

STUDY ON MICROBIOLOGICAL CONTAMINANTS OF  
EGYPTIAN EDIBLE MUSHROOM

Amra<sup>1</sup>, H.A.S. ; Bakr<sup>2</sup>, A.A. ; Abdulfatah<sup>1</sup>, A.S.  
and \*Nogaim<sup>2</sup>, Q.A.

1- Food Toxicology and Contaminants Department, National Research Center,  
Cairo, Egypt.

2- Food Science and Technology Department, Faculty of Agriculture, Minufiya  
University, Egypt.

(Received: 31 / 12 / 2008)

ABSTRACT

A total of 40 samples of *Agaricus* and *Pleurotus* were collected from local market during winter 2007 and spring 2008 and analyzed. The results revealed that, the percentages of moisture content were 89.30 and 89.12% in winter and spring seasons, for *Agaricus* respectively, and 90.26 and 88.80% for *Pleurotus* mushroom samples in same order. The total bacteria count were  $1.35 \times 10^5$  and  $0.94 \times 10^5$  CFU/g in winter and spring seasons, for *Agaricus* respectively, and  $0.278 \times 10^5$  and  $1.4 \times 10^5$  CFU/g, for *Pleurotus* mushroom samples, in same order. The coliform bacteria in these samples were  $3.44 \times 10^3$  and  $1.1 \times 10^3$  CFU/g in winter and spring seasons, for *Agaricus* respectively, and  $0.31 \times 10^3$  and  $4.7 \times 10^3$  CFU/g, for *Pleurotus* mushroom samples in same order. Among the coliform bacteria, *E. coli* bacteria was detected; their count being  $10 \times 10$  and  $0.8 \times 10$  CFU/g. in winter and spring seasons, for *Agaricus* respectively, and  $1 \times 10$  and  $20 \times 10$  CFU/g, for *Pleurotus* mushroom samples in same order. The pathogenic bacteria like *E. coli* 0157 and *Salmonella* were not found in any of the samples. The average count of spore-forming bacteria were  $0.8 \times 10^2$  and  $0.48 \times 10^2$  CFU/g in winter and spring seasons, for *Agaricus* respectively, and  $5.7 \times 10^2$  and  $2.4 \times 10^2$  CFU/g, for *Pleurotus* mushroom samples in same order. On the other hand the total count of fungi and yeasts in these samples were  $2.5 \times 10$  and  $1.1 \times 10$  in winter and spring seasons, respectively for *Agaricus*, and  $5.3 \times 10$  and  $4.7 \times 10$  CFU/g, for *Pleurotus* mushroom samples in same order. It is noted that analysis of all mushroom samples in winter and spring seasons, have not confirmed the existence of any mycotoxins.

## INTRODUCTION

Since earliest times, mushroom has been treated as a special kind of food. It also has been considered as the oldest microbial food. The Greeks regarded mushroom as providing strength for warriors in battle. The Romans regarded mushroom as the (food of the God), which was served only on festive occasions. The Chinese treasured mushroom as health food, the (elixir of life), at first such mushroom collected from their natural growing habitats, but with the passing of time numerous attempts have been made to establish practical cultivation techniques so far about some genera. There are about 5000 mushroom species, of which approximately 50–100 are known to be poisonous to man, however, only 200 to 300 varieties have been clearly established to be safely edible [Kyan et al., (2005)].

As reported by [Caglarirmak (2007)], mushrooms are good source of vitamins and minerals. Increasing consumption of mushroom is good for preventing malnutrition, although mushrooms cannot be an alternative protein source instead of meat, fish, and egg. Elibuyuk (2007), mentioned that, the cultivation of various species of mushrooms have been conducted throughout the world. Worldwide there are 200 edible fungi of which only 25 species are widely accepted as human food and are cultivated. However, *Agaricus bisporus* (white button mushroom) is the most widely cultivated species of mushrooms comprising approximately 32% of world production. Other cultivated mushrooms are in descending order: *Lentinus edodes* (shiitake mushroom), *Pleurotus* spp. (oyster mushrooms), *Volvariella volvacea* (straw mushroom), *Auricularia* spp. (wood ear or Jew's ear mushroom), *Flammulina velutipes* [Flammulina velutipes] (velvet foot, velvet stem or enokitake mushroom), *Tremella fuciformis* (white jelly or silver ear mushroom) and *Pholiota nameko* (nameko mushroom). Edible mushrooms are characterized by a short shelf life (1–3 days at room temperature) or (5–7 days at 5°C), linked to the occurrence of post-harvest changes. These changes are due to the high moisture content of the carpoforus and to the high activity of enzymes such as protease or polyphenol oxidase, responsible for protein and sugar decrease and for a browning reaction during storage [Pamela Manzi et al., (2004)].

Sokovic & Griensven (2006) found that, bacterial and fungal diseases are major problems in mushroom cultivation; a high percentage of product is lost due to lower productivity, decrease of quality and

shortened shelf life. The white button mushroom *Agaricus bisporus* (Lange) is highly sensitive to bacterial, fungal and viral diseases. Major pathogens are the bacterium *Pseudomonas tolaasii*, fungi *Verticillium fungicola* and *Trichoderma harzianum* and La France virus; all are highly infectious and cause serious damage. In Western countries the average annual damage due to those four diseases accounts for approximately 25% of the total production value. Although careful farm management and extreme hygiene may prevent major attacks, some diseases are very difficult to control on the farm. While, in African countries, [Gbolagade (2005)] carried out many studies on bacteria which usually infect spawns and culture plates of *Psathyrella antroumbonata* and *Schizophyllum commune*, two Nigerian edible mushrooms. During the vegetative propagation of these higher fungi, six different bacterial species were isolated and characterized from 14 day old spawns and mycelial ramified PDA culture plates. These bacteria include *Bacillus licheniformis*, *Bacillus subtilis*, *Leuconostoc mesenteroides*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus*. The average of bacterial count was  $1.0 \times 10^6$  CFU/ml and these bacteria grew within pH range of 5.0 and 9.0. The optimum temperature range of growth is between 30°C and 37°C. In Egypt, total microbial count of different mushroom strains either fresh or dried was determined by [Hussein (2002)], and the data showed that fresh mushroom samples had the highest total count being  $5.1 \times 10^3$ ,  $7.2 \times 10^3$  and  $5.3 \times 10^3$  CFU/g for *P. ostreatus*, *A. bisporus* (X<sub>25</sub> strain) and *A. bisporus* (G<sub>2</sub> strain), respectively. Total microbial count in *P. ostreatus* dried samples (sun and oven) ranged between (1.4 – 1.9) and (1.1 – 1.5) CFU  $\times 10^3$  /g, in succession. While, it was found to be ranged between (1.3 – 1.7) and (1.2 – 1.4) CFU  $\times 10^3$  /g for *A. bisporus* (X<sub>25</sub>) samples, consecutively. As for *A. bisporus* (G<sub>2</sub> strain) total microbial counts ranged between (1.2 – 1.9) and (1.1– 1.4) CFU  $\times 10^3$  /g for dried samples, in succession.

This study aims to evaluate microbiological contaminants of marketable Egyptian edible mushrooms, and also, testing its contamination by toxigenic strains of fungi in addition to mycotoxins determination.

## MATERIALS AND METHODS

### Materials:

**Samples:** 40 samples of both *Agaricus* and *Pleurotus* Egyptian edible cultivated mushroom were purchased randomly from markets of Greater Cairo Governorate Egypt, during winter and spring seasons 2007 and 2008.

**Medium:** Standard plate count (SPC) agar, Malt extract agar, MacConkey agar, Sorbitol MacConkey agar, Selenite Cystine broth and Salmonella Shigella agar were obtained from Oxoid Ltd, Basingstoke, UK.

**Mycotoxins Standards:** The standards of Aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>, Ochratoxin A and Zearalenone were obtained from Sigma Chemical Company, P.O. 145508, St. Louis, USA.

**Solvents:** Acetonitrile for high performance liquid chromatography was obtained from Sigma Chemical Company, P.O. 145508, St. Louis, USA.

### Methods: Microbiological examination:

The total number of bacteria, yeast and molds, were measured by inoculating dilutions of the samples suspension into various cultures and incubating them for fixed periods at optimum temperatures. The resulting colonies are then calculated as colony forming unit per gram of mushroom CFU/g. Total viable bacteria counts were determined using the SPC agar medium according to the method recommended by the [FDA (2001)]. For bacterial counts plates were incubated at 35 °C for 48h.

**Aerobic spore-form count.** were determined by aseptically transferring 10 mL dilutions of suspensions of the sample to each of two sterile, plugged test-tubes. A thermometer was inserted into one of the test-tubes through the cotton-wool plug, so that the thermometer bulb was completely immersed in the sample. Both tubes were placed in a water bath at 80°C and allowed to remain in the bath for 15 min after the temperature in the control tube attained a maximum (usually just below the temperature of the water bath). The tubes were removed and cooled quickly in cold water. Plates of SPC agar were inoculated with the inoculums in duplicate sets and incubated at 30°C.

**Yeast and mold counts**, were determined using the diluting plating technique described by (FDA, 2001) using malt extract agar medium. The medium was sterilized by autoclaving at 121°C for 15 min. To inhibit bacterial growth, antibiotics or sterile tartaric acid solution was added immediately to the agar after it had been tempered before pouring into plates. The plates were incubated in the dark at 22–25°C for 5 days.

**Enterobacteriaceae count:** Total *Enterobacteriaceae* count (glucose fermentation) using selective medium (MacConkey Agar) was enumerated according to the method recommended by the [FDA (2002)]. The incubation was carried out at 37 °C for 48 h. These organisms indicate the standard of hygiene used in the production or cultivation of mushroom.

**Detection of *Salmonella* spp. and *E.coli* 0157:** *Salmonella* spp. were detected according the method described in the [Oxoid Manual (1998)], using Salmonella-Shigella Agar medium. The plates were incubated at 37°C for 18–24 h. *E.coli* 0157 was determined according to the method described in the Oxoid Manual. Twenty-five grams of mushroom sample was diluted with 225 ml buffer peptone water and 1 ml was spread on Sorbitol MacConkey agar medium, which was sterilized, by autoclave. The inverted dishes were incubated at 37°C and examined after 18 - 24 h for typical colonies of *E.coli* 0157.

**The identification of fungi:**

The isolated fungi were identified to species level by aid of Pest and Plant Protection Laboratory, National Research Center, Dokki, Cairo. All isolated fungi were identified according to the procedures of [Gilman (1957); Barnnett & Hunter (1972) and Nelson *et al.*, (1983)]. The members of the *Aspergillus* species were classified according to the key published by [Raper & Fennell (1965)], while for species of other isolated fungi, the methods described by [Count *et al.*, (1954)] were used.

**Toxicity of isolated fungi and mycotoxins detection:**

*Aspergillus flavus*, *Asp. ochraceus* and *Fusarium graminearum* isolated from both *Agaricus* and *Pleurotus* mushroom were inoculated on Yeast Extract Sucrose medium (YES), and incubated at 25°C, for 15 days to determine the toxicity of certain genera, whether the fungal toxin was produced or not. After that, mycotoxins were detected in YES

medium and in mushroom samples, according to multi technique, [FDA (2001) and A.O.A.C. (1995)].

## RESULTS AND DISCUSSION

### The microbiological analysis of edible Egyptian mushroom:

The results presented in table (1) clearly indicate that, the percentages of moisture content were 89.30 and 89.12% in winter and spring seasons, for *Agaricus* respectively, and 90.26 and 88.80 % for *Pleurotus* mushroom samples in same order. While, the total of bacteria plate count were  $1.35 \times 10^5$  and  $0.94 \times 10^5$  CFU/g in winter and spring seasons, for *Agaricus* respectively, and  $0.278 \times 10^5$  and  $1.4 \times 10^5$  CFU/g, for *Pleurotus* mushroom samples, in same order. The coliform bacteria in these samples were  $3.44 \times 10^3$  and  $1.1 \times 10^3$  CFU/g in winter and spring seasons. for *Agaricus* respectively, and  $0.31 \times 10^3$  and  $4.7 \times 10^3$  CFU/g, for *Pleurotus* mushroom samples in same order. In coliform bacteria, *E. coli* bacteria was detected, and their count were  $10 \times 10$  and  $0.8 \times 10$  CFU/g in winter and spring seasons. for *Agaricus* respectively, and  $1 \times 10$  and  $20 \times 10$  CFU/g, for *Pleurotus* mushroom samples in same order. The pathogenic bacteria like *E. coli* 0157 and *Salmonella* were not found in any of the analyzed samples of edible Egyptian mushroom. Finally, average of total count of spore-form bacteria at of these samples were  $0.8 \times 10^2$  and  $0.48 \times 10^2$  in winter and spring seasons, for *Agaricus* respectively, and  $5.7 \times 10^2$  and  $2.4 \times 10^2$  CFU/g, for *Pleurotus* mushroom samples in same order.

These results are in agreement with the findings of [Reyes et al., (2004)], who analyzed a total of 95 samples of *Agaricus bisporus*, *Lentinula edodes* and *Pleurotus ostreatus* to quantify the *Enterobacteriaceae* and to identify the species isolated. The host pathogenicity test was used to verify their mycopathogenic potential. The genus *Pseudomonas* was also quantified, since it is the predominant bacterial group in cultivated mushrooms. The counts of *Enterobacteriaceae* ranged from 2.88 to 3.66 log<sub>10</sub> CFU/g, which was significantly lower than the counts of *Pseudomonas* spp. (4.52 - 7.80 log<sub>10</sub> CFU/ g). Also, result which recorded by [Hussein (2002)], are coincide with those reported by [Pattnaik et al., (1998)], they studied microorganisms associated with fresh and dried *P. sajor-caju*, *P. sajor-caju* bed and spawn. They reported that, microbial load of fresh *P. sajor-*

*caju* was  $\log_{10} 6.13 \pm 0.63$  CFU/ml. The bacterial isolates obtained included *Bacillus* sp., *Escherichia coli*, *Pseudomonas* sp., *Alcaligenes* sp., *Klebsiella* sp., *Staphylococcus* sp., *Streptococcus* sp. and *Acinetobacter* sp. [Acinetobacter]. These results are in agreement with the result recorded by [Yousef (1997)] in Egypt, who found that total microbial count in dried *A. bisporus* ranged between (1.1–1.6) and (1.3–1.8) CFU  $\times 10^3$  /g for hot air and vacuum-dried samples, respectively compared to  $8.0 \times 10^3$  CFU /g for fresh samples. We think that, our results are slightly upper than the other results in Egypt, because our samples collected from markets (under poor control during handling and storage), while most Egyptian researchers cultivate mushroom and directly analyze fruiting- bodies. The variation between our slightly high counts and the other results can be attributed to the difference in packaging materials, the storage temperature and storing period.

Data in Table (2) show the total count of fungi which isolated from mushroom and percentage of genus distribution. While, data in Tables (3,4, and 5) show, the determination of toxicity of some fungi which able to produce mycotoxins in foods like (Aflatoxins, Ochratoxin and Zearalenone).

Table (1): Microbiological analysis of edible Egyptian mushroom.

Samples*/ Tests	<i>Agaricus</i> Winter Mean	<i>Agaricus</i> Spring Mean	<i>Pleurotus</i> Winter Mean	<i>Pleurotus</i> Spring Mean
Moisture %	89.30	89.12	90.26	88.80
Total Bacterial Count CFU/g	$1.35 \times 10^5$	$0.94 \times 10^5$	$0.278 \times 10^5$	$1.4 \times 10^5$
<i>E. coli</i> CFU/g	$10 \times 10$	$0.8 \times 10$	$1 \times 10$	$20 \times 10$
Coliform group CFU/g	$3.44 \times 10^3$	$1.1 \times 10^3$	$0.31 \times 10^3$	$4.7 \times 10^3$
Spore-form Bacteria CFU/g	$0.8 \times 10^2$	$0.48 \times 10^2$	$5.7 \times 10^2$	$2.4 \times 10^2$
Pathogenic Bacteria <i>E.coli</i> . 0157 <i>Salmonella Spp.</i>	Negative	Negative	Negative	Negative

\* we collect 10 samples from each species in two seasons the total sample was 40 .

Table (2): Total count of Fungi in mushroom and their genera % distribution:

Samples Fungi	<i>Agaricus</i> Winter	<i>Agaricus</i> Spring	<i>Pleurotus</i> Winter	<i>Pleurotus</i> Spring
Total count fungi CFU/g	2.5 X 10	1.1 X 10	5.3 X 10	4.7 X 10
<i>Aspergillus</i>	25 %	15 %	70 %	50 %
<i>Penicillium</i>	65 %	70 %	20 %	30 %
<i>Fusarium</i>	5 %	10 %	10 %	5 %
<i>Rhizopus</i>	5 %	5 %	Zero	10 %

Table (3): Natural occurrence of *A.flavus* producing Aflatoxin in Egyptian mushroom.

Samples of mushroom	No. of tested <i>Aspergillus</i> spp. isolates	Frequency of <i>A. flavus</i> isolate %	Frequency of <i>A. flavus</i> isolate producing Aflatoxins %*	Aflatoxins Concentration mg/liter	
				Range	Mean
<i>Agaricus</i>	100	20	10	5.5-8.7	7.1
<i>Pleurotus</i>	100	35	20	6.8-15.4	11.1

\* These percentages were counted for *A. flavus* isolates producing Aflatoxins from 10 isolates of *A. flavus*.

Table (4): Natural occurrence of *A. ochraceus* producing Ochratoxin A in Egyptian mushroom.

Samples of mushroom	No. of tested <i>Aspergillus</i> spp. isolate	Frequency of <i>A. ochraceus</i> isolate %	Frequency of <i>A. ochraceus</i> isolate producing Ochratoxin A %*	Ochratoxin A concentration mg/liter	
				Range	Mean
<i>Agaricus</i>	100	15	--	0.0	0.0
<i>Pleurotus</i>	100	25	10	4.3-6.7	5.5

\* These percentages were counted for *A. ochraceus* isolates producing Ochratoxin A from 10 isolates of *A. ochraceus*.



Table (5): Natural occurrence of *F. graminearum* producing Zearalenone in Egyptian mushroom.

Samples of mushroom	No. of tested <i>Fusarium</i> spp. isolate	Frequency of <i>F. graminearum</i> isolate %	Frequency of <i>F. graminearum</i> isolate producing Zearalenone %*	Zearalenone concentration mg/litter	
				Range	Mean
<i>Agaricus</i>	40	20	10	7.2-10.3	8.7
<i>Pleurotus</i>	40	10	--	0.0	0.0

\* These percentages were counted for *F. graminearum* isolates producing Zearalenone from 10 isolated of *F. graminearum*.

The results of *Aspergillus*, *Penicillium*, *Fusarium*, and *Rhizopus* that presented in Tables (2) show that, the total count of fungi and yeasts at the average in these samples were  $2.5 \times 10$  and  $1.1 \times 10$  CFU/g in winter and spring seasons, for *Agaricus* respectively, and  $5.3 \times 10$  and  $4.7 \times 10$  CFU/g for *Pleurotus* mushroom samples in same order. From Table (2), % distribution of fungal genera in *Agaricus* mushroom samples at winter season 2007, we found that, 25%, 65%, 5% and 5% of genera represents *Aspergillus*, *Penicillium*, *Fusarium*, and *Rhizopus*, respectively.

Whereas, % distribution of fungal genera in *Pleurotus* mushroom samples at winter season 2007, were 70%, 20%, 10% and 0.0% genera of represents *Aspergillus*, *Penicillium*, *Fusarium*, and *Rhizopus*, respectively. On the other hand % distribution of fungal genera in *Agaricus* mushroom samples at spring season 2008, were 15%, 70%, 10% and 5% genera of represents *Aspergillus*, *Penicillium*, *Fusarium*, and *Rhizopus*, respectively. Finally in *Pleurotus* mushroom samples at spring season 2008, were 50%, 30%, 5% and 10% genera of represents *Aspergillus*, *Penicillium*, *Fusarium*, and *Rhizopus*, respectively.

Identification of certain genera, have been done, Table (3) show % distribution of *Aspergillus flavus* in the genus *Aspergillus* in both *Agaricus* and *Pleurotus* mushroom. We took strains *Aspergillus* isolated from both *Agaricus* and *Pleurotus* mushroom on Yeast Extract Sucrose medium (YES) to determine whether the fungal are toxin-producing or not. We found that, 20% and 35% of strains isolated from both *Agaricus* and *Pleurotus* mushroom are *Aspergillus flavus* respectively, only 10%

and 20 % aflatoxin-producing toxin in mean reached to 7.1 to 11.1 mg/L of the medium on the same previous arrangement. As also, Table (4) show % distribution of *Aspergillus ochraceus* in the genus *Aspergillus* in both *Agaricus* and *Pleurotus* mushroom, we found that, 15 % and 25 % of strains isolated from both *Agaricus* and *Pleurotus* mushroom are *Aspergillus ochraceus* respectively, only 10 % of these strains produced ochratoxin by an average of 5.5 mg/L of the medium of the strains isolated from *Pleurotus* mushrooms. While, isolates of the fungus *Agaricus* not able to producing any ochratoxin. Table (5) show % distribution of *Fusarium graminearum* in the *Fusarium* isolated from both *Agaricus* and *Pleurotus* mushroom on Yeast Extract Sucrose medium (YES) to determine whether the fungal are toxin-producing or not. We found that, 20 % and 10 % of strains isolated from both *Agaricus* and *Pleurotus* mushroom are *Fusarium graminearum* respectively, only 10 % of these strains produced zearalenone by an average of 8.7 mg/L of the medium of the strains isolated from *Agaricus* mushrooms, while the isolates from *Pleurotus* mushrooms were not able to producing any toxins.

With respect to the mycotoxins analysis of samples of *Agaricus* and *Pleurotus* mushrooms in both winter and spring seasons, our results have not confirmed the existence of any of the studied mycotoxins (Aflatoxin, Ochratoxin and Zearalenone). This result may be due to many reasons, the very important is, the mushroom natural contamination by fungi occurred during the handling or after harvest, in addition, the total counts of fungi in our study was not high, and the competition between different micro-organisms in mushroom affected to prevent beginning mycotoxins production by fungi. Furthermore, the time between collecting the samples or of infection and analysis was short (4 to 5 days).

Finally the chemical structure of mushroom had very important role in these result of [Engelhardt (2002)], in his study reported that, the degradation of ochratoxin A (OTA) and ochratoxin B(OTB) during solid state fermentation was compared after a four-week incubation period of selected fungi on contaminated barley, in the presence of the soil fungus (*Rhizopus japonicus*) and white rot fungi (*Panerochaete chrysosporium*), more than 60% of mycotoxins remained stable. On the other hand, 23% OTA and 3% OTB were detected in the presence of another white rot fungus, *Pleurotus ostreatus* after four weeks. Kinetic studies on mycotoxins degradation by *P. ostreatus* demonstrated the formation of

ochratoxin alpha and presumably ochratoxin beta as intermediate products. This indicates that hydrolysis is the first step in OTA and OTB degradation followed by further degradation of the intermediate products.

Nineteen fungi were tested by [Motomura *et al.*, (2003)] for their ability to degrade Aflatoxin B1 (AFB1). An extracellular enzyme from the edible mushroom *P. ostreatus* showed Aflatoxin-degradation activity detected by thin-layer chromatography (TLC). The apparent molecular mass of the purified enzyme was estimated to be 90 KDa by SDS-PAGE. Optimum activities in pH (4 to 5) and at 25°C. Based on these data, they suggest that this enzyme is a novel enzyme with aflatoxin-degradation activity. Fluorescence measurements suggest that the enzyme cleaves the lactone ring of aflatoxin. At the same point, Tekiel (2005) found that, greenhouse experiments were conducted to determine the different pathogenic fungi infecting *Agaricus bisporus*. The number and composition of microorganisms which accompany mushroom cultivation depends on the healthiness of the compost, casing and *A. bisporus* spawns. The presence of pathogenic fungi in the cultivation halls at the beginning of the production cycle is a serious threat to the cultivation of mushroom because their rapid development shortens the span of fruiting body harvests. Many studies reported that, the early good treatment of compost which used for mushroom cultivation is very important for good quality. Saifullah *et al.*, (2005) study the effect of pasteurization time on the control of mycoflora present in the casing material used for growing *Agaricus bisporus* was investigated. The casing soil materials were pasteurized for two, three and four hours at 85°C. Significant differences were recorded among different treatments. The recovery of fungi was negatively correlated to the pasteurization time and best control was achieved with 4 h pasteurization. The fungi recovered were species of *Aspergillus*, *Coprinus*, *Fusarium*, *Penicillium*, *Rhizopus*, *Sclerotinia*, *Trichoderma*, and *Verticillium*. *Trichoderma* and *Rhizopus* spp. were the most frequently recovered fungi followed by *Penicillium* spp., whereas *Fusarium* spp. were the least associated fungi recovered.

## CONCLUSION

It can already conclude that, the mushrooms as a raw food containing relatively high-microbial contamination, it must be cooked or processed in good conditions; appropriate high temperatures to be safe and healthy. Do not advise applying simple treatments such as washing and surface roasting or fresh addition for salads. It must be going through the whole processing of cooking such as meat.

## REFERENCES

- A.O.A.C. (1995):** Official Methods of Analysis. Association of Official Analytical Chemists, 16<sup>th</sup> ed., vol. 2. Washington, DC.
- Barnett, H.L. and B.B. Hunter (1972):** Illustration Genera of Imperfect Fungi. Burgess Publishing Company, Minn-Eapolis 15 Min. U.S.A., pp. 225.
- Caglarlrmak, N. (2007):** Analytical, Nutritional and Clinical Methods. The nutrients of exotic mushrooms (*Lentinula edodes* and *Pleurotus* species) and an estimated approach to the volatile compounds. Food Chemistry .vol.105. (2007) pp. 1188–1194.
- Count, N.F.; F.S. Martin; D.T. Smith; R.F. Baker and J.L. Gallaway (1954):** Manual of Clinical Mycology. Philadelphia, W.B. Saunders Company., pp.11-15.
- Elibuyuk,-I-O. (2007):** Virus diseases on cultivated mushrooms. Ondokuz Mays-Universitesi, Ziraat Fakultesi Dergisi. 2007. 22(1): 105-115. Samsun, Turkey.
- Engelhardt,-G. (2002):** Degradation of ochratoxin A and B by the white rot fungus *Pleurotus ostreatus*. Mycotoxin Research. 2002; 18(1): 37-43. Mainz, Germany.
- F.D.A.(2001):** U.S. Food and Drug Administration. Bacteriological Analytical Manual (8th Edition).AOAC,. Revision A, (1998). FDA Home Page. (Bacteriological Analytical Manual *Online*, January 2001).

(Chapter 3 Aerobic Plate Count) and (Chapter 18 Yeasts, Molds and Mycotoxins ).

**FDA, (2002):** U.S. Food and Drug Administration. (department of Health and Human Services) FDA Home Page.(Bacteriological Analytical Manual *Online*, September 2002). (Chapter 4 Enumeration of *Escherichia coli* and the Coliform Bacteria).

**Gbolagade, J.S. (2005):** Bacteria associated with cultures of Nigerian edible mushrooms, *Psathyrella antroumbonata* (Pegler) and *Schizophyllum commune* (Fr. ex Fr.). Acta Phytopathologica et-Entomologica-Hungarica. 2005; 40(3/4): 333-340. Ibadan, Nigeria.

**Gilman, J.E. (1957):** "A Manual of Soil Fungi". Low State College Press. Anes, Iowa, U.S.A. pp. 392.

**Hussein, F.R. (2002):** Studies On The Bioconversion Of Some Agricultural Wastes Using *Pleurotus* and *Agaricus* Mushrooms .Ph.D. Thesis Cairo university.

**Kyan J. Berger, and David A. Guss. (2005):** MYCOTOXINS REVISITED: PART II. The Journal of Emergency Medicine, Vol. 28, No. 2, pp. 175–183. Copyright © 2005 Elsevier Inc. Printed in the USA.

**Motomura, M. ; Toyomasu, T. ; Mizuno, K. and Shinozawa, T. (2003):** Purification and characterization of an Aflatoxin degradation enzyme from *Pleurotus ostreatus*. Microbiological Research. 2003; 158(3): 237-242. Jena, Germany.

Nelson, P.E. ; T.A. Toussoun, and F.O. Marasan, ,(1983). An Illustrated Manual for Identification. Published by the Pennsylvania State, University Press, University Park and London.

Oxoid Manual, 8th edition. Oxoid, Basingstoke, UK (1998).

**Pamela Manzi ; Stefania Marconi ; Altero Aguzzi, and Laura Pizzoferrato. (2004):** Commercial mushrooms: nutritional quality and effect of cooking. Food Chemistry.vol. 84 (2004). pp 201–206.

**Pattnaik, P.; Patra, A.K and Pattnayak, N.B.(1998):** Serratia marcescens from oyster mushroom spawn a case study. Journal of Dairying, Foods and Home Sciences. 1998; 17 (3/4): 205-210. India.

**Raper, J.P. and D.T. Fennell (1965):** The genus *Aspergillus williams* and *Aspergillus wilkins*. Baltimore, U.S.A.

**Reyes, J.E ; Venturini, M.E ; Oria, R. and Blanco, D. (2004):** Prevalence of *Ewingella americana* in retail fresh cultivated mushrooms (*Agaricus bisporus*, *Lentinula edodes* and *Pleurotus ostreatus*) in Zaragoza (Spain). FEMS Microbiology Ecology. 2004; 47(3): 291-296. Amsterdam, Netherlands.

**Saifullah, ; Zahid-Iqbal, ; Hakim-Khan, and Shah, M.A.Q.(2005):** Effect of pasteurization time on the recovery of mycoflora of casing material used for growing *Agaricus bisporus* (Lange) Sing. Sarhad-Journal-of-Agriculture. 2005; 21(2): 275-280. Peshawar, Pakistan.

**Sokovic, M and Griensven, L.J.L.D-van. (2006):** Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, *Agaricus bisporus*. European Journal of Plant Pathology. 2006. Vol. 116(3). pp 211-224.

**Tekiela, A. (2005):** The pathogenic fungi in mushroom cultivation of *Agaricus bisporus* (Lange.) Imbach. Acta-Agrobotanica. 2005; 58(2): 189-195. Warszawa. Poland.

**Yousef, N. S. (1997):** Studies on the processing of mushroom cultivation on some agro-industrial wastes. Ph.D. Thesis. Ain-shams. university.

دراسة تواجد الملوثات الميكروبيولوجية في عيش الغراب المصري

\* حسن أحمد السيد عمرو ، \*\*على عبد الهال بكر ، \* أبو بكر سالم  
و \*\* قيس عبد الله نجيم.

\* قسم سموم وملوثات الغذاء - المركز القومي للبحوث - القاهرة.

\*\* قسم علوم وتكنولوجيا الأغذية - كلية الزراعة - جامعة المنوفية .

يعتبر عيش الغراب (المشروم) مادة غذائية ممتازة نظرا لنسبة البروتين الجيدة فيه وأن بروتينه كامل القيمة الغذائية، كما أن إمكانية زراعته الاقتصادية واستثمارية، ووفرة غذاءه ملائما لمرضى السكر ومن يتبعون الحميه الغذائية نظرا لمحتواه المنخفض جدا من الدهون وبنوعيه الألياف التي يحتويها، كما انه يحتوي على مركبات مفيدة مثل السكرات العديدة (البيتا - جلوكان وغيرها) التي ثبت أن لها تأثيرات صحية ممتازة، ونظرا للظروف القلبية البسيطة التي يتم بها إنتاج عيش الغراب ولأن فترة صلاحيته لا تتجاوز ٦ أيام بسبب محتواه العالي من الرطوبة الذي يصل الي ٩٠% فإنه يتعرض للتلف. ولذا أيضا تسليط الضوء على بعض الملوثات الميكروبية في عيش الغراب الذي يباع في الاسواق المصرية بنوعيه العادي من جنس (الأجاريكس) أو النوع الآخر المحارى من جنس (البلوروتس) وخلال موسم البيع في شتاء وربيع عامي ٢٠٠٧ و ٢٠٠٨. وقد تم تجميع العينات من الأسواق المحلية بمحافظات القاهرة الكبرى، وقد تم إجراء الاختبارات الميكروبيولوجية التالية: العدد الكلى للبكتريا، عدد الفطريات والخمائر، عند البكتريا المكونة للجراثيم، عدد بكتريا القولون، فحص البكتريا المرصية من جنس السالمونيلا والإشريشيا كولاي، تقدير أحاس الفطريات التي تنتج السموم وأخيرا فحص تواجد بعض السموم الفطرية في عينات عيش الغراب نفسها. مما سبق يمكن أن نخلص الى القول أن عيش الغراب كمادة غذائية خام يحتوي حمل ميكروبي على نسبيا وبرتغم انه لم يسجل تواجد السموم الفطرية على العينات قيد الدراسة فإنه لا بد من معاملة عيش الغراب بطوروف طبخ أو تصنيع ملائمة تتضمن تبريده لدرجات حرارة مرتفعة نسبيا لكي يكون غذاءا آمنا، ولا ننصح بمعاملات مثل الانسيل أو التخمير البسيطة والأسطحية أو إضافته طازجا مع السلطات بل يجب أن يمر بعملية طبخ كامل ملته مثل اللحوم أو الخضروات المطبوخة.