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CONTRIBUTION OF THE FUNGAL FLORA AND MYCOTOXINS OF SOME CANNED TOMATO PASTE SAMPLES

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الفلورا الفطرية وسموم الفطريات في بعض عينات صلصة الطماطم

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تم في هذا البحث عزل وتعريف ٢٢ نوعا تنتمي إلى سبعة أجناس فطرية من ٢١ عينة من صلصة الطماطم وذلك على نوعين من الأوساط الغذائية عند ٢٨ ± ٢°م . باستخدام الوسط جلوكوز – كزابكس أجار تم عزل ١٤ نوعاً تنتمي إلى خمسة أجناس بينما على الوسط جلوكوز – كزابكس أجار المزود بتركيز ١٠٪ كلوريد صوديوم تم عزل ١٧ نوعاً تنتمي إلى ست أجناس فطرية . كان التعداد الكلي للفطريات في جميع العينات المختبرة على الوسط الأول ٦١٨٩٠ مستعمرة لكل جرام بينما على الوسط الثاني كان التعداد الكلي ٢٢٢٥٠ مستعمرة لكل جرام . كانت أكثر الفطريات انتشارا على الوسط الأول هي **اسبرجيلس فيوميجاتس ، اسبرجيلس فلافس ، اسبرجيلس نيجر ، وبنيسيليوم أوكراليكم** أما على الوسط الثاني فكانت الفطريات الأكثر انتشارا هي **اسبرجيلس نيجر ، اسبرجيلس فلافس ، اسبرجيلس سيداوي ، وايروتيم مونتيفيدنسس** .

تم استخلاص السموم الفطرية الموجودة في هذه العينات بواسطة الكلوروفورم . درست سمية تلك المستخلصات بالإستعانة ببرقات قشريات الملاحات رخوية الهيكل **(ارتيميا ساليني)** وهي شديدة الحساسية للتركيزات الضئيلة من سموم الفطريات . وقد تم التعرف على السموم الموجودة بطرق التحليل الكروماتوجرافي باستخدام رقائق السليكا وعديد من المذيبات والكواشف اللونية . وقد ثبت أن مستخلص ١٣ عينة لها تأثير سام على هذه اليرقات . كما أظهر التحليل تلوث العينات ذات التأثير السام على اليرقات بسموم الافلاتوكسين G_1 , B_1 بتركيزات تتراوح ما بين ٤٠ إلى ١٢٠ ميكروجرام لكل كيلو جرام .

Key Words: Tomato paste, Mycoflora, Fungi, Mycotoxins, Aflatoxins.

ABSTRACT

Twenty-two species belonging to seven genera were isolated and identified from 21 tomato paste samples on glucose-Czapek's (5 genera and 14 species) and 10% NaCl-glucose-Czapek's (6 genera and 17 species) agar media, at 28 ± 2°C. On glucose-Czapek's agar medium the gross total count of fungi was 61890 colonies/g in all samples and the most common fungi were *Asperigillus fumigatus, A. flavus, A. niger* and *Penicillum oxalicum*. On 10% NaCl-glucose-Czapek's agar, the gross total count of halophilic and/or halotolerant fungi was 22250 colonies/g and the most common species were *Aspergillus niger, A. flavus, A. sydowii* and *Eurotium montevidensis*.

The chloroform extracts of 13 samples of tomato paste were toxic to brine shrimp *(Artemia salina)* larvae. Thin-layer chromatographic analysis revealed that the toxic samples were naturally contaminated with aflatoxins B_1 and G_1 (40-120 µg/kg).

INTRODUCTION

Since the discovery of aflatoxin in 1960-1961, extensive investigations concerning contamination of human food-stuffs by toxins and toxin producing fungi have been conducted in many areas of the world. As a continuity to the previous investigations achieved in this laboratory regarding the mycoflora and mycotoxins of different food sources in Egypt [1-10], this study was carried out to: a- determine the types of fungi which may exist as contaminants in the different local and imported brands of tomato paste. b- determine whether a potential hazard may exist due to contamination of tomato paste with mycotoxins.

MATERIALS AND METHODS

Collection of samples

Twenty one samples of canned tomato paste were collected from shops and markets of different sanitation levels at Assiut and Sohag Governorates in Egypt. These samples include three samples which represent the major brands available for sale in the local market. The different brands tested are:

Brand I: Samples 1-3, Balkan tomato paste, Bulgaria.
Brand II: Samples 4-6, Five stars tomato paste, Greece.
Brand III: Samples 7-9, Fondana tomato paste, Greece.
Brand IV: Samples 10-12, Foodico tomato concentrate, Egypt.
Brand V: Samples 13-15, Kaha tomato concentrate, Egypt.
Brand VI: Samples 16-18, Edfina tomato concentrate, Egypt.
Brand VII: Samples 19-21, Prodexport tomato paste, Romania.

All canned tomato paste samples were opened under aseptical conditions in the laboratory immediately before mycological and mycotoxins analysis. Visual examination of the samples revealed that all samples examined were of normal color, odor and taste.

Determination of fungi

This was made by using the dilution plate method as described by Christensen [11], but with some modification as employed by Moubasher *et al.*, [12]. Two types of media were used:

(i) Glucose-Czapek's agar, which consisted of (g per liter of distilled water): sodium nitrate, 2.0; potassium dihydrogen phosphate, 1.0; magnesium sulphate, 0.5; potassium chloride, 0.5; ferrous sulphate, 0.01; glucose, 10.0 and agar, 15.0.

(ii) Glucose-Czapek's agar supplemented with 10% sodium chloride.

Streptomycin (20 µg/ml) and rose bengal (30 µg/ml) were applied to suppress bacterial growth [13, 14]. Ten plates were used for each sample (5 plates for each type of medium). The plates were incubated at 28 ± 2°C for 8-15 days during which the developing colonies were counted and identified and the numbers were calculated per g of each sample.

Identification

Purified fungal isolates were identified, whenever possible, in the original Petri-dish culture. When this was not possible, fungi were subcultured and stored for later identification, according to references [15-25].

Mycotoxins analysis

Twenty g of each sample were transferred into a blender jar. 100 ml of chloroform were added. The contents were homogenized for five min. at low speed and three min. at high speed. The extract was filtered through a fluted filter paper (Whatman No. 4). The extraction procedure was repeated three times. The combined chloroform extracts were washed with equal volume of distilled water, dried over anhydrous sodium sulphate, filtered then concentrated under vacuum to near dryness. The residue was diluted with chloroform to one ml.

Chromatographic analysis of the chloroform extracts were achieved on precoated silica gel plate type 60 F 254 (Merck) for the presence of aflatoxins B_1 B_2 ,G_1 & G_2 , citrinin, diacetoxyscirpenol, ochratoxins, patulin, rubratoxins, sterigmatocystin, T-2 toxin, and zearalenone according to [16-28].

The presence of aflatoxins in the chloroform extracts was confirmed by derivative methods of Przybylski [29] and quantitatively determined according to the method of Jones [30].

Bioassay of toxins

Brine shrimp (*Artemia salina* L.) larvae were used for toxins bioassay according to the method described by Korpinen [31].

a) 15-20 drops of brine shrimp eggs (HADLOW, KENT, ENGLAND) were hatched in artificial sea water (NaCl, 30 g; $CaSO_4$, 2 g; $MgSO_4$, 7 H_2O, 3g; MgCl, 8.5 g; KCl, 0.8 g and MgBr, 0.1 g; per liter distilled water and adjusted to pH 10 with NaOH) and kept at room temperature (22-24°C). Air is usually conducted into the water in small bubbles through a tube. Three days after the emergence, the hatched larvae were used as test animals. In order to obtain the desired concentration of the larvae, they were filtered through ordinary filter paper and resuspended in a known volume of sea water.

b) 0.05 ml of chloroform extract was placed in each test tube, the chloroform was evaporated and about 20-40 shrimp larvae in one ml sea water were transferred into the tubes. The tubes were kept at room temperature. Control tubes with 0.05 ml of chloroform were also made.

c) After 24 h, the mortality was determined with a stereoscopic microscope.

Source of mycotoxin standards

All of mycotoxin standards used throughout this study were kindly provided by Prof. Dr. I.A. El-Kady, Botany Dept., Fac. Sci., Assiut Univ., Egypt.,

RESULTS AND DISCUSSION

Mycoflora of tomato paste samples

The total count of filamentous fungi in the different samples tested was widely fluctuated between 1666-3849 and 845-1215 colonies/g on glucose-Czapek's and 10% NaCl-glucose-Czapek's agar media, respectively. Twenty-two species belonging to seven genera were isolated and identified from 21 tomato paste samples on plates of glucose-Czapek's (5 genera and 14 species) and 10% NaCl-glucose-Czapek's (6 genera and 17 species) agar media at 28 ± 2°C. (Tables 1 & 2). The gross total counts of glucophilic and halotolerant or halophilic fungi in all samples tested were 61890 and 22250 colonies /g fresh weight, respectively. All of these fungi were previously recovered from different food sources in this laboratory [1-10, 12, 32-34].

Aspergillus was the common genus on the two types of media used, and recovered from all samples constituting 89% and 77.2% of total fungi on glucose-Czapek's and 10% NaCl-glucose-Czapek's agar, respectively. The genus was represented by nine species of which *Aspergillus flavus* and *A. niger* were the common on the two types of media. *A. fumigatus* was isolated with high and low frequencies of occurrence on glucose-Czapek's and 10% NaCl-glucose-Czapek's agar media, respectively. *A. sydowii* was isolated with moderate occurrence on 10% NaCl-glucose-Czapek's agar and from one sample only on the other type of media. The remaining five *Aspergillus* species were less frequent (Table 1). All of these species were isolated previously, but with variable densities and frequencies, from different food sources [2, 4, 5, 10, 12, 32, 34]. El-Kady *et al.,* [3] isolated *Aspergillus flavus* var. *columnaris* and *A. niger* from tomato paste.

Eurotium was isolated with high frequency on 10% NaCl-glucose-Czapek's agar medium only. It was recovered from more than 50% of the samples matching 7.46% of the gross fungal count. It was represented by five species of which *E. montevidensis* was isolated with moderate frequency of occurrence while, *E. amstelodami, E. halophilicum, E. chevalieri* and *E. repens* were isolated with low or rare frequencies of occurrence (Table 1). Moubasher *et al.,* [35] classified these species as highly halophilic fungi, which grow on 5% to 20% or 25% NaCl. El-Kady *et al.,* [36] isolated *Eurotium amstelodami, E. chevalieri* var. *intermedium, E. montevidensis* and *E. rubrum* from four types of seed in Egypt on 15% NaCl-water agar medium as a halophilic species.

Penicillium was recorded in moderate frequency of occurrence on the two types of media used and recovered from 38.57% and 33.3% of the samples constituting 9.95% and 13.75% of total fungi on glucose-Czapek's and 10% NaCl glucose-Czapek's agar media, respectively. Four species of *Penicillium* were isolated and identified: *P. oxalicum* and *P. chrysogenum* on glucose-Czapek's; *P. expansum* on 10% NaCl-glucose-Czapek's; and *P. citrinum* on the two types of media (Table 1). These four species were isolated previously from different food sources in this laboratory [1-5, 10, 34].

Table 1

Average total counts (ATC, calculated per g fresh weight in all samples), number of cases of isolation (NCl, out of 21 samples) and occurrence remarks (OR) of fungal genera and species isolated from tomato paste samples on glucose-Czapek's and 10% NaCl-glucose-Czapek's agar media at 28 ± 2°C

Genera & Species	Glucose-Czapek's agar		10% NaCl glucose-Czapek's agar	
	ATC	NCl & OR*	ATC	NCl & OR*
Aspergillus	55090	21 H	17170	21 H
A. fumigatus Fresenius	42490	21 H	120	4 L
A. flavus Link	3960	19 H	7760	19 H
A. niger Van Tieghem	8080	18 H	7950	21 H
A. awamori Nakazawa	320	3 L	0	0
A. candidus Link	40	2 R	0	0
A. ochraceus Wilhelm	20	1R	80	3 L
A. sydowii (Bain. & Sart.) Thom & Church	20	1 R	340	9 M
A. terreus Thom	160	1 R	120	2 R
A. versicolor (Vuill.) Tiraboschi	0	0	800	2 R
Eurotium	0	0	1660	11 H
E. montevidensis Talice & Mackinnon	0	0	280	10 M
E. amstelodami Mangin	0	0	120	4 L
E. halophilicum Christensen, Papavizas & Benjamin	0	0	1220	3 L
E. chevalieri Mangin	0	0	20	1 R
E. repens De Bary	0	0	20	1 R
Penicillium	6160	8 M	3020	7 M
P. oxalicum Currie & Thom	6020	8 M	0	0
P. citrinum Thom	100	2 R	220	4 L
P. chrysogenum Thom	40	1 R	0	0
P. expansum (Link) Thom	0	0	2800	4 L
Emericella nidulans (Eidam) Vuillemin	440	2 R	340	2 R
Giberella fujikuroi (Sawada) Wollenw.	160	2 R	40	1 R
Trichoderma viride Pers. ex S.F. Gray	40	1 R	0	0
Scopulariopsis halophilica Tubaki	0	0	20	1 R
Gross total count	61890		22250	
Number of genera (7)	5		6	
Number of species (22)	14		17	

* OR : Occurrence remarks:
H : High occurrence, between 11-21 cases (out of 21).
M : Moderate occurrence, between 5-10 cases.
L : Low occurrence, 3 and 4 cases.
R : Rare occurrence, 1 and 2 cases.

Table 2
The total counts (TC, calculated per g fresh weight in each samples), number of genera (NG), number of species (NS) and the dominant species of fungi isolated from tomato paste samples on glucose-Czapek's and 10% NaCl-glucose-Czapek's agar media at 28 ± 2°C

S. No.	glucose-Czapek's agar medium				10% NaCl-glucose-Czapek's agar			
	TC	NG	NS	The dominant species	TC	NG	NS	The dominant species
1	3849	2	5	*Aspergillus flavus, A. niger*	1123	2	5	*A. flavus, A. niger*
2	3364	1	4	*A. flavus, A. fumigatus, A. niger*	1198	2	5	*A. flavus, A. niger*
3	3548	1	4	*A. flavus, A. fumigatus, A. niger*	1136	2	4	*A. flavus, A. niger*
4	2938	1	2	*A. niger, A. terreus*	1069	3	4	*P. expanasum. A. niger*
5	2929	2	4	*Penicillim oxalicum, A. fumigatus*	1111	2	3	*P. expanasum, A. niger*
6	3146	2	6	*a. flavus, A. fumigatus, A. niger*	1177	2	3	*P. expanasum, A. niger*
7	2173	2	4	*P. oxalicum, A. fumigatus, A. niger*	936	2	5	*A. versicolor, A. niger*
8	1666	2	4	*P. oxalicum, A. fumigatus*	1023	2	5	*A. versicolor, A. niger*
9	1876	2	4	*P. oxalicum, A. fumigatus, A. niger*	939	2	6	*A. flavus, A. niger*
10	2857	1	4	*A. flavus, A. fumigatus*	961	2	7	*A. flavus, A. niger*
11	2736	2	3	*Emericella nidulans, A. fumigatus*	938	2	4	*E. nidulans, A. niger*
12	2518	2	4	*E. nudulans, A. fumigatus, A. awamori*	845	2	4	*A. flavus, A. niger*
13	2755	1	3	*A. flavus, A. niger*	926	2	3	*A. flavus, A. niger*
14	3137	1	3	*A. flavus, A. fumigatus*	1075	2	6	*A. flavus, A. niger*
15	2683	1	3	*A. fumigatus, A. niger*	1036	2	4	*A. flavus, A. niger*
16	3178	1	3	*A. flavus, A. fumigatus*	1034	2	5	*E. halophilicum, A. niger*
17	2988	1	3	*A. flavus, A. fumigatus*	1066	2	6	*E. halophilicum, A. flavus*
18	3351	1	4	*A. flavus, A. fumigatus*	1149	2	3	*E. halophilicum, A. flavus*
19	3435	3	5	*A. flavus, A. niger, P. oxalicum*	1133	2	3	*A. flauvs, A. niger*
20	3535	3	4	*A. flavus, A. niger, P. oxalicum*	1215	2	3	*A. flavus, A. niger*
21	3228	2	5	*A. flavus, A. niger, P. oxalicum*	1160	2	3	*A. flavus, A. niger*

Emericella nidulans and *Giberella fujikuroi* were isolated in less frequencies on the two types of media used. *Trichoderma viride* was recorded in one sample only on glucose-Czapek's agar while *Scopulariopsis halophilica* was isolated from one sample on 10% NaCl-glucose-Czapek's agar only as shown in Table 1. The preceding genera and species were previously isolated, but with variable densities and frequencies, from different food sources in this laboratory.

In conclusion, it could be said that there were no specific fungal flora for tomato paste, since these mycoflora were recovered from different food sources.

Table 3
Toxicity test (Tt*) and natural occurrence of mycotoxins detected in chloroform extracts of different tomato paste samples

Sample No.	Tt*	Toxins detected	µg/kg	Sample No.	Tt*	Toxins detected	µg/kg
1	a	Aflatoxins B_1 + G_1	120	12	d	- ve	—
2	b	Aflatoxins B_1 + G_1	80	13	b	Aflatoxins B_1 + G_1	50
3	b	Aflatoxins B_1 + G_1	70	14	b	Aflatoxins B_1 + G_1	60
4	d	-ve	—	15	d	- ve	—
5	d	- ve	—	16	c	Aflatoxins B_1 + G_1	80

Table 3 Contd.

Sample No.	Tt*	Toxins detected	µg/k g	Sample No.	Tt*	Toxins detected	µg/k g
6	c	Aflatoxins B_1 + G_1	40	17	a	Aflatoxins B_1 + G_1	90
7	d	-ve	—	18	b	Aflatoxins B_1 + G_1	80
8	d	- ve	—	19	a	Aflatoxins B_1 + G_1	100
9	d	- ve	—	20	a	Aflatoxins B_1 + G_1	110
10	b	Aflatoxins B_1 + G_1	80	21	B	Aflatoxins B_1 + G_1	90
11	D	- ve	—				

*Tt (Toxicity test):
a = High toxicity; more than 75% mortality of larvae test.
b = Moderate toxidity; between 50-74% mortality of larvae test.
c = Low toxicity; between 25-49% mortality of larvae test.
d = non toxic; less than 25% mortality of larvae test.

Natural occurrence of mycotoxins

The toxicity test using brine shrimp larvae revealed that the chloroform extracts of 13 samples of tomato paste were toxic to the test organism. Thin-layer chromatographic analysis of the chloroform extracts of the different tomato paste samples revealed that 13 out of the 21 samples (more than 60% of the samples) were naturally contaminated with aflatoxins B_1 and G_1 at concentrations ranged between 40 and 120 µg/kg (Table 3). Although several samples proved to be contaminated by various toxic fungi, however only aflatoxin was detected in the crude extract of some samples. This clearly shows that the presence of mycotoxic fungi in a product does not automatically indicate the presence of mycotoxins, especially if growth has not occurred.

According to the available literatures, this is the first record about contamination of tomato paste by aflatoxin. Based on laboratory studies and surveillance reports of aflatoxin detected in commercial products, the commodities most likely to serve as substrates for aflatoxin production are pea-nuts, brazil-nuts, pecans, wal-nuts, almond, filberts, pistachio-nuts, cotton seeds, copra, corn, sorghum, millet and figs [37].

Most of the positive samples were highly infected with *Aspergillus flavus*. From the view point of direct hazard to health, aflatoxins are the most important among the known mycotoxins [38]. Aflatoxins are mutagenic, carcinogenic, teratogenic and actually toxic to most experimental and domesticated animal and man [39, 40].

Citrinin, diacetoxyscirpenol, ochratoxins, patulin, rubratoxins, sterigmatocystin, T-2 toxin, and zearalenone were not detected in any sample of tomato paste tested.

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