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MOULD MYCOFLORA OF SOME SAUDI ARABIAN POTATO CHIPSY

(With 2 Tables)

By

LAILA A. NASSER

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الفلورا الفطرية لبعض أنواع الشيبسي في المملكة العربية السعودية

ليلى أحمد ناصر

يهدف هذا البحث الى التعرف على الفلورا الفطرية المصاحبة لبعض أنواع الشيبسى التي تباع في أسواق الرياض بالمملكة العربية السعودية . لقد تمت الدراسة على ٣٥ عينة تمثل ١١ نوعاً من الأنواع شائعة الإستعمال بالمملكة العربية السعودية وذلك بإستخدام ثلاثة أوساط غذائية للعزل وهى جلوكوز ــ مستخلص النشا ــ ١٠% كلوريد صوديوم ــ تشابكس آجار والتحضين عند ٢٨°م. ولقد لوحظ أن أكثر العينات تلوثاً بالفطريات هى تسالى ، غندور ، فشفاش ، بينما عينات شامبيون، زيزو ، أطايب ، أتينيتيكو ، باهلسين باكنترى ، جاكن جيل كانت أقلها تلوثاً بالفطريات. تم عزل وتصنيف ٢٥ نوعاً فطرياً تمثل ١١ جنساً وذلك من العينات المختبرة على الأوساط الثلاثة المستخدمة . كانت أكثر الفطريات إنتشاراً وتعداداً هى فطر أسبرجيللس أنواع فلافس ، فيوميجاتس ، نيجر ، بنسيليوم أنواع كريزوجينم ، كوريللوفيلم وأيروتيم أمستيلودامي. وقد وجد أن بعض الأنواع ظهرت على نوع واحد من الوسط الغذائي ولم تظهر على الآخر مثل أكريمونيم ستريكتم ، أسبيرجيللس أوكريشيس ، وأستس ، كوشيلوبلس سبيسيفير ، نيوروسبورا كراسا بنسيليوم دبكلوكسى وأوكساليكم التي أمكن عزلها فقط على الوسط الغذائى النشا الآجارى بينما أيميرسيلا نيديولانس وأيروتيم أمستيلودامي عُزلت فقط على الوسط الغذائى ١% جلوكوز ــ تشابكس آجار والمضاف اليه ١٠ % كلوريد صوديوم. تم إختبار بعض الفطريات والتي عُزلت بترددات عالية فى هذا البحث وذلك لدراسة مقدرتها على إفراز إنزيم الأميليز . وقد وجد أن أكثر من ٦٠% من العزلات المختبرة لها القدرة على إفراز هذا الإنزيم . ولقد لوحظ أيضاً أن معظم العزلات لها نشاط عالي على إفراز هذا الإنزيم ، بينما العزلات الباقية لها نشاط متوسط أو ضعيف على إفراز الإنزيم .

SUMMARY

Survey of mycoflora in 35 samples representing 11 kinds of chipsy commonly consumed in Saudi Arabia was evaluated using 1% glucose-, 1% starch-, and 10% sodium chloride-Czapek's agar at 28°C. The most contaminated samples were Tasali, Gandour, and Fesh fash with high incidence of mycoflora while samples of Champion, Zizo, Ataib, Authentico, bahlesen picantrie and Jack'n jill were less contaminated with fungi. Twenty-five species representing 11 genera were isolated from the samples tested on the three media used. The most frequently isolated fungi were *Aspergillus flavus, A. fumigatus, A. niger, Penicillium chrysogenum, P. corylophilum* and *Eurotium amstelodami*. The starch-decomposing species such as *Acremonium strictum, A. ochraceus, A. ustus, Cochliobolus spicifer, Neurospora crassa, Penicillium dauclauxi* and *P. oxalicum* were only isolated on starch agar plates whereas the halophilic or halotolerant *Emericella nidulans* and *Eurodium chevalieri* were only encountered when using 1% glucose-Czapek's agar medium supplemented with 10% sodium chloride. The high frequently encountered fungi in the current study were tested for their ability to amylase production. 60% of the isolates could produce this enzyme and the most isolates exhibited high amylolytic activity and the remaining isolates showed moderate or weak production.

Key words: *Mould mycoflora, Potato*

INTRODUCTION

Mould spoilage of baked foodstuffs was confined to the crust, where moulds like *Aspergillus, Penicillium* and *Cladosporium* are capable of rapid growth (Seiler, 1986, 1987). Since baking process kills moulds and mould spores, mould contamination occurs after baking by long storage in high temperature, during processing and transporting of food products (Odell, 1983). The identification of the mycoflora of foodstuffs helps to estimate the probable types of mycotoxins that might be produced (King et al., 1981; Leistner, 1984; Megalla et al., 1985; Lacey, 1988; Zohri et al., 1995 and Siame et al., 1998).

Chipsy is one of the most popular food products consumed in Saudi Arabia especially by children as well as in the other countries. They are prepared mainly from baked potatoes and other ingredients such as vegetable oils, natural flavours, salts and spices. Contamination of

foodstuffs with spoilage fungi leading to great economic losses (Beuchat, 1978), besides constituting a major public health hazard by producing a wide variety of mycotoxins (FAO, 1979).

This study assessed the mould populations associated with different kinds of chipsy and the production of the enzyme amylase by the fungi that could lead to degradation of the ingredients was also evaluated.

MATERIAL and METHODS

Thirty-five samples of eleven different trade marks of chipsy were collected from shops/markets in Riyadh, Saudi Arabia. The samples were transferred to the laboratory and kept in a refrigerator (5-7°C) until identification of the fungi had been undertaken.

Determination of fungi:

Three media were used for this propose: 1% glucose-, 1% starch and 10% sodium-chloride-Czapek's agar. Five segments of snack (chipsy) were put on the surface of each agar plate, nine plates for each sample (3 each medium) were used. The plates were incubated at 28°C for 1-2 weeks, and the growing fungi were identified (Raper and Fennell, 1977; Pitt, 1979; Domsch et al., 1980; Kozakiewicz, 1989).

Screening for amylase enzyme production:

Starch hydrolysis was detected on Czapek's agar with the sucrose replaced by 10g soluble starch. A positive reaction was indicated by the appearance of a clear zone around the colony after flooding with Gram's iodine solution (Bridge, 1985).

RESULTS

Results are obtained at Tables 1 and 2.

DISCUSSION

A total viable counts of fungi in the samples tested fluctuated from 140-219 colonies/525 segments in all samples, on glucose- (151 colonies), starch- (140 colonies) and 10% NaCl-Czapek's agar (219 colonies) at 28°C. Tasali, Gandour and Fesh Fash snacks had the highest number of fungi and Champion, Zizo, Ataib, Authentico, bahlesen Picontrie and Jack's Jill the lowest (Table 1).

Twenty-five species representing 11 genera were isolated from the samples tested on glucose- (16 species + 7 genera), starch- (16+6) and 10% NaCl-Czapek's agar (13+4) at 28°C. In this respect, most of these fungi have been reported previously from different food materials such as snacks (Zohri et al., 1995), Biscuits (Abdel-Sater and Ismail, 1993) some grains and seeds (El-Kady et al., 1982; Abdel-Hafez, 1984; Megalla et al., 1985; Lund et al., 1996; Viljoen and Holy, 1997) as well as from other foods (Samson et al., 1995; Scholte, 1995; Hartog and Kuik, 1984)

Aspergillus (10 species) was the most prevalent genus encountered in 86%, 91% and 69% of the samples comprised 68.2%, 75.7% and 52.9% of total fungi, on glucose-, starch- and 10% NaCl-Czapek's agar, respectively. The most frequently encountered species from the genus were: *A. flavus, A. fumigatus* and *A. niger*. They were isolated from 37-60%, 23-74% and 49-51% of the samples on the three isolation media, respectively. Some fungal species were recovered on two media and not recovered on the third such as: *A. fumigatus, A. sclerotiorum*, on glucose- and starch and not on 10% NaCl., whereas *A. ochraceus* on starch and 10% NaCl, *A. terreus* on glucose- and 10% NaCl. On the other hand, *A. egyptiacus* and *A. versicolor* were isolated from the tested samples on glucose- or on the three isolation media in rare occurrence, (Table 1). There are records which showed that some of these fungi produced several toxic substances (Debeaupuis and La Font, 1978; Charles et al., 1979; Wyllie and Morehouse 1977) and such foodstuffs could possibly be unsuitable for human consumption, if heavily contaminated. The results of the present work are in close agreement with those recorded by Megalla et al. (1985) and Aran and Eke (1987) who noticed that *A. niger* and *A. flavus* were the most common in the Egyptian and the Turkish foodstuffs, respectively. Also, *Aspergillus* species were isolated from snacks and biscuits substrates in high occurrence as reported by Zohri et al. (1995); Abdel Sater and Ismail (1993). All *Aspergillus* species isolated in the current study were encountered previously in various types of foodstuffs, seeds and grains in Egypt (El-Kady et al., 1982; Abdel-Hafez, 1982; Zohri, 1990) and in many parts of the world (Salcado and De Carvalho, 1980; Supriaman and Palmer, 1981 and Abdel-Hafez, 1984; Lund et al., 1996; Viljoen and Holy, 1997; Siame et al., 1998).

Penicillium (five species) was the second common genus behind *Aspergillus* representing 51%, 40% and 43% of the samples constituting 23.2%, 20% and 18.7% of the total fungi on the three media used, respectively. From the five species identified *P. chrysogenum* and *P. corylophilum* were the prevalent species. They were isolated from 11-43%,

17-29% and 14-26% of the samples contributing 2.6-16.6%, 5.7-10.7% and 5.9-7.8% of total fungi, respectively (Table 1). *Penicillium oxalicum* and *P. aurantiogriseum* were only isolated, in low or rare occurrence from two media and not encountered from the third medium (Table 1). The obtained results were nearly similar to those obtained by Zohri et al. (1995) and Abdel-Sater and Ismail (1993). They reported that the genus *Penicillium* was the most prevalent behind *Aspergillus* in some foodstuffs, snacks and biscuits. Also, *Penicillium* species were previously reported as common fungi in several food materials or grains and seeds or some breads as indicated by Megalla et al., 1985; Aran and Eke, 1987; Pitt and Hocking, 1985; Lund et al., 1996; Viljoen and Holy, 1997; Siame et al., 1998.

Eurotium (2 species) as halophilic genus, which was isolated only from one sample on glucose-Czapek's agar and not on starch agar, and was in high occurrence on medium supplemented with 10% NaCl. It was represented in 54% of the samples comprising 24.7% of total fungi. *E. amstelodami* (43% of the samples) and *E. chevalieri* (31%) were only identified using 10% sodium chloride-Czapek's agar (Table 1). This genus was also previously isolated from different food, seeds and grains on media containing 20-50% sucrose or supplemented with different concentrations of sodium chloride (Abdel-Sater and Ismail 1993; Zohri et al. 1995; Lund et al., 1996; and several authors).

The remaining identified fungi were rare in frequency of occurrence on one or two medium. These were: *Acremonium strictum* (6%), *Cochliobolus spicifer* and *Neurospora crassa* (3% each) on starch-Czapek's agar; *Altrenaria alternata* and *Gibberella fujikuroi* (6% and 3%) on glucose-; *Emericella nidulans* (9%) on 10% NaCl-; *Cladosporium cladosporioides* (9% and 6%, respectively) on glucose- and 10% NaCl whereas *Rhizopus stolonifer* (9% and 3%) on glucose- and starch-Czapek's agar (Table 1). These fungi were isolated from other food and foodstuffs (Pitt and Hocking, 1985; Megalla et al., 1985; Aran and Eke, 1987; Abdel-Sater et al., 1993; Zohri et al., 1995; Hartog, 1981; King et al., 1981; Odell, 1983; Samson et al., 1995; Scholte, 1995; Ismail, 1993; King et al., 1979).

In the present study the most prevalent species were screened for their ability to produce amylase enzyme as shown in Table (2). The results indicated that among 60 isolates, representing 19 species appertaining to 9 genera, 36 (60%) isolates could produce amylase and degraded the soluble starch into glucose (Table 2). Of the positive fungal isolates 10 isolates (27.7%) showed high amylase activity and these related to *A. flavus, A. niger, A. ustus, A. versicolor, C. cladosporioides* and *P. aurantiogriseum*.

Of the remainder, 26 isolates had moderate and weak activity (13 isolates each) and these are the fungi belonging to *A. strictum, A. ochraceus, A. sydowii, C. spicifer, E. amstelodami, P. chrysogenum* and *P. oxalicum* (Table 2). These results agree with those obtained by El-Kady et al. (1984), who concluded that about 88% of cultures tested were amylase producers. Also, Ismail (1993) showed that about 75% of 248 isolates had the ability to produce amylase. More recently Abdel-Sater and Ismail (1995) noticed that among 69 isolates tested for their ability to produce amylase enzyme, 61 isolates could produce this enzyme.

REFERENCES

Abdel-Hafez S.I.I. (1984): Composition of the fungal flora of four cereal grains in Saudi Arabia. Mycopathologia 85: 53-57.

Abdel-Sater M.A. and Ismail M.A. (1993): Ecological and enzymatic studies on fungi associated with biscuits in Egypt. International Biodeterioration and Biodegradation 31: 277-292.

Aran N. and Eke D. (1987): Mould mycoflora of some Turkish cereals and cereal products. Mircen J. 3: 281-287.

Beuchat R.L. (1978): Food and Beverage Mycology. Avi. Pub. Co. Inc., Wastport, Connecticut.

Bridge P.D. (1985): An evaluation of some physiological and biochemical methods as an aid to the characterization of species of *Penicillium* sub-section Fasciculata. J. Gen. Microbiol. 131: 1887-1895.

Charles P.; Grost-Allman and Steyn P.S. (1979): Structural elucidation of the nigerones, four new naphthopyrones from cultures of *Aspergillus niger*. J. Chem. Soc. Perkin 1: 2474-2479.

Debeaupuis J.P. and La Font P. (1978): Fumitoxins, new mycotoxins from *Aspergillus fumigatus* Fres. Appl. and Environ. Microbiol. 35: 8-10.

Domsch K.W.; Gams W. and Anderson T.H. (1980): Compendium of soil fungi. Academic Press London.

El-Kady I.A.; Abdel-Hafez S.I.I. and El-Maraghy S.S. (1982): Contribution to the fungal flora of cereal grains in Egypt. Mycopathologia 77: 103-109.

El-Kady I.A.; Mazen M.B. and Saber S.M. (1984): Some enzymatic activities of fungi isolated from cotton seeds and cotton seed products. Qatar Univ. Sci. Bull. 4: 85-93.

FAO (1979): Food and Nutrition, paper No. 13 presespective on mycotoxins. Food and Agricultural Organization of the United Nations, Rome.

Hartog B.I. (1981): The detection and quantification of fungi in Food. In Introduction to food-borne fungi. (ed. R.A. Samson, E.S. Hoekstra and C.A.N. Van Oorschot) Centraalbureau Voor Schimmelcultures, Baarn, The Netherlands, pp. 206-211.

Hartog B.J. and Kuik D. (1984): Mycological studies on Dutch rye bread. In Microbial Associations and Interactions in Food. (ed. Kiss; Deak T. and Incze K.) pp. 241-246. Dordrecht: D. Reidel Publishing Company.

Ismail M.A. (1993): Degratitive enzymes and fungal flora associated with the Egyptian foodstuffs kishk. Inter. Biodet. Biodeg. 31: 143-157.

King A.D.; Hocking A.D. and Pitt J.I. (1979): Dichloran-rose-bengal medium for the enumeration and isolation of molds from foods. Appl. Environ. Microbiol. 37: 959-964.

King A.D.; Hocking A.D. and Pitt J.I. (1981): The mycoflora of some Australian foods. Food Technol. Aust. 33: 55-60.

Kozakiewicz Z. (1989): Aspergillus species on stored products (Mycological papers No. 161) International Mycological Institute, Kew, Surrey, UK.

Lacey J. (1988): The microbiology of cereal grains from areas of Iran with a high incidence of oesophageal cancer. J. Stored Prod. Res. 24 (1): 39-50.

Leistner L. (1984): Toxigenic Penicillia occurring in feeds and foods: a review. Food Technol. Aust. 36: 404-406.

Lund F.; Filtenborg O.; Westall S. and Frisvad J.C. (1996): Associated mycoflora of rye bread. Letter in Applied Microbiology 23: 213-217.

Megalla S.E., Abdou R.F. and Bagy M.M.K. (1985): Fungal flora of Egyptian baladi bread with special reference to the mutagenic effects of their toxic metabolites. Mycopathologia 89: 35-41.

Odell D.E. (1983): Fungi isolated from incubated commercial wheat germ produced in the UK during 1982-83. FMBRA Bull. 6: 281-286.

Pitt J.I. (1979): The genus *Penicillium* and its teleomorphic states, *eupenicillium* and *Talaromyces*. Academic Press, London.

Pitt J.I. and Hocking A.D. (1985): Fungi and Food spoilage Academic Press, Sydney, New South Wales, Australia.

Raper K.B. and Fennell D.I. (1977): The genus *Aspergillus*. R.E. Krieger Publishing Company, Washington, D.C.

Salcado T.M. and De Carvalho J.B. (1980): Toxigenic fungi associated with grains. I- Survey of microflora associated with corn, wheat and rice. Rev. Microbiol. 11: 60-63.

Samson R.A.; Hoekstra E.S.; Frisvad J.C. and Filtenborg O. (1995): Introduction to food-borne fungi. 4th edn. Bearn: Centraalburea voor Schimmelculturs.

Scholte R.P.M. (1995): Spoilage fungi in the industrial processing of food. In Introduction to Food-borne fungi, 4th edn, Samson R.A.; Hoekstra, E.S.; Frisvad J.C. and Filtenborg O. pp. 275-288. Baarn: Centraalbureau voor Schimmel culures.

Seiler D.A.L. (1986): Preventing mould growth on bakery products using ovenable. Lined fibreboard containers. FMBRA Bull. 6: 281-285.

Seiler D.A.L. (1987): The mould-free shelf life of bread. FMBRA Bull. 5: 133-136.

Siame B.A.; Mpuchane S.F.; Gashe B.A.; Allotey J. and Teffera G. (1998): Occurrence of aflatoxins, Fumonisin B1, and zearalenone in foods and feeds in Botswana. J. Food Protect. 61 (12): 1670-1673.

Supriaman J. and Palmer L.T. (1981): Seed-borne fungi of rice in Indonesia. Contributions of Central Research Institute for Agriculture, Bogor D (570: 1-12.

Viljoen C.R. and Holy A. (1997): Microbial populations associated with commercial bread production. J. Basic Microbiol. 37 (6): 439-444.

Wyllie T.D. and Morehouse L.G. (1977): Mycotoxic fungi, Mycotoxins, Mycotoxicoses. Vol. I. Mycotoxic fungi and chemistry of Mycotoxins. Marcel Dekker, Inc., New York.

Zohri A.A. (1990): Mycoflora and mycotoxins of some meat products. Ph.D. Thesis, Botany Department, Faculty of Science, Assiut University, Assiut, Egypt.

Zohri A.A.; Abdel-Sater M.A. and Ismail M.A. (1995): Incidence of aflatoxins and mould flora in corn snacks. J. Food Science and Technology 32: 289-294.

Table (1): Total counts (TC per 15 segments each sample), number of cases of isolation (NCI, out of 35 samples) and occurrence remarks (OR) of fungi isolated from different kinds of chips on glucose-, starch-, and 10% NaCl-Czapek's agar at 28°C.

Genera & species	Glucose-Czapek's agar		Starch-Czapek's agar		10% NaCl-Czapek's agar	
	TC	NCI & OR	TC	NCI & OR	TC	NCI & OR
Acremonium strictum W. Gams	-	-	2	2 R	-	-
Alternaria alternata (Fries) Keissler	3	2 R	-	-	-	-
Aspergillus	103	30 H	106	32 H	116	24 H
A. egyptiacus Moubahser & Moustafa	1	1 R	-	-	-	-
A. flavus Link	55	21 H	59	26 H	35	18 H
A. fumigatus Fresenius	18	13 M	12	8 L	-	-
A. niger Van Tieghom	20	14 M	24	13 M	49	17 M
A. ochraceus Wilhelm	-	-	3	3 R	14	6 L
A. sclerotiorum Huber	3	1 R	2	2 R	-	-
A. sydowii (Bain. & Sart.) Thom & Church	1	1 R	2	2 R	11	2 R
A. terreus Thom	2	2 R	-	-	6	3 R
A. ustus (Bain.) Thom & Church	-	-	2	1 R	-	-
A. versicolor (Vuill.) Tirab.	1	1 R	2	1 R	1	1 R
Cladosporium cladosporioides (Fries.) de Vries	5	3 R	-	-	3	2 R
Cochliobolus spicifer Nelson	-	-	2	1 R	-	-
Emericella nidulans (Eidam) Vuillemin	-	-	-	-	5	3 R

Table (1): Continued.

Genera & species	Glucose-Czapek's agar		Starch-Czapek's agar		10% NaCl-Czapek's agar	
	TC	NCI & OR	TC	NCI & OR	TC	NCI & OR
Eurotium	1	1 R	-	-	54	19 H
E. amstelodami Mangin	1	1 R	-	-	36	15 M
E. chevalieri Mangin	-	-	-	-	18	11 M
Gibberella fujikuroi (Sawada) Ito	1	1 R	-	-	-	-
Neurospora crassa Shear & Dodge	-	-	1	1 R	-	-
Penicillium	35	18 H	28	14 M	41	15 M
P. aurantiogriseum Dierckx	6	3 R	-	-	2	2 R
P. chrysogenum Thom	25	15 M	8	6 L	13	5 L
P. corylophilum Dierckx	4	4 L	15	10 M	17	9 M
P. duclauxii Delacroix	-	-	2	2 R	-	-
P. oxalicum Currie & Thom	-	-	3	3 R	9	L
Rhizopus stolonifer (Ehrenb.) Lindt	3	2 R	1	1 R	-	-
Total counts	151		140		219	
Number of genera = 11	7		6		4	
Number of species = 25	16		16		13	

Occurrence remarks (OR) H = high occurrence, 19-35; M = moderate occurrence, 9-18; L = low occurrence, 4-8; R = rare occurrence, 1-3.

Table (2): Ability of common fungal species isolated from chipsy to produce amylase enzyme .

Organisms	NIT	Degree of production			
		+++	++	+	-ve
Acremonum strectum	2	-	-	2	-
Alternaria alternata	1	-	-	-	1
Aspergillus flavus	6	1	1	-	4
A. fumigatus	3	-	-	-	3
A. niger	5	3	2	-	-
A.ochraceus	3	-	3	-	-
A.sydowii	3	-	1	2	-
A. terreus	4	-	-	3	-
A.ustus	4	1	-	3	-
A.versicolor	3	1	1	-	1
Cladosporium cladosporioides	2	1	-	-	1
Cochliobolus spicifer	2	-	2	-	-
Emericella nidulans	6	-	-	1	5
Eurotium amstelodami	2	-	1	1	-
Gibberella fujikuroi	3	3	-	-	-
Psenicillum aurantiogriseum	3	3	-	-	-
P. chrysogenum	4	-	1	1	2
p. corylophilum	3	-	-	-	3
P. oxalicum	2	-	1	-	1
Total isolates	60	10	13	13	24

NIT = number of isolates tested .

+++ = high enzyme producers ; ++ = moderate producers ; + = weak producers ; - ve = negative isolates .