Study the pollution by mycoflora and aflatoxins of some of black tea kindes in Iraqi markets

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Abstract

This study was aimed to investigate the fungal contamination and aflatoxins occurrence in Twenty two samples of some black tea types that collected from the local markets in Iraq. Results were indicated that found as contaminated by mycoflora, and isolated the *A. niger* as the most dominant in all the tea brands samples, and the other fungi species isolated were as *A. flavus, A. parasiticus, A. ochraceus, Alternaria* and *penicillium.*

Bacteria speices were isolated an aerobic bacteria count, spor forming, coliform. However *Salmonella* and *Staphyllococcus aurous* were not detected.

Aflatoxin were detected in 37 % of the samples with averages of 4.54 – 11.98 µg /kg of black tea. The highest concentration of aflatoxin B1 was production in Apple, Merd and AL-Qeethara tea samples and the lowest quantity was produced in Golden and AL-Sanabul tea.

Introduction

Aflatoxin biosynthesis from *Aspergillus flavus* was recorded in all kinds of tea after 20 day of storage at 45% m.c. and 28°C. The highest aflatoxin concentration was produced in Al-Fares, Ezi-Nasser, Lipton and Tayseer tea were found by their isolates of *A. flavus* when cultured on Potato dextrose broth, and the lowest quantity produced by the same isolates was found in Khan El-Khallily and Massgeed tea Hassan and Abdel-Sater (1993). Twenty-eight herbal medicinal products from Thailand were investigated for aflatoxin (AF) contaminations, the results revealed that five (18%) of herbal samples were contaminated with detectable amount of the total AFs ranging from 1.7 to 14.3 ng/g,(wongwiwat, 2004). Storage fungi are important factors responsible for tea deterioration. There were several contaminants during initial tea preparation. This was possible because the isolated fungi are common aerial contaminants which are able to proliferate very rapidly especially when remmants of the leaves can provide a rich food source for growth (Ciegler, 1975).

The evaluated of ninety-one samples from medicinal plants to contains fungal contamination by Adriana *et al* (2006), the results indicated that predominant mycoflora was distributed in 10 genera from these, 89.9% of the isolates corresponded to genera *Aspergillus* and *Penicillium*.. EL-Shafie *et al* (1999) found five fungal species were isolated with *A. niger* as the most dominant in all the brands samples and having percentage contamination ranging between 0.66% to 30.34%, other fungi isolated were *Aspergillus flavus*. Significant differenceswere found among the batches contaminated by *A. niger.* Ostry *et al* (2001) when collected Twenty five commodities from twelve collection places in the Czech Republic (300 food samples). The presence of potentially toxinogenic fungi was found as *Aspergillus flavus* and observed in 28% of the sampled foods, black pepper,caraway seeds, fruit tea, black tea, oat flakes, fine flour,rolled oat flakes and semolina in the year 1999, and in 25% of the sampled foods black pepper, black tea, fine flour in the year 2000.

For the microbial status of dried herbal materials including an aerobic bacterial counts, spore-forming, Coliform, *E. coli, Staphylococcus aureus,* Salmonella, yeast and molds, Moreover, fungi were found in all of the collected samples. *Aspergillus,Fusarium* and

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Penicillium **genera were more frequently detected than other genera** ***Alternaria, Absidia spp.,Mucor spp., Rhizoctonia*** **and** ***Cladosporium spp.*****(Abu dunya, 2008)**

Materials and methods

Twenty two of different kinds of black tea samples, commonly used in iraq, were collected from retail markets. These samples were transferred to the laboratory in food science department- College of Agriculture- University of Tikrit and kept at room temperature untill detect and identified of fungi and toxins analysis.

Mycological studies:

Ten grams of each sample were added to 90 ml portion of sterile saline solution (85%) in 500 ml Erlenmeyer flask and homogenized thoroughly on an electric shaker at constant speed for 15 minutes. Five fold serial dilutions were then prepared Aziz and Youssef (1991). One ml portion of suitable dilutions were used to inoculate Petri dishes containing 15 ml of media. Plates were incubated at 28 ºC for 3-7 days and examined for the growth of molds, Raper and Fennel (1977) and Pitt (1985). Fungi were isolated and identified according to key classification, Robert *et al* (1981). For the detection and enumeration of microorganisms, standard media are prepared. For aerobic and sporeformer bacterial count, the medium was standard nutrient agar and mackongy agar. For coliform bacteria count, the medium used was mackongy broth and EMB. For *Staphylococcus aureus* was used the Manitol salt agar and for *Salmonella,* the medium was used as the S. S. Agar and Tetra thionate broth. For yeast and molds, the medium was used the Malt extract agar, Harrigan and McCance (1976) and APHA,(1960). Gram stain and microscopic examination was for confirmation. Plates of the bacterial growth was incubate at 35 Cº for 48 h.

Aflatoxins analysis:

Aflatoxigenic ability of isolated strains of *A flavus* and *A parasiticus* were tested according to the Direct Competitive Method (Romer Company) 20 g of dried tea was extracted with 100 ml methanol and filteration by Whatman No.1, aflatoxin was determination by ELIZA. Optical density was reader by Stat Fax 3200 on length wave 450 nm.

Results and Discussion:

Bacteria contamination:

The microbiological quality of black tea samples which collected from Iraqi markets is shown in table 1.This results was indicated that founds among differents bacteria, total viable counts were noticed in different kinds at different levels. The highest mean count was detected in ALGhazaleen followed by, Golden tea and Ahmed tea. However, the lowest mean counts were detected in Alton kaya followed by Al-Sanabul. Spor-forming bacteria were detected in some of the analyzed samples allready. The highest mean count was detected in Gold leaf, Golden tea, and ALGhazaleen. On the other hand, coliform bacteria were detected in some of the samples except Golden and ALWazah tea samples. Salmonella and *Staphyllococcus aureus* was not detectable in all the analyzed samples. These results were in agreement with that reported by, Abou Donia, (2008) he found the microbial status of dried herbal materials including an aerobic bacterial count, sporeforming, Coliform, *Staphyllococcus aureus*, and *Salmonella* were detected. In other studies, Martins *et al*

(2001) reported that 96.8% of the collected medicinal plants were contaminated with *Bacillus cereus*,19.2% of them contained levels higher than 10 spores/g. Frank, (1989) was summarized data that investigation analyzed 578 samples of crude plant and he reported that total viable count <10 to >10 CFU/g, coliform bacteria up to 10 , *Staph. aureus* detected in <1.3% of the samples and Salmonella were detected in 1.3% of the samples.

Table 1. Mean counts of microorganisms detected in tea kinds samples.

No.	Tea kinds	Total count	Sporform Bac.	Coliform bac.
1-	Lira	$13.5*10^5$	$1.5*10^3$	---
2-	Al-Sanabul	$6*10^5$	$1*10^3$	$5*10^4$
3-	Alton kaya	$5*10^5$	$1.5 * 10^3$	$2*10^4$
4-	Lipton	$14*10^5$	$2*10^3$	$3*10^4$
5-	Gold leaf	$20*10^5$	$5*10^3$	$4.5*10^4$
6-	Rabeea tea	$23*10^5$	$5*10^3$	$6.5*10^4$
7-	Ahmed	$22*10^5$	$3.5*10^3$	---
8-	Merd	$17*10^5$	$3.5*10^3$	$4*10^4$
9-	Dorra AL-ottuor	$11*10^5$	$2*10^3$	$2*10^4$
10-	ALWazah	$20*10^5$	$4.5*10^3$	$4.5*10^4$
11-	ALGhazaleen	$25.5*10^5$	$5*10^3$	$3*10^4$
12-	Gihan	$5.5*10^5$	$2.5*10^3$	---
13-	Balabel	$12*10^5$	$2*10^3$	$4*10^4$
14-	Barari	$8*10^5$	$3*10^3$	$2*10^4$
15-	Apple	$14*10^5$	$2*10^3$	$2.5*10^4$
16-	Mahmood	$19.5*10^5$	3*103	---
17-	Bent AL-Azhaar	$8*10^3$	$2.5*10^3$	$4*10^4$
18-	AL-Qeethara	$10*10^5$	$3*10^3$	$3*10^4$
19-	Seven Elephants	$11.5*10^5$	$2*10^3$	$1.5*10^4$
20-	AL-Badea	$10*10^5$	$4*10^3$	$2*10^4$
21-	AL-Inab	$16*10^5$	$3*10^3$	$6*10^4$
22-	AL-Hilal	$13.5*10^5$	$3*10^3$	$1.5*10^4$

Fungal contamination:

Table 2 Indicated the frequency of distribution of the 23 samples of black tea samples according to the fungi contamination. The results showed the fungi were founds in all samples, and were the *A. niger* isolate was appear as the most dominant in all the brands, and the other fungal isolated were *A. flavus, A. parasiticus, A. ochraceus, Alternaria and penicillium*. These result are in agreement with those of previous studies Aziz, *et al* (1998) : EL-shafie, *et al* (1999) and Mandeel, (2005) whom presence of a wide range of storage fungi indicates that considerable improvements could be made during post-harvest storage. These results approximate with previous reports that showed *Aspergillus flavus*, in particular, was the main contaminant of different herbal and spices samples.

Aflatoxins production:

Table 3 Illustrated the frequency of distribution of the 22 Samples of black tea samples according to the aflatoxin produced. The results showed the highest concentration of aflatoxin was production in Apple, and Merd at (11.98 and 10.86) µg/kg respectively, and the lowest concentration were produced in Golden, and AL-Sanabul at (4.54 to 7.32) µg/kg respectivily. These results agree with the finding of Abdel-Hafez and El-Maghraby,(1992) they proved that tea powder was contaminated by 72 µg aflatoxin per kg dry tea samples. The highest quantity was produced on Al-Fares, Ezi-Nasser, Al- Nakhil and Blue tea Pot brands of tea samples and the lowest one was produced on Shimto, Yaquot, Brooke Bond and Khan El-Khallily. In other studies, Hassan and Abdel-Sater 1993 found the amount of aflatoxin varied from 26-81 µg/kg dry tea. The highest aflatoxin quantity produced in Al-Fares, Ezi-Nasser, Lipton and Tayseer tea. The lowest quantity produced on Khan El-Khallily and Massgeed tea.

Table 2. Distribution of the fungi detectedin samples of tea kinds samples.

No.	Tea kinds	A. niger	A. flavus	A. parasiticus	A. ochraceus	Alternaria	Penicillium spp.
1-	Lira	$1*10^5$	---	---	---	---	$1*10^5$
2-	Al-Sanabul	$1.5*10^5$	$2*10^5$	$1*10^5$	---	---	---
3-	Alton kaya	$2.5*10^5$	$1*10^5$	---	$1*10^5$	---	---
4-	Lipton	$3.5*10^5$	$1*10^5$	---	$1*10^5$		
5-	Gold leaf	$4*10^5$	$2*10^5$	$2*10^5$	---	---	$1*10^5$
6-	Rabeea tea	$4*10^5$	$1*10^5$	$1*10^5$	$1*10^5$	---	---
7-	Ahmed	$2.5*10^5$	---	---	---	$1*10^5$	---
8-	Merd	$1.5*10^5$	$1*10^5$	---	---	---	---
9-	Dorra AL-ottuor	$1*10^5$	$1*10^5$	---	---	---	$1*10^5$
10-	ALWazah	$1*10^5$	$1*10^5$	---	$1*10^5$	---	---
11-	ALGhazaleen	$1*10^5$	$1*10^5$	---	---	---	---
12-	Gihan	$3*10^5$	---	---	---	---	---
13-	Balabel	$3*10^5$	---	$1*10^5$	---	---	$1*10^5$
14-	Barari	$2*10^5$	$1*10^5$	---	---	---	---
15-	Apple	$2*10^5$	$3*10^5$	---	---	$1*10^5$	$1*10^5$
16-	Mahmood	$1.5*10^5$	$1*10^5$	---	---	---	---
17-	Bent AL-Azhar	$1*10^5$	$2.5*10^5$	---	---	---	$1*10^5$
18-	AL-Qeethara	$3*10^5$	2*105	$1*10^5$	---	---	---
19-	Seven Elephants	$2.5*10^5$	$1*10^5$	---	$1*10^5$	---	---
20-	AL-Badea	$1.5*10^5$	$1*10^5$	$1*10^5$	---	---	---
21-	AL-Inab	$2*10^5$	---	$3*10^5$	---	---	$1*10^5$
22-	AL-Hilal	$2*10^5$	$1*10^5$	$1*10^5$	$1*10^5$	---	$2*10^5$

Table 3. Distribution of Aflatoxin B1 analyzed in this study.

No.	Tea kinds	Aflatoxin B1 (µg/kg)Dry tea	No.	Tea kinds	Aflatoxin B1 (µg/kg)Dry tea
1-	Lira	-----	12-	Gihan	-----
2-	Al-Sanabul	7.32	13-	Balabel	10.34
3-	Alton kaya	-----	14-	Barari	-----
4-	Lipton	-----	15-	Apple	11.98
5-	Gold leaf	9.5	16-	Mahmood	-----
6-	Rabeea tea	4.54	17-	Bent AL-Azhar	8.58
7-	Ahmed	-----	18-	AL-Qeethara	10.5
8-	Merd	10.86	19-	Seven Elephants	-----
9-	Dorra AL-ottuor	-----	20-	AL-Badea	-----
10-	ALWazah	-----	21-	AL-Inab	-----
11-	ALGhazaleen	-----	22-	AL-Hilal	-----

دراسة التلوث الميكروبي وانتاج سموم الافلا في بعض انواع الشاي الاسود في الاسواق العراقية

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الخلاصة

هدفت هذه الدراسة الى محاولة معرفة مدى التلوث الميكروبي لعينات بعض انواع الشاي الاسود وكذلك تركيز سموم الافلا فيها. اذ تم جمع اثنان وعشرون عينة من انواع الشاي الاسود من الاسواق المحلية في العراق. اظهرت النتائج انه تم عزل الفطر *A. niger* من كل انواع العينات قيد الدراسة، كما تم عزل الانواع الاخرى من الفطريات مثل *A. flavus, A. parasiticus, A. ochraceus, Alternaria ,penicillium.* اما بالنسبة للانواع البكتيرية فتم عزل انواع منها لاسيما المنتجة للسبورات وبكتريا القولون، بينما لم تظهر بكتريا *Salmonella, Staphyllococcus aurous*

اما سموم الافلا فقد وجدت في 37 % من العينات وبمعدل 4.54 – 11.98 ميكروغرام \ كغم من الشاي الاسود. واظهرت النتائج ان اعلى تركيز من سموم الافلا كان في الشاي نوع التفاحة يليه شاي مرد ثم القيثارة، اما اقل التراكيز فقد كان في شاي ربيع يليه شاي السنابل.

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