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Research Article

MYCOLOGICAL ANALYSIS OF RAISINS RETAILING IN SUPERMARKETS OF BOTSWANA

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ABSTRACT
The fungal infestation of raisins may pose hazard to human health because of implication of aflatoxins. Raisins are vulnerable to fungal infections due to its moisture and sugar contents. Ninety raisin samples were purchased randomly from three supermarkets (Shop A, Shop B and Shop C) in Gaborone and assessed for moisture contents, colony forming unit per gram (cfu/g), fungal contaminations and their implication on mycotoxins discussed. The moisture contents of the raisin samples from shop A, shop B and shop C were 14.2±0.1%, 14.5±0.1% and 14.1±0.1% respectively, and were significantly different. Although moisture contents were within the range for good quality raisins they showed considerable amount of fungal infestations. Average CFU/g values were 5×10^5, 4×10^5 and 7×10^5 from the three shops respectively, higher than the acceptable limit of $<10^5$. A total of 810 isolates belonging to 34 species of 12 genera was recovered from ninety raisin samples from three different shops. *Aspergillus*was the most frequently encountered genus of fungi constituting 26.9% of its species of total fungi isolated, 5.7% being *A. flavus*, the main producer of Aflatoxins. This was followed by *Mucor* (20.1%), *Penicillium* (16.5%), *Cladosporium* (11.9%) and *Rhizopus* (10.6%)n in that order. The other genera with low incidence were *Neosartorya* (3%), *Alternaria* (2.2%), *Endomyces* (1.7%), *Cunninghamella* (1.2), *Geotrichum* (1%), *Trichoderma* (1%) and *Fusarium* (1%).

Key Words: *Mycological Analysis, Raisins, Colony Forming Unit, Moisture Contents*

INTRODUCTION
Raisins (dried grapes) are consumed as energy dried fruits and they are produced in the most regions of the world. Dried fruits including raisins are susceptible to fungal growth and mycotoxins production because of their favourable moisture and sugar contents (Saeed and Rahman, 2004). Raisins can be infected with fungi and other contaminants either already presents on the fruits or during the drying process that occur under unhygienic conditions. Further contamination can take place during storage, handling and transport until sale (Mandeel, 2005). The main genera that attack and produce mycotoxins in food and dried fruits are *Aspergillus*, *Fusarium* and *Penicillium* (Pitt, 2000). Mycotoxins are hazardous to consumers' health and affect the food quality leading to economic losses (Bhat and Vashanti, 1995; Otzuki *et al.*, 2001). Aflatoxins are considered the most toxigenic metabolites from mycotoxins classes and are a potential risk to human and animal health. At present no regulations for mycotoxins are in force in Botswana except in some other African countries (Fellinger, 2006). In the European Union, the current legislation is 4 µg/kg for total aflatoxins in dried fruits for direct human consumption (Commission, 2003). The literature on mycological analysis; moisture contents, fungal load and presence of mycotoxins are few (Saxena and Mehrotra, 1990; Herry and Lemetayer, 1992; Abdel-Sater and Saber, 1999; Saeed and Rahman, 2004; Trucksess and Scott, 2008). As for as Botswana is concerned this information is lacking. Packaged raisins imported to Botswana are retailed in different supermarkets. Raisins, like other food commodities, are susceptible to aflatoxins contamination and if such raisins are consumed it may pose hazard to human health (Garbutt, 1997). Due to significant health risks associated with aflatoxins in foods and also because raisins are consumed directly, there is need for surveillance of food for the presence of mycotoxins (Fingani *et al*., 2004). A preliminary study also revealed that some of the raisin packets bought from the supermarket for consumpson had whitish mouldy growth which on examination

proved to be a *Fusarium s*pecies. The present study was therefore, undertaken to assess the fungal contamination and load in raisins samples sold in supermarkets in Gaborone, Botswana.

MATERIALS AND METHODS

Sampling

Five hundred gram each of ninety raisin samples were purchased randomly from Shop A (30 samples), Shop B (30 samples) and Shop C (30 samples) in Gaborone, Botswana, between July and August, 2009. All samples had been processed and packaged by one same company and they had not reached their expiry dates. In all the three shops, raisin packets were stored on the shelves at room temperature. The samples were collected into sterile Whirl-Par sampling bags and their moisture content was immediately determined after they were brought to the laboratory. Then, the samples were stored in a cold room at 4º C until mycological analysis.

Moisture Content

The moisture content was determined by oven-drying the ground samples (5 g each sample) for six hours at 70±1°C under reduced pressure (≤ 100 mmHg) (Association of Official Analytical Chemists (AOAC), 1995)). The samples were analysed in triplicate and the average recorded

Colony Forming Units (CFU) Per Gram

Five grams of ground samples was mixed with 225 ml of 0.1% peptone water (1016577 (Bio lab Diagnostics (Pty) Ltd, Gauteng, South Africa)) to obtain 10^{-1} stock. Further 1:10 dilutions up to 10^{-5} were prepared using 0.1% peptone water. Then, 0.1 ml of each dilution was plated onto duplicate plates of dichloran 18% glycerol agar (DG18) (CM 729 (Oxoid Ltd., Hampshire, UK)). The plates were then incubated at 25°C for 5-7 days. Total counts of fungi were taken on the plates having between 15 and 150 and used in calculating the Colony Forming Units (CFU) per gram of the samples.

Isolation and Identification of Fungi

For mycological analyses, raisins from each sample were externally disinfected with 1% sodium hypochlorite (13181 (Minema Chemicals Ltd, South Africa)) for 2 minutes and the disinfected raisins (8 to 10 raisins per plate) were plated directly onto dichloran 18% glycerol agar (DG18) (CM 729 (Oxoid Ltd., Hampshire, UK)) according to Pitt and Hocking (1997). The plates were incubated at 25ºC for 5-7 days. Spores and hyphae that developed were subcultures onto malt extract agar (MEA) (CM 59(Oxoid Ltd., Hamshire, UK)), czapek yeast extract agar (CYA and 25% glycerol nitrate agar (G25N). Isolates obtained from dilution technique as mentioned above and direct plate method were sub cultured and identified using Pitt and Hocking (1997) and other relevant literature.

Statistical Analysis

The moisture content, CFU/g and total aflatoxins content of the ninety raisin samples were analyzed by analysis of variance (ANOVA). Means were compared using the Fisher's protected LSD test. The analysis was conducted using Stigmata 3.1.

RESULTS AND DISCUSSION

The average moisture contents of Shop A, Shop B and Shop C were found to be14.2±0.1, 14.5±0.1and 14.1±0.1% respectively (Table 1, Khooanyana *et al*., 2012)). There was a statistically significant difference among the moisture contents of the raisin samples from the three super markets ($P < 0.05$; $P=0.048$). Adams and Moss (1995) stated that the moisture content of a food product can have important implications for its microbial ecology, and the rate and nature of its spoilage. Good quality raisins should have moisture contents between 14 and 16% (Christeen, 2000). Most samples from the three supermarkets were in the range between 14 and 16%, but they showed considerable amount of fungal infestation and thus were of not good quality.

The average CFU/g of the samples from Shop A, Shop B and Shop C were found to be 5×10^5, 4×10^5 and 7×10^5 respectively (Table 2). One-way ANOVA analysis of CFU/g of the samples from the three supermarkets were statistically insignificant ($P>0.05$; $P=0.562$). The Colony Forming Unit per gram

(CFU/g) is a general test to determine the overall microbiological quality of food or food crops (Rockliff and Khan, 2003).Regulations governing microbiological standards for foodstuffs and related matters in South Africa (R. 692 of 16 May, 1997) state that the standard plate count of yeast and moulds in raw fruits should be < 10^5/g. The average CFU/g values of samples from the three supermarkets were higher than the acceptable limit of 10^5/g.

Table 3 shows that a total of 810 isolate belonging to 34 species of 12 genera were recovered from the ninety raisin samples in three supermarkets of Gaborone, Botswana. *Aspergillus* was the most frequently encountered genus of fungi constituting 26.9% of its species of total fungi isolated, 5.7% and 7.1% being *A. flavus and A. niger*, the main producer of aflatoxins and ochratoxin A respectively. This was followed by *Mucor* (20.1%), *Penicillium* (16.5%), *Cladosporium* (11.9%) and *Rhizopus* (10.6%) n in that order. The other genera with low incidence were *Neosartorya* (3%), *Alternaria* (2.2%), *Endomyces* (1.7%), *Cunninghamella* (1.2), *Geotrichum* (1%), *Trichoderma* (1%) and *Fusarium* (1%). *Aspergillus* was recovered from most of the samples studied. *Aspergillus Niger*, *Aspergilluscandidus* and *Aspergillus flavus* were the most prevalent. The other species of *Aspergillus* identified were *Aspergillususstus, Aspergillusochraceous, Aspergillusoryzae, Aspergillusfumigatus, Aspergillus japonicas* and *Aspergillussydowii*. *Mucor*, which ranked second, was represented by M*ucorcircinelloides* and *Mucorhiemalis*. In this study, *M.circinelloides* was recovered with the highest incidence. *Penicillium* was the third most frequent genus. *Penicilliumbrevicompactum, Penicilliumdecumbens* and *Penicilliumthomi* were the most prevalent. *Penicillium* species recovered with low incidence were *Penicilliumcitrinum, Penicilliumsimplicissimum, Penicilliumdigitatum* and *Penicilliumexpansum.*

Table 1: Moisture content of raisin samples collected from different supermarkets in Gaborone (After Khooanyana *et al*., 2012)

	Super Markets		
	Shop A	**Shop B**	**Shop C**
Average Moisture Content (%)	14.2±0.1	14.5±0.1	14.1±0.1
Range (%)	12.9–15.6	13.1–15.8	12.7–15.1
LSD[1] (0.05)	0.355[a]	0.355[b]	0.355[c]
Significance	NS	*	NS

[1]Least Significant Difference
**Significant at 5% Level*
NS, Not Significant
[a]Comparison between Shop A and Shop B
[b]Comparison between Shop B and Shop C
[c]Comparison between Shop C and Shop A

Table 2: CFU/g of raisin samples collected from different supermarkets in Gaborone

	Super Markets		
	Shop A	**Shop B**	**Shop C**
Average CFU/g	$5x10^5$	$4 x10^5$	$7x10^5$
Range (%)	$1.x10^{2}$ -$5x10^6$	$1.x10^2$-$5x10^6$	$1x10^2$-$5x10^6$
LSD[1] (0.05)	$6.73x10^{5a}$	$6.73x10^{5b}$	$6.73x10^{5c}$
Significance	NS	NS	NS

[1]Least Significant Difference
**Significant at 5% level*
NS, Not Significant
[a]Comparison between Shop A and Shop B
[b]Comparison between Shop B and Shop C
[c]Comparison between Shop C and Shop A

Table 3: Fungi isolated from raisin samples collected from the three supermarkets (30 samples/supermarket)

Species	Number of Isolates from the Species (%)			Total Incidence of Supermarkets
	Shop A	Shop B	Shop C	
Alternariaalternata	07	09	02	2.2%
Apsergilluscandidus	21	05	24	6.2%
Aspergillus flavus	22	09	15	5.7%
Aspergillusfumigatus	00	05	03	1.0%
Aspergillus japonicas	02	03	01	0.7%
Aspergillus niger	17	21	20	7.1%
Aspergillusochraceous	05	02	05	1.5%
Aspergillusoryzae	01	00	07	1.0%
Aspergillussydowii	02	01	01	0.5%
Aspergillususstus	07	11	08	3.2%
Cladosporiumcladosporiodes	13	16	03	4.0%
Cladosporiumherbarum	08	17	13	4.7%
Cladosporiummacrocarpum	14	21	17	6.4%
Cunninghamellaelegans	01	04	05	1.2%
Endomycesfibuliger	07	01	06	1.7%
Fusariumsemitectum	05	00	01	0.7%
Geotrichumcandidum	00	01	07	1.0%
Mucorcircinelloides	63	35	47	17.9%
Mucorhiemalis	07	03	08	2.2%
Neosartoryafischeri	11	09	04	3.0%
Penicilliumbrevicompactum	06	06	08	2.5%
Penicilliumchrysogenum	08	11	09	3.4%
Penicilliumcitrinum	04	04	02	1.2%
Penicilliumcorylophilum	01	03	04	1.0%
Penicilliumdecumbens	11	05	02	2.2%
Penicilliumdigitatum	01	03	02	0.7%
Penicilliumexpansum	03	01	01	0.6%
Penicilliumimplicatum	03	03	04	1.2%
Penicilliumphoeniceum	00	01	03	0.5%
Penicilliumsimplicissimum	02	03	03	1.0%
Penicilliumthomi	04	10	04	2.2%
Rhizopusmicrosporus	00	04	04	1.0%
Rhizopusoryzae	31	19	28	9.6%
Trichodermaharzianum	03	00	05	1.0%

Cladosporium species which were identified were *Cladosporiummacrocarpum*, *Cladosporiumherbarum* and *Cladosporiumcladosporiodes*. *Rhizopus* species recovered were *Rhizopusoryzae* and *Rhizopusmicrosporus*. The other species from other genera which were recovered were *Neosartoryafischeri*, *Alternariaalternata*, *Endomycesfibuliger*, *Cunninghamellaelegans*, *Geotrichumcandidum*, *Trichodermaharzianum* and *Fusariumsemitectum*. They further reported the wide occurrence of *Penicillium* species, with *Penicilliumchrysogenum* being the most common species in the raisin samples analyzed. *Cladosporiumherbarum*, *Cladosporiumoxysporum* and *Cladosporiumsphaerospermum* were recovered from raisin samples in the incidence of 0.2%, 0.1% and 6.9% respectively (Saeed *et al*., 2008). The other fungal species recovered in the present study were

isolated from various fruits, seeds and vegetables by several researchers (Abdel-Sater and Saber, 1997; Ozay *et al.*, 1995; Elhalout and Debevere, 1997 and Herry and Lemetayer, 1992). The results on the incidence of fungi obtained are similar to those in the existing literature, but there are some variations. The variations may be due to sampling variability, related environmental factors, divergent processing and storage practices (Lugauskas *et al*., 2005). *Aspergillus flavus*, *Aspergillus niger*, *Aspergillusfumigatus* were amongst the most frequently encountered fungi in 100 samples of raisins collected from retail markets in Egypt (Youssef *et al*., 2000). Zohri and Gawad (1993) isolated *Mucorcircinelloides* in the raisin samples.

Fungi such as *Aspergillus Niger*, *Aspergillus flavus*, *Penicilliumcitrinum* and *Penicilliumchrysogenum* isolated from the raisins in this study are known to produce mycotoxins. Recent evidence suggests that some *A Niger* strains produce ochratoxin (Abarca *et al.,* 1994). Many strains of *A. flavus* produce significant quantities of aflatoxins, a carcinogenic and acute toxic compound (Klich, 2007). In raisins, strains of *P. citrinum*are known to produce citrin (Pitt and Hocking, 2009). Citrin causes mycotoxins nephropathy in livestock and has been implicated as a cause of Balkan nephropathy and yellow rice fever in humans (Bennet and Klich, 2003). Samson *et al.*, (1995) stated that *P. chrysogenum*is a potential producer of a wide range of toxic compounds that could be considered as a hazard to human health. The literature shows the presence of aflatoxins in contaminated raisins of different magnitude (30-350 µg/kg, Saeed and Rahman, 2004; 2 - 550 µg/kg, Saxena and Mehrotra, 1990; Juan *et al*., 2007; Trucksess and Scott, 2008). Khooanyana *et al.,* (2012) working with the same raisin samples in Botswana reported that raisin samples contained total aflatoxins in the range between 1.5 and 9.1µg/kg, and 8.9% of the samples had aflatoxins above the maximum limit of 4 µg/kg as set by EU regulations.

The results of this study clearly showed that raisinsharbor fungal contaminants especially *Aspergillus, Mucor, Penicillium, Cladosporium* and *Rhizopus.* Some of the fungi isolated are capable of producing mycotoxins (including aflatoxins), and thus there may be risks of human exposure to these mycotoxins through consumption of raisins sold in Gaborone, Botswana. Therefore, strict hygiene microbiological measures must be applied during different steps of harvest, transport, storage and drying to avoid contamination of raisins by fungi and mycotoxins particularly aflatoxins which are harmful to human health.

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