The Annals of the University Dunarea de Jos of Galati
Fascicle VI – Food Technology 35(2) 9-15

ORIGINAL RESEARCH PAPER

INCIDENCE OF MOULDS AND PRESENCE OF AFLATOXIN ON TOASTED CASHEW NUTS (*ANACARDIUM OCCIDENTALE L)* IN VENEZUELA

ALEJANDRA ACEVEDO, JAMES SMITH, ANA Y. RAMOS-VILLARROEL*
University of Oriente , Núcleo Monagas. Av. University, Campus Los Guaritos, 6201, Maturín, Venezuela
**Corresponding author: ay2170@gmail.com*

Received 6 June 2011
Revised 2 August 2011

The main objective of this work was to determine the incidence of fungal growth in commercial cashew nuts. The highest mould count in cashew nuts was 658.05 UFC/g (sales point 1). The incidence of moulds in cashew nuts in the first testing period was between 91,67 and 31.25% and in the second period it was between 89.58 and 62.5% for sales points 1, 2, 3 and 4. The incidence of *Aspergillus flavus* and *Aspergillus parasiticus* in cashew nuts was 5.74% and 0.49%, respectively, and the differences were not significant. The concentrations of aflatoxins recovered from cashew nuts were between 20.67 and 11.33 ppb, for all sales points.

Keywords: aflatoxin, cashew nuts, *Aspergillus flavus*, *Aspergillus parasiticus*

Introduction

Cashew plant (*Anacardium occidentale L*) is a small tree, 7 - 20 m length, and real fruits are seeds which have a reniform shape covered with a hard grey-green pericard, 0.025 - 0.03m length and 0.02 - 0.025m width. It represents about 8 - 12% of the total fruit. Also it has a red or yellow pseudo-fruit with dimensions of 0.04 -0.12m length. (Perozo *et al.*, 2006). The pseudo-fruit is consumed like fresh fruit for its high level of vitamin C, and seeds have industrial uses in cosmetology, resins, varnishes, pastry, and others (Avilán *et al*., 1992 and Román, 1991 mentioned by Perozo *et al*., 2006).

Worldwide cashew nuts are known for being expensive and are recognized by their delicate and delicious flavor. In Venezuela they are commonly cultivated at a small scale and consumed roasted, which means the process of burning off the shell, of cooking the seed and of removing the pericarp. Cashew nuts are also included in many Venezuelan desserts such as nougats or marzipans (Sindoni *et al*., 2005).

Besides, cashew nut is characterized by the high percentage of lipids and carbohydrates that makes them vulnerable to the attack of moulds, specifically the lipolytics kind, found in the genre of *Aspergillus* and *Penicillium* (Doyle *et al.*, 2001*). Aspergillus flavus* and *Aspergillus parasiticus* are two of the most important toxigenic moulds (Requena *et al*., 2005). Only, some of the isolates of *A. flavus* originate B_1, B_2 and cyclopiazonic acid. *A. parasiticus* originates aflatoxin B_1, B_2, G_1, and G_2 and all the isolates are toxigenic (Kosalec and Pepeljnjak, 2005).
Aflatoxin B_1 and M_1 are the most harmful, being M_1 a secondary metabolic derivate from aflatoxin B_1, followed by G_1, M_2, B_2, M_2 (Bolet and Socorrás, 2005). Aflatoxins are classified into heterocycles, which are extremely heat-resistant above 373 K (100ºC) (Hayes, 1993). They are some of the most studied and controlled heterocycles, by their toxicity related to cell damage, carcinogenic, teratogenic and mutagenic effects in animals and humans (Bogates *et al*., 2004).
Studies on the levels of fungal contamination for cashew nuts in Venezuela are scant, and there are hardly any reports on toxigenic moulds and their hazard on human health. Knowing this deficient, this investigation had as principal objective to determine the incidence of mould growth and the presence of aflatoxins in toasted and retail cashew nuts sold in Maturín, Monagas state, Venezuela.

Materials and methods

Samples

Samples were taken in two period of time; the first one began in May. A total of 3 samples of 0.2 kg from each sales point of retail cashew nuts was obtained. The sales points were: point 1 (Furrial town), 2 (Candelaria town), 3 (Corozo town) and 4 (downtown of Maturin), that were within the municipality. The second period of analysis began in July, following the procedure used in the first period.

Total mould count

We followed the methodology described by COVENIN 1126-89 (1989) and COVENIN 1337-90, (1990). The physical conditions of the packaging and the external appearance of the nuts were noted. The initial dilution was prepared by adding 0.05 kg of sample to 0.05 L of diluents (peptone water 0.1%), and shaking vigorously for 50 times in an angle of 45 degrees. Decimal dilutions (0.011 + 0.099 L) were prepared down to 10^{-5}, and spread plated in duplicate, 0.001 L per plate. Potato Dextrose Agar (PDA) (2% chloramphenicol) was the only medium used, because it showed better results at pilot assays. Plates were incubated upright at 293 - 298 K (20 - 25ºC) for 5 days.

Incidence of mould growth

For the incidence of mould growth we followed the method mentioned by Adebajo and Diyalou (2003). Twenty whole nuts were obtained randomly for the assay. The cotyledons (2 per nut), were hand separated, then the surfaces were disinfected with an aqueous solution of sodium hypochlorite 2% for 60 seconds. This was followed by three washes with sterile distilled water, before four cotyledons were

plated together equispaced on the medium (PDA agar plus chloramphenicol 2%). The plates were incubated upright 293-298 K (20 - 25ºC) for 7 days. The fungal incidence was expressed as a percentage of the total of samples evaluated.

Macroscopic and microscopic identification of Aspergillus flavus and Aspergillus parasiticus

Colonies of *A. flavus* and *A. parasiticus* were identified according to the report by Abarca (2000) and Singh *et al.*, (1991) respectively. Before the identification, a microculture was prepared in a plate following the procedure described by Granados and Villaverde (2002), using Potato Dextrose (PDA) agar with 2% of chloramphenicol and a slide was equipped with cotton-blue indicator to observe the microscopic characteristics of the moulds.
Microscopic identification was done using the Samson and Hoekstra's key (1995), and Onions *et al.,* (1981) for both microorganisms, taking into account the difference in the surface of the conidia between the moulds. *A. parasiticus* has equinulated conidia and *A. flavus* presents smooth conidia.

Incidence of Aspergillus flavus and Aspergillus parasiticus and determination of aflatoxins

The incidence of *A. flavus* and *A. parasiticus* was expressed as a percentage, calculated with the positive colonies found after the microscopic identification and the total of the samples. A RIDA® QUICK Aflatoxin kit was used to determinate the presence of aflatoxin in the sample. The test was done following the indications of the kit and the results were expressed as a ppb (parts per billion) value. The results were taken by the time that last the sample in react with the kit and appear the red band.

Experiments design

A randomized block design (4x2) was employed, the factors taken into account being sales points (4) and sampling periods (2). The statistical data were analyzed by means of an ANOVA using Statistix 8.0 program with a probability level of $P<0.05$. If statistical differences were determined, the MSD was determined. For the samples of aflatoxin the Kruskal-Wallis test was employed.

Results and discussion

Total fungal count

In many cases it is possible to find certain levels of microorganisms in fruits directly taken from tree like the cashew nuts (Davis *et al*., 1996). However, when seeds are entirely covered by pericard, this works like a perfect barrier against the attack of microorganisms, but if the pericard is injured by animals or insects, contamination is always present, and fungal invasion quickly happens (ICMFS, 2001). The manipulation during transportation from fields to the processing industry may damage the shell and help to contaminate the rest of the healthy seeds. Besides, the contamination provided by an unclean transportation may be

significant (ICMFS, 1985). However, no information exists in Venezuela about the total fungal count in cashew nuts, as, for example, it is found for corn or peanut.
Table 1 describes the total fungal count for samples of retail cashew nuts. Statistical differences were found for media values per all sales points ($p<0.05$). The total fungal count ranged between 658.05 – 58.92 UFC/g, sales point 1 being the most contaminated (658.05 UFC/g). This value is above those reported by COVENIN 1337-90 (maximum value 100 UFC/g) for total fungal count in foods, and also reported by FRUTAL ES (2002) for cashew nuts (500 UFC/g). This fact reflects a contamination post-heat treatment and a deficient hygienic practices during storage (Adams and Moss, 1997). Moisture and temperature conditions are important to avoid mould growth on seeds. Frazier and Westthoff (2000) mention that most moulds require a low quantity of water, and a 14-15% in dry fruits and flours are needed to maintain them without mould growth.

Table 1. Average of total mould count (UFC/g) for retail cashew nuts sold in Maturin, Venezuela

Nut	Nº of samples	Sales points				Total average	C.C.V
		1	2	3	4		
Cashew nut	24	658.05	99.00	58.92	88.25	226.05	98.29

C.C.V: Comparison of critical values.

Incidence of mould growth

Temperature and moisture are factors that trigger the mould growth, toxins dissemination and production. In other hands, conditions during harvest, storage and transportation are also important (Cerovich and Miranda, 2004). The incidence of mould growth per 4 sales points in two periods of time, May and July, for retail cashew nut are shown in Table 2. Significant differences were found between the testing periods of time and the interaction sales points-assays period ($p< 0.05$). The results for period 1 ranged between 91.67 - 31.25%, and for period 2 were between 89.58 - 62.50%.

Table 2. Incidence of moulds (%) in cashew nuts studied in 4 sales points per two periods of time (May and July)

Sales points	Period	
	1	2
1	58.33 %	72.92 %
2	91.67 %	62.50 %
3	52.08 %	89.58 %
4	31.25 %	77.08 %
Comparison of critical values 31.01		

An increase of mould incidence in period 2 (July) compared to period 1 (May) can be explained by the weather conditions between May and July, registering 300,3 K(27.3ºC) temperature, 64.1mm monthly rainfall, and relative humidity of 73% in

May. But July presented 299.7K (26.7ºC) of temperature, 249.7mm of monthly rainfall and 82% of relative humidity (Estación Meteorológica de Maturin, 2009). The increase of rainfall and relative humidity could help to raise the incidence of moulds.

Incidence of Aspergillus flavus and A. parasiticus

Table 3 shows the incidence (%) of both micotoxigenic moulds in 24 samples of cashew nuts and the results were 5,74% and 0,49%, respectively, not being significant ($p<0.05$). Although the there are not similar investigations in Venezuela, Mazzani *et al*., (2000) reported presence of *Aspergillus flavus* in corn seeds, from Guarico state. Also, Adebajo and Diyaolu (2003), denoted 3% in MA40 and 9 % in MY50G media for incidence of *Aspergillus flavus* in cashew nuts in Nigeria, and Adbel-Gawad and Zohri (1993) found the presence of *A. flavus* and *A. parasiticus* in five types of dry fruits, including cashew nuts.

Table 3. Average incidence of *Aspergillus flavus* and *Aspergillus parasiticus* (%) in seeds of cashew sold at retail in Maturin, Venezuela.

Cashew nuts	Nº of samples	Total average
Aspergillus flavus	24	5.74 %
A. parasiticus	24	0.49%

Even if of these values were not statistically significant, this kind of retail products count with ideal conditions for fungal growth, and without the control of temperature and humidity, *A. flavus* or *A. parasiticus* may rapidly reproduce representing a food safety problem, and a dormant risk for the production of aflatoxins. Furthermore, Aspergillus genre can easily grow up to 40ºC, pH between 2.2 and 8 and water activity above 0.77-0.88, and optimum conditions for development of aflatoxins are 27-33ºC, pH 5-6 and water activity between 0.82-0.99 (Richard, 2007).

Presence of aflatoxins

Table 4 describes the values of aflatoxins expressed in ppb (parts per billion) found during the analysis of cashew nuts. Statistical differences were not found between sales points neither the ANOVA applied to the ranges. Aflatoxin content was between 11.33 and 20.67ppb beating in all cases the value reported by FRUTAL ES (2002) (10ppb).

Aflatoxins are secondary metabolites produced by a series of micotoxigenic moulds, including *A. flavus* and *A. parasiticus* (Izquierdo *et al*,. 1995). When microorganisms produce the toxin, it is difficult to eliminate it from food because of its thermo-resistance (Richard, 2007). These results reaffirm that even though seeds presented a low incidence of *A. flavus* and *A. parasiticus*, aflatoxins may be present. Thus the consumption of mouldy cashew nuts could be a potential hazard for public health.

Table 4. Presence of aflatoxins in cashew nut (ppb) according to sales points

Nut	N° of samples	Sales points				Kruskal-Wallis Statistics	P-value Chi square
		1	2	3	4		
Cashew nut	12	11.33	20.33	20.67	15.00	4.2706	0.2337

Conclusions

The interaction between sales points and the period of assay was significant, being the most influenced testing. The incidence of *A. flavus* and *A. parasiticus* in cashew nuts was not significant, but at the same time, is an alert about the presence of aflatoxins. And these secondary metabolites are harmful for humans as it they are to animals. Thus, the presence of aflatoxins in cashew nuts is a huge hazard for public health, and is independent of the presence of *A. flavus* and *A. parasiticus*.

Acknowledgements

To Professors Nilda Alcorcéz and Julio Colivet, from University of Oriente, Núcleo Monagas. To Central University of Venezuela, professor Claudio Mazzani, expert in micotoxigenic moulds in Venezuela, for providing the material necessary for the identification of *A. flavus* and *A. parasiticus.* We also acknowledge Simón Barreto, part of Empresas Polar, who helped to the training of Aflatoxin kit.

References

Abarca, M. 2000. Taxonomía e identificación de especies implicadas en la aspergilosis nosocomial. *Revista Iberoamericana de Micologia,* (17), 79-84.

Abdel-Gawad, K. and Zohri, A. 1993. Fungal flora and mycotoxins of six kinds of nut seeds for human consumption in Saudi Arabia. *Mycopathologia*, 124(1), 55-64.

ADAMS, M y MOSS, M. 1997. *Microbiología de alimentos,* 161-294. Acribia, España.

Adebajo, L. and Diyaolu, S. 2003. Mycology and spoilage of retail cashew nuts. *African Journal of Biotechnology*, 2 (10), 369-373

Bogantes, P. Bogantes, D. y Bogantes, S. 2004. Aflatoxinas. *Acta Médica Costarriquence*, 46(4), 174-178.

Bolet, M. y Socorrás, M. 2005. Micotoxinas y cáncer. *Revista Cubana de Investigación Biomédica*, 24(1), 54-59.

Cerovich, M. y Miranda, F. 2004. Almacenamiento de semillas: estrategia básica para la seguridad alimentaria. *Ceniap hoy,* 4, 32.

COVENIN. Comisión Venezolana de Normas Industriales. 1989. Alimentos. Identificación y preparación de muestras para el análisis microbiológico. Norma 1126-89. Fondonorma. Venezuela.

COVENIN. 1990. Comisión Venezolana de Normas Industriales. Alimentos. Método para recuento de mohos y levaduras. Norma 1337-90. Fondonorma. Venezuela.

Davis, D., Dulbecco, R. y Ginsberg, H. 1996. *Tratado de microbiología*. Mansson, España.

Doyle, M., Beuchat, L. y Montville, T. 2001. *Microbiología de alimentos. Fundamentos y Fronteras*, 413-425. Acribia. España.

Estación Meteorológica de Maturín. 2009. *Datos de temperatura, humedad relativa, velocidad y dirección del viento, precipitación, insolación y evaporación, período (2008)*.

Frazier, W. y Westhoff, P. 2000. *Microbiología de alimentos*. Acribia. España.

Frutal es. 2002. Boletín Mercado de la Nuez de Marañón. Instituto Interamericano Cooperación para la agricultura y Ministerio de Agricultura y Ganadería, 1(3).

Granados, R. y Villaverde, M. 2002. *Microbiología. Tomo II. Bacteriología. Medios de cultivo y pruebas bioquímicas. Micología general. Parasitología general.* Paraninfo. España.

Hayes, P. 1993. *Microbiología e higiene de los alimentos*, 55-57. Acribia. España.

ICMSF. International Commission on Microbiological Specifications for Foods. 2001. *Microorganismos de los alimentos 6, ecología microbiana de los productos alimentarios*. Acribia. España.

ICMSF. International Commission on Microbiological Specifications for Foods. 1985. *Ecología Microbiana de los Alimentos 2. Productos alimenticios*. Acribia. España.

Izquierdo, P., Rojas, E., Rangel, L. y Márquez, E. 1995. Presencia de aflatoxinas en algunos alimentos. *Revista de la Facultad de Agronomia (Universidad del Zulia)*, 13, 485-492.

Kosalec, I. and Pepeljnjak, S. 2005. Mycotoxigenicity of clinical and environmental *Aspergillus fumigatus* and *Aspergillus flavus* isolates. *Acta pharmaceutica*, 55 (4), 365-375.

Mazzani, C., Borges, O., Luzón, O., Barrientos, V. y Quijada, P. 2000. Fusarium moniliforme, fumonisinas y *Aspergillus flavus* en granos híbridos de maíz en el estado Guárico, Venezuela. *Revista de la Facultad de Agronomía (Universidad del Zulia)*, 17, 185-195.

Onions, A., Allsopp, D. and Egging, H. 1981. *Smith's Introduction to Industrial Mycology.* Edward Arnold. Great Britain.

Perozo, A., Ramírez, M., Gómez, A. y Buitrago, N. 2006. Germinación y características morfológicas de plántulas de merey (*Anacardium occidentale L*) tipo amarillo*.) Revista de la Facultad de Agronomía (Universidad del Zulia)*, 23 (1), 134-140.

Richard, J. 2007. Some major mycotoxins and their mycotoxicoses- and overview. *International journal of food microbiology*, (119), 3-10.

Requena, F., Saume, E. y León, A. 2005. Micotoxinas: enemigas silenciosas de la salud. *Ceniap hoy*, 9, 32.

Samson, R., Hoekstra, S., Frisvad, J. and Filtenborg, O. 1995. *Introduction to Food-Borne Fungi*. Centralalbureau Voor. Holand.

Sindoni, M., Hidalgo P., Chauran, O., Chirinos, J., Bertorelli, M, Salcedo F., García M., Martínez F., Romero A. y Silva F. 2005. El cultivo de merey en el oriente de Venezuela. *Series Manuales de Cultivos. INIA* (3), 86.

Singh, K., Frisvad, J., Thrane, U. and Mathur, S. 1991. *An Illustrated Manual on Identification of Some Seed-Borne Aspergilli, Fusaria, Penicilia and their Micotoxins.* Jordbrugsforlaget. Denmark.