are a widely consumed, rich source of phyto-chemicals; epidemiological studies have linked the consumption of apples with reduced risk of some cancers, cardiovascular disease, asthma, and diabetes due to strong antioxidant activity **(Marchand et al., 2000).**

The most important of the citrus fruits (*Citrus* sp.) (Family Rutaceae or orange family) are commonly eaten fresh, juiced, and in processed products. Citrus fruits are acidic fruits and they are well known for their health promoting compounds. Citrus is a good source of vitamin C (L-ascorbic acid). Citrus fruits also contain an impressive list of other essential nutrients, including glycaemic and non-glycaemic carbohydrates (sugars and fiber), potassium, folate, calcium, thiamin, niacin, vitamin B6, phosphorus, magnesium, copper, riboflavin, pantothenic acid and a variety of phytochemicals. In addition, citrus contains no fat or sodium and, being a plant food, no cholesterol. The rind, which contains numerous oil glands, and the fragrant blossoms of some species are also a source of essential oils used for perfumes and as a flavoring agent in foods. The most important use of citrus for processing is the production of frozen concentrated orange juice **(Milind, 2008).**

Fruits contain high levels of sugars and nutrients and their low pH values make them vulnerable to fungal decay**.** It has been estimated that about 20-25% of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries**.** Plant pathogens may infect fruits either prior to harvest under field conditions or after harvest during transit and storage. Many pathogens may remain dormant for varying periods until favorable conditions

become available for their development, leading to visible symptoms **(Droby, 2006, Zhu, 2006** and **Singh,** and **Sharma 2007)**. It should be noted that for a total of 100,000 fungi, less than 10% are pathogenic for plants and around 100 species are responsible for the majority of postharvest damage **(Singh and Sharma, 2007).**

The common postharvest and storage fungi of fruits are *Alternaria* spp., *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp. **(Bhale, 2011).** The major mycotoxin-producing fungi are not aggressive pathogens in plants; however, mycotoxins are produced by several genera in plants during the growing season when portals of entry are provided and environmental conditions are appropriate and be continued or initiated in postharvest and stored products**.** The majority of these toxins are produced by fungi of the genera, *Aspergillus, Penicillium* and *Fusarium***(Barkai-Golan** and **Paster, 2008** and **Ammar, and El-Naggar, 2014)**.

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)**. The surface of apple fruits harbors microorganisms depending upon the mechanical handling of the fruits. Microbes can adhere to surface, invade/penetrate fruits surface and multiply within the tissue. Contamination could be from human handling, transport vehicles, insects, dust, and rinse water, harvesting equipment, soil, faeces, irrigation water, water used to apply fungicides and insecticides, manure, wild and domestic animals **(Buck et al., 2003)**. Besides nutritional merits, apple may get contaminated during growth, harvest, transportation and further processing & handling with microbes from soil, air, water or animal wastes. Handling in stores and retail markets could also add more microorganisms to the surface of the fresh produce. Among microbes, *Penicillium expansum*, a fungus is a major causative agent of post harvest decay in apple, since it produces patulin, a mycotoxin known to cause harmful effects in humans **(Chen et al., 2004).** The mycotoxin patulin is a secondary metabolite produced by different species of fungi in the genera *Penicillium, Aspergillus, Gymnoascus, Paecilomyces* and *Byssochlamys. P. expansum* is the most important patulin producer **(Pitt & Hocking, 1997).** Although patulin can occur in several fruits, grains and other foods infected by fungi, the main concern are with apples, apple cider and apple juice due to their higher consumption. As mentioned before, patulin can be found in human food and beverages, animal feed, biological and environmental samples **(Begum, 2012).**

The post-harvest diseases caused to about 50% losses in citrus fruits stored in poor storage conditions, especially under high humidity. The most important

fungi causing the post-harvest diseases include: *Penicillium* spp., *Aspergillus* spp., *Alternaria* spp., *Botrytis cinerea, Monilinia lax* and *Rhizopus stolonifer***(Agrios, 2005).** Most post-harvest pathogens are weak such as (*Penicillium, Alternaria, Diplodia* and *Phomopsis*) and invade through wounds and when the host defense is weak. Citrus fruits have a pH lower than 4, so most of the fungi attack these fruits **(Palou et al., 2001; Milind, 2008 and Zamani et al., 2009).**

Thermal processing is the most widely used technology for pasteurization of fruit juices and beverages. Juice pasteurization is based on a 5-log reduction of the most resistant microorganisms of public health significance **(USFDA 2001** and **Rupasinghe, and Juan, 2012).** Fruit juice has been traditionally pasteurized by batch heating at 63-65°C for relatively long time **(D'Amico et al. 2006** and **Rupasinghe, and Juan, 2012).** High temperature short time (HTST) pasteurization is the most commonly used method for heat treatment of fruit juice. 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 **(Moyer & Aitken 1980** and **Rupasinghe, and Juan, 2012).** Sterilizing in an autoclave at 121°C for 20 minutes **(Rupasinghe, and Juan, 2012).**

In Egypt and under local markets, there is relatively little information related to the natural occurrence of fungi and mycotoxins in fruits**.**The investigation aimed to survey the fungal plant diseases in some fruit juices (freshly and pasteurized prepared juices i. e. apple, grape-fruit and navel orange) and their possibility of mycotoxins secretion that may be harmful to humans and its control.

Material and Method

Sample collection:

Fresh juice of washington navel orange,(*Citrus sinensis* L.), grapefruits, (*Citrus paradisi* Macf) and apple fruits (*Malus domestica*) as well as pasteurized apple and citrus juices which were used for this work were collected from different supermarket in two different localities i. e. Kalubia and Gharbia Governorates, Egypt. Freshly ripe of apple and citrus fruits (grape-fruit and washington navel orange) were collected in polythene bags and stored at 4°C till used in the laboratory, Microbiology Department, fac. of Sciences, Zagazige University, for further work.

1-Juice preparation for analysis:

Three juices belong to the commonly citrus fruit consumed such as navel orange, and grapefruits as well as apple fruits were selected for microbiological study. Each sample was washed, peeled, and cut into slices,

crushed by a processor to obtain the fruit juices and juice were extracted using the sterile stainless steel blender, then filtered by 1 mm^2 sieve made of stainless steel to obtain 30 L of juice. Concentrated apple and orange juices were diluted with a ratio of 1:1. 2:l of the juice and poured into sterile beaker then left in room temperature for 5 days to elevate the microorganism count in the specimen according to **Torkamani,** and **Niakousari, (2011)**and**Kamal, et al., (2014).**

2-**Microbiological analysis:** Microbial contaminant of apple and citrus juices were done by serial dilution agar plate technique. Ten mL of juice sample was diluted with 90mL of sterilized water **(Tournas, et al., 2006).** One milliliter of fresh and pasteurized of apple and citrus juices sample dilutions was pipetted to Petri dishes containing PDA supplemented with antibiotic (at pH 5.5) for enumeration of fungi in duplicates. These plates were then incubated at 25± 2°C for five days. All tests were performed and the total count experiments were expressed as colony forming units per 1 ml of the sample (CFU/ml) **(Torkamani,** and **Niakousari, 2011**and **Iva Dolezalkova, et al., 2012).** Mould isolates were purified by single spore isolation and maintained on potato dextrose agar, further sub cultured on slants for further study and microscopic examination. Moulds were identified on the basis of morphological and cultural characteristics according to the methods described in "Fungi and Food Spoilage **(Kamal, et al., 2014** and **Vermani, et al., 2014)** These fungi were observed under a microscope and identification in Plant Pathology Departmeny, National Research Centre, Cairo, Egypt with the help of available literature by **Raper, and Fennell, (1965), Ainsworth, et al., (1972),Biligrami et al., (1991), Barnett and Hunter, (1999) Grizzle, (2006), Singh, (2009).** Different frequencies of fungi were noted.

3-Mycotoxin production: The different fungal isolates were propagated as pure culture in 100 ml SMKY broth (Sucrose 200 g, $MgSO_4$ $7H_2O$ 0.5 g, KNO_3 3 g, yeast extract 7 g) for 10 days. All fungal isolates incubated in dark condition at 25±2°C.

3.a-The determination of aflatoxins was carried out by using HPLC according to (AOAC, 2007). The HPLC instrument used was waters (474) system, equipped with quaternary pump. The fluorescence detector system was set at 360 nm excitation and 440 nm emission wavelengths. The chromatography column was phenomenex c18 (250x 4.6 mm), 5 µm. The mobile phase system (H_2O: MeOH: CH_3CN, 30:60:10 v/v/v) was isocratically at flow rate of 1 ml /min. The data were collected and integrated using Totalchrom Navigator Chromatography Manager Software. according AOAC**, (2007); Han, et al., (2004)** and **Embaby, et al., (2007** and **2012).**

3.b-Fusarium mycotoxins assay: The samples were analyzed using the Fumonisin test (FB_1) by using HPLC, **(Ammar, and El-Naggar, 2014).**

3.c-Penicillium mycotoxins assay:Patulin in apple juice was tested by Then Layer Chromatography (TLC) with the advantages of being simple and low cost. One official method of the Association of Official Analytical Chemists **(AOAC)**for the analysis of mycotoxins **(2007)** uses TLC followed by silica gel column chromatography as sample preparation procedures. The analysis was carried out in silica gel plates, using, for detection, the reaction of patulin with 3-methyl-2-hydrazone benzothiazolinone and HCl (MBTH). The limit of detection (LOD) was described as 20 µg/L **(Shephard & Leggott, 2000).** Alternative detection and quantification methods were described using fluorodensiometry, or absorbance–transmittance followed by densitometry at 275nm. Reversed phase TLC was despite the patulin elution with a wide variety of solvents **(Shephard & Leggott, 2000).**

Penicillium isolate were examined to produce Patulin. To extract patulin from apples, A small portion of apple was blended for 2 min using a high speed homogenizer in a C_{18}-bonded silica solid support and filtered using glass filter paper. Patulin was extracted from homogenized filtrate using acetonitrile: water (5:95 v:v) solution. The solvent was then evaporated at 35°C under vacuum. The dried residues were dissolved in 1 ml of acetonitrile: water (5:95 v:v) solution according to **Begum, (2012) and Ammar, and El-Naggar, (2014).**

4-Effects of heat treatments on germination (viability)*in vitro*:-

4.a-Hot water treatment: Sterile glass tubes containing 1.8 ml distilled water were placed in water baths at 45, 50, and 55°C, and allowed to equilibrate for 5 min. afterwards; 0.2 ml of a concentrated spore suspension was added to the tubes, to achieve a final concentration of 2 x 10^5spore's 1mlL. After 5min., tubes were removed from the water baths and placed immediately on ice. Aliquots of the spore suspensions were transferred to Potato Dextrose Agar (PDA), according to **Plaza, *et. al.*, (2004), Fatemi and Borji (2011)** and **Embaby** and **Hagag (2014).** All Petri dishes were incubated for 48 or 72 h at 25±°C in darkness.

4.b- Pasteurized apple and citrus fresh juices under agitation to 80°C **De Donno, et al., (1998)** and **Sant'Ana, et al., (2009 & 2010).** Orange juice is processed by High temperature short time (HTST) at 90°C for 30 s. And apple juice is treated by High temperature short time (HTST) at 80°Cfor 30 s according to **Moyer &Aitken (1980)** and **Rupasinghe, and Juan, (2012)** then

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transferred to Potato Dextrose Agar (PDA)**.** All Petri dishes were incubated for 48 or 72 h at 25±°C in darkness.

4.c- Sterilizing apple and citrus fresh juices in an autoclave at 121°C for 20 minutes then transferred to Potato Dextrose Agar (PDA)**.** All Petri dishes were incubated for 48 or 72 h at 25±°C in darkness. The numbers of colony-forming were counted after 3 days of incubation. and the number of viable spores was calculated for each treatment compared with un-treated (as control).

Results and discussions

1-Mycoflora analyses of juices

a-Isolation: Total fungal isolates associated with some Fresh and Pasteurized juices were tabulated in Table (1). Data in this table show that, fungal isolation yielded Three hundred and eighteen fungal colonies were isolated from all the three tested of fresh juices i. e. Apple, Grape fruit and Navel orange and some yeast. Grape-fruit juice was higher total fungal count compare with others, which record 161 fungal colonies equal 50.63% followed by Navel orange juice which gave 126 fungal colonies equal 39.62%. Apple juice was less total fungal count and gave 31 fungal colonies equal 9.75%. On the other hand the same table indicated that, no any fungal colonies were appeared when diluted the same pasteurized juices (Apple, Grape-fruit and Novel-orange) on the same medium. **Droby, (2006) and Zhu, (2006)** reported that, plant pathogens may infect fruits either prior to harvest under field conditions or after harvest during transit and storage. It is estimated that about 20-25% of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries.

Table (1): Percentage of total fungal isolates from different juice samples

	Type of juices						Total
	Apple		Grape-fruit		Navel orange		
	F	P	F	P	F	P	
Number of samples	10	10	10	10	10	10	60
Total fungal isolates	31	NF	161	NF	126	NF	318
%	9.75	00.00	50.63	00.00	39.62	00.00	100.00

F = Fresh juice P = Pasteurized juices NF=Not found

b-Fungal frequency: Data in Table (2) presented that, seven fungal genera belong to nine fungal species were isolated and identified from the three tested of fresh juices i. e. Apple, grapefruit and navel orange **Fig. (1)**. These are

Alternaria citri, Aspergillus flavus, A. parasiticus, Botryodiplodia theobromae, Colletotrichum gloeosporioides, Fusarium sp., *Penicillium expansum, P. digitatum, P. italicum* and *Rhizopus stolonifer*. Also, data in this table show that *P. italicum* was the most fungal frequency which record 37.42% followed by *P. digitatum* 22.64%, *B. theobromae* 22.01%, *Rhizopus stolonifer* 5.97%, *Alternaria citri* 4.40%, *P. expansum* 3.77%, *Aspergillus flavus* record 1.59%, *A. parasiticus* 1.26% and *Colletotrichum gloeosporioides* 0.63%. Less fungal frequency was recorded with *Fusarium* sp. fungus which gave 0.31%.

Nunes et al., (2010) and Munoz et al., (2011) reported that, green and blue moulds, due to the pathogenic action of *Penicillium digitatum* and *Penicillium italicum* respectively, are the main causes of citrus fruit losses during postharvest. *Penicillium expansum* causes a soft rot of apples. This particular species has a special significance because of its ability to produce the mycotoxin patulin which has been detected as a contaminant in unfermented apple juices but not in cider. Commercially produced and properly handled bread generally lacks sufficient amounts of moisture to allow for the growth of any microorganisms except moulds. One of the most common is *Rhizopus stolonifer*, of the referred to as the "bread mould".

Colletotrichum acutatum and *C. gloeosporioides* (Penz.) Penz. & Sacc. are the two members of the genus that are most commonly associated with fruit rots in the literature. *Colletotrichum gloeosporioides* is considered a cumulative species and is found on a wide variety of fruits, including apple, almond, avocado, citrus, mango, olive, and strawberry **(Arauz, 2000; Embaby, and Abd-Ellatife 2013 and Embaby, et al., 2014)**. The *Colletotrichum acutatum* and *C. gloeosporioides* caused anthracnose in a wide range of hosts found mainly in temperate, subtropical and tropical areas, which causes anthracnose and blight in agriculturally important hosts including many dicotyledonous plants such as apple, strawberry, citrus, and stone fruits **(Talhinhas et al., 2005; Lakshmi, et. al., 2011; Embaby,** and **Abd-Ellatife 2013** and **Embaby, et al., 2014).**

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Table (2): Percentage of fungal frequency contaminated some fresh juices

Fungal isolates	Type of juice			
	Apple	Grape-fruit	Navel-orange	Total
Alternaria citri	00	00.00	4.40	4.40
Aspergillus flavus	1.59	00.00	00.00	1.59
A. parasiticus	1.26	00.00	0.00	1.26
B. theobromae	0.63	21.38	0.00	22.01
C. gloeosporioides	0.63	00.00	0.00	0.63
Fusarium sp.	0.31	0.00	0.00	0.31
P. expansum	3.77	0.00	0.00	3.77
P. digitatum	0.00	10.06	12.58	22.64
P. italicum	0.00	16.67	20.75	37.42
R. stolonifer	1.59	2.52	1.89	5.97
Total	9.75	50.63	39.62	100.00

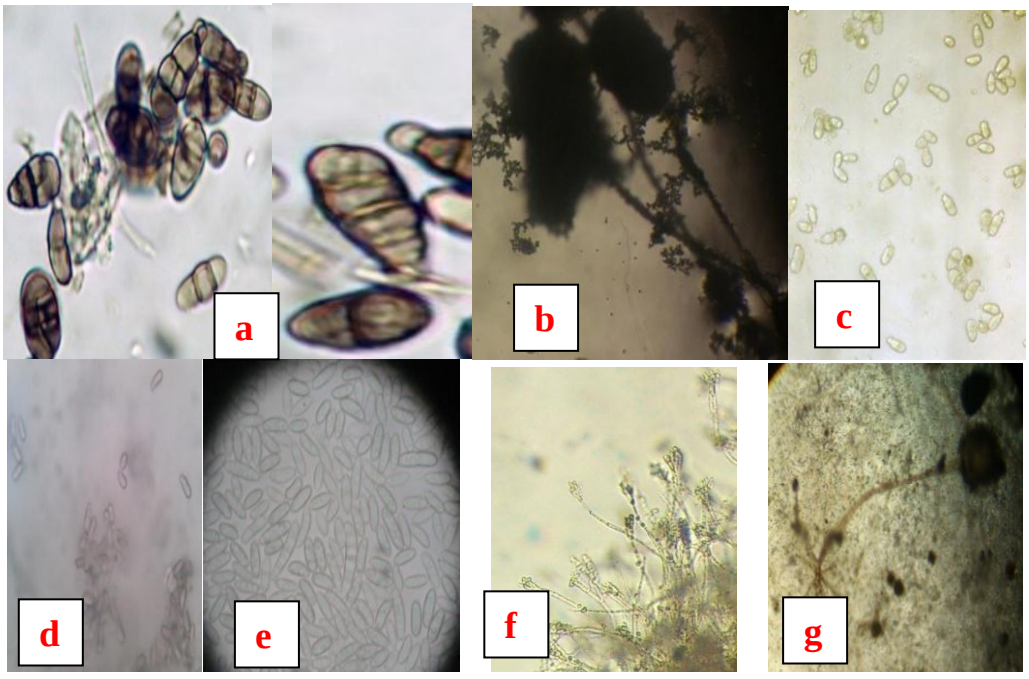

Fig. (1): See seven fungal genera which were isolated and identified from different fresh juices i. e. *Alternaria* (a), *Aspergillus* (b), *Botryodiplodia* (c), *Colletotrichum* (d), *Fusarium* (e), *Penicillium* (f) and *Rhizopus* (g) respectively

2-Mycotoxin production: All the obtained mycoflora i. e. *Alternaria citri*, *Aspergillus flavus*, *A. niger*, *A. parasiticus*, *A. terries*, *Fusarium* sp., *Penicillium degetatum*, *Penicillium italicum* and *Penicillium expansun* were tested for producing mycotoxin(s) by using thin layer chromatography (TLC). Data were recorded in **Table (3).** Data in this table show that, only one isolate of *Aspergillus flavus* (No. 20) which isolated from apple fresh juice Gharbia sample gave positive producer of Aflatoxin. Also, only one isolate of *Aspergillus parasiticus* (No. 4) isolated from apple fresh juice Gharbia sample gave positive producer of Aflatoxin. Neither *A. niger* nor *A. terries* produced any Aflatoxin and record negative reaction. **Kakde, and Kakde (2012)** reported that**,** molds, which are of importance in food because of potential mycotoxin production, include members of the genera *Aspergillus*, *Trichothecium*, *Fusarium*.**Drusch, and Ragab, (2003)** and **Monso, (2004)** Stated that, some potent fungal toxins like aflatoxins, ochratoxinA, patulin have been detected in fruits during storage**.**

Table (3): Tested of mycotoxin production

Fungal isolate(s)			Localities	
	mycotoxin	juice	Gharbia	Kalubia
A. flavus	Afs	apple	+	ND
A. niger		apple	ND	ND
A. parasiticus		apple	+	ND
A. terries		apple	ND	ND
P. degetatum	pat	citrus	ND	ND
P. italicum		citrus	ND	ND
P. expansun		apple	+	+
Fusarium sp.	F B_1	apple	ND	ND

Afs = Aflatoxins F B_1 = Fumonisin B_1 pat = patulin
ND: Not detected

Determination of mycotoxin(s)

Identification and determination of aflatoxions could easily be deduced from the constant retention time compared with the standard spiked in the HPLC chromatogram **(Fig. 1)**. Determination of aflatoxins produced by *Aspergillus flavus* and *A. parasiticus* after inoculated artificially and incubated

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for two weeks at 28 ± 2ºc were tabulated in **Table (4).** Data in this table presented that, aflatoxin content after inoculated with the tested of *Aspergillus flavus* isolate No. 20 which isolated from apple fresh juice Gharbia sample was found to be produced only 0.10 ng/ml of aflatoxin AFB_1**(Fig. 2)** while, *A. parasiticus* isolate No. 4 isolated from apple fresh juice Gharbia sample was found to be produced all types of aflatoxins which gave 0.52 ng/g of aflatoxin AFB_1, 0.10 ng/g of aflatoxin AFB_2, 0.32 ng/ml of aflatoxin AFG_1 and 0.10 ng/ml of aflatoxin AFG_2**(Fig. 3). Pande, et al., (2012), Sarmah, and Sarma, (2012), Ahire, and Sangale, (2012) and Vermani, et al., (2014)** reported that, the presence of fungi is a serious health hazard for workers as well as consumers in markets. Quantification of airborne fungi in these markets is essential to identify major pathogenic fungi causing post harvest diseases of fruits. It is crucial for the post-harvest quality management of a wide range of high value fruit crops**.**

Table (4): Quantitively of aflatoxins ng/g HPLC

Type of			Concentration of aflatoxin (ng/ml)				Total
Juice	Sample	Isolate	AFB_1	AFB_2	AFG_1	AFG_2	
Apple	Gharbia	*A. flavus*	0.10	ND	ND	ND	0.10
		A. parasiticus	0.52	0.10	0.32	0.10	1.04
	Kalubia	*A. flavus*	ND	ND	ND	ND	ND
		A. parasiticus	ND	ND	ND	ND	ND
		Fusarium sp.	ND	ND	ND	ND	ND

ND = Not detectedd

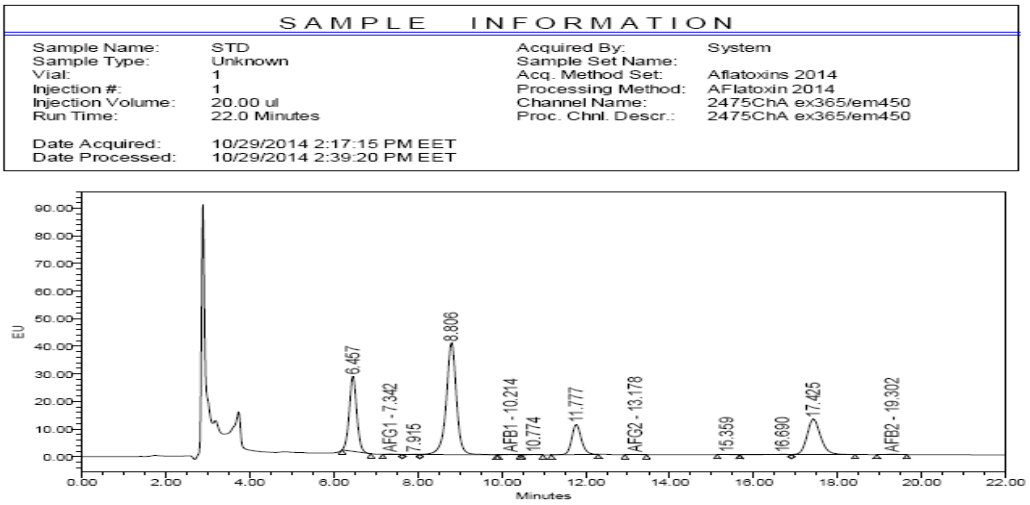

Fig. (1): Standard spiked in the HPLC chromatogram of aflatoxins AF G_1, B_1, G_2& B_2

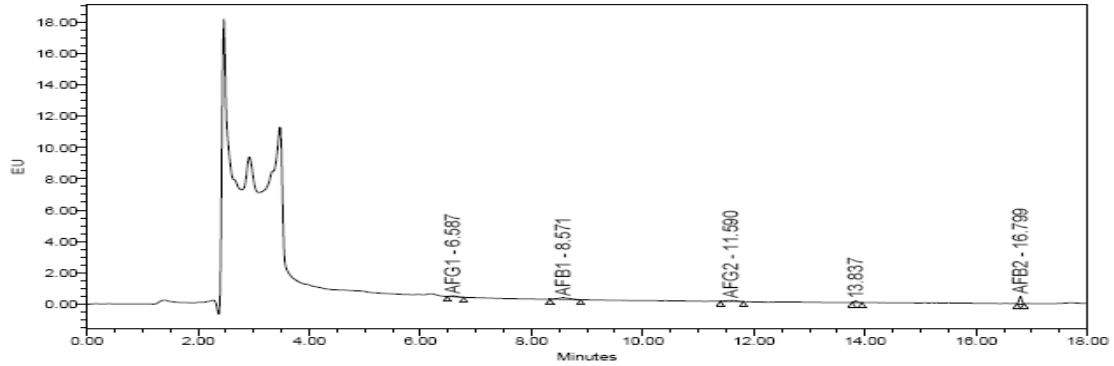

Fig. (2): HPLC chromatogram of aflatoxins produced by *Aspergillus flavus* (No. 20) isolated from apple fresh juice Gharbia sample

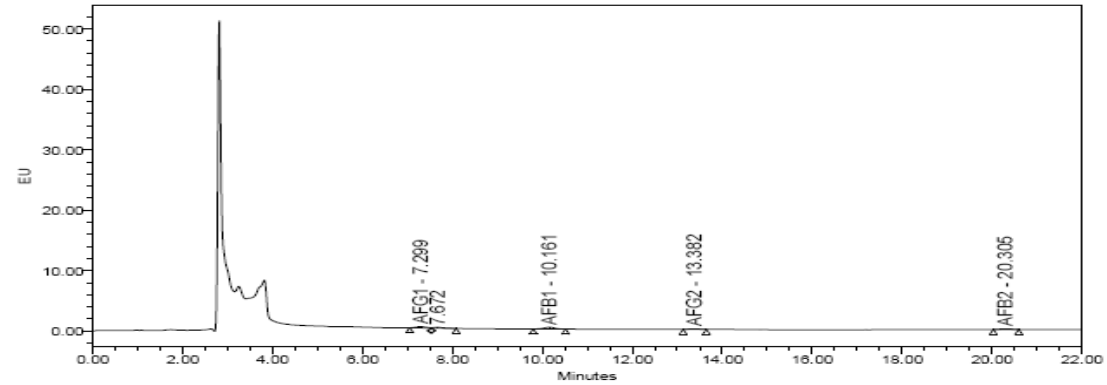

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Fig. (3): HPLC chromatogram of aflatoxins produced by *Aspergillus parasiticus* (No.4) isolated from apple fresh juice Gharbia sample

3-Effect of heat treatments on spore viability of the tested fungi

a-Effect of different hot water treatments on spore viability of the three tested fungi i. e. *Aspergillus flavus, A. parasiticus* and *Pencillium expansum* were recorded in **Table (5).** Data show that, all hot water treatments were found to reduce the number of spores' viability of the tested fungi. The numbers of spore viability were decreased with increasing the temperature degree used. Spores viability of *A. flavus* was found to be decreased from 51 to 24 when treated with hot water treatment at 45^0c with 52.94 %reduction, *A. parasiticus* was found to decrease from 64 to 40 with 37.50% reduction and *P. expansum* from 73 to 6 with 91.78 reduction percent. Hot water treatment at 50^0c was found to be decreased Spore viability of *A. flavus* from 51 to 13 with 74.51% reduction, A. parasiticus from 64 to 40 with 37.50% reduction while, completely decreased was found with *P. expansum* which record hundred percent of reduction. Enhanced effect was recorded when treated Spores viability of *A. flavus* with hot water treatments at 55^0c which decreased from 51 to 8 with 84.31 %reduction, *A. parasiticus* from 64 to 10 with 84.38 % reduction and *P. expansum* from 73 to zero which gave completely reduction(100%) .

b-Also, effect of pasteurization and sterilization treatments on spore viability of the tested fungi i. e. *Aspergillus flavus*, *A. parasiticus* and *Pencillium expansum* were recorded in Table (5). Data presented that, both pasteurization and sterilization treatments have a great effect on spore viability of the three tested fungi. Data also show that either pasteurization or sterilization gave completely inhibition of spore viability of the three tested fungi with hundred percent of reduction. In case of *A. flavus* , Pasteurization and sterilization effected on spore viability as spores are completely reduced from (51x10-4 spores/ml), *A. parasiticus* from (64 $x10^{-4}$ spores/ml) and *P. expansum* from ($73x10^{-4}$ spores/ml) to zero with 100% reduction.**USFDA (2001)** and **Rupasinghe, and Juan, (2012) stated that,** thermal processing is the most widely used technology for pasteurization of fruit juices and beverages. Juice pasteurization is based on a 5-log reduction of the most resistant

microorganisms of public health significance**.**Fruit juice has been traditionally pasteurized by batch heating at 63-65^{0}c (Low temperature long time (LTLT) for relatively long time **(D'Amico et al. 2006** and **Rupasinghe, and Juan, 2012).** Pasteurization is the most commonly used method for heat treatment of fruit juice. For example, orange juice is processed by high temperature short time (HTST) at 90 to 95^{0}c for 15 to 30 s. And apple juice is treated by HTST at 77 to 88^{0}c for 25 to 30 s **(Moyer & Aitken 1980** and **Rupasinghe, and Juan, 2012)** andsterilizing in an autoclave at 121^{0}c for 20 minutes **(Rupasinghe, and Juan, 2012).**

Table (5). Effect of heat treatments on spore viability of the tested fungi

Tested fungi		Type of treated/ ^{0}c									
		Hot water						Pasteurized		Sterilized	
		45^{0}c	%R	50^{0}c	%R	55^{0}c	%R	80°C	%R	121°C	%R
A. flavus	51	24	52.94	13	74.51	8	84.31	00	100	00	100
A. parasiticus	64	40	37.50	19	70.31	10	84.38	00	100	00	100
P. expansum	73	6	91.78	00	100.0	00	100.0	00	100	00	100

Conclusion: Juices squeezed from fresh fruits contain microorganisms which are potentially hazardous to public health. Juices were spoiled with high level of moulds and yeasts which is attributable to low pH of juices. The selling and consumption of juices are never stopped on nutritional grounds as well as livelihood of street vendors. It is alarming situation for suitable agency to take some necessary action, make guidelines to prevent potential food poisoning from juices that contain pathogenic microorganisms and find natural antimicrobials from plants that control spoilage and pathogenic microorganisms in juices. Mycotoxin analysis is an important subject with social relevance. The most suitable analytical methods respecting accuracy, low detection limits and simple procedures for application for the determination of mycotoxins in food products, especially apple juice and its derivatives techniques were thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Heat treatments i. e. waterbath(at 45, 50 and 55°C), pasteurization (at 80°C) and sterilization (at 120°C) treatments were the most suitable for elemination all Juice microbial, this work will contribute to a better characterization and quantification of its presence in human diet.

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