Mycobiology 37(3) : 215-217 (2009)

Grading of Fermented and Dried Cocoa Beans Using Fungal Contamination, Ergosterol Index and Ochratoxin a Production

S. O. Aroyeun[1]*, G. O. Adegoke[2], J. Varga[3] and J. Teren[4]

[1]*Cocoa Research Institute of Nigeria, PMB, 5244, Ibadan, Nigeria*
[2]*Department of Food Technology, University of Ibadan, 200001, Ibadan, Nigeria*
[3]*Department of Microbiology, University of Szeged, Szeged 6728, Hungary*
[4]*Animal Health and Food Control Station, Szeged 6728, Hungary*

(Received May 13, 2009. Accepted August 24, 2009)

Sixty four samples of cocoa beans replicated in quadruplicates were collected from five warehouses from southwest Nigeria and examined for fungal loads, ergosterol and ochratoxin A The levels of all the variables obtained were further used as indices for cocoa grading into food quality, FoQ (erg < 5 mg/kg; OTA < 1 μg/kg), feed quality, FeQ (erg = 5~10 mg/kg; OTA in the range of 1.1~3.11 μg/kg), Screen for mycotoxin, SFM (erg = 10~20 mg/kg; OTA from 3.12 μg/kg and above) with fuel quality, FuQ having erg > 20 mg/kg and OTA > 6.12 μg/kg. Using these ergosterol indices, 18.75% of the cocoa beans examined was classified with the FoQ, 18.75% with the FuQ while 31.25% was classified with both the FeQ and the SFM, respectively. In conclusion, ergosterol can be used as a rapid index to grade fermented, dried cocoa beans meant for export.

KEYWORDS : Feed, Food, Fuel, Mycotoxin, Quality

Molds are common contaminants of agricultural commodities used for foods and feeds. Fungal development on the alimentary substrates can lead to different detrimental effects, alteration of technological properties, decrease of nutritive value and synthesis of mycotoxins (Pitt and Hocking, 1985). Evaluation of mold development is of interest to estimate global quality of raw materials and may be useful to take decision on their potential use. Ergosterol is considered as the principal sterol of fungi and it plays an important role as cell membrane component. Therefore, it has been proposed as a global indicator of mycological quality of foods and feeds (Bailly *et al.*, 1999; Cahagnier, 1998; Schnurrer, 1993; Schwardorfk and Muller, 1980; Seitz *et al.*, 1977, 1979). Ergosterol levels are commonly used as quality parameters in ecological (Sashdhar *et al.*, 1989), industrial, (Hippelein and Rugermer, 2004), and agronomic environments (Kadakal and Artik, 2004; Sashdhar *et al.*, 1989). Moreover, significant correlations were found between ergosterol and the major mycotoxins notably Fumonisin B_1, Zearalenone, Deoxynivalenol, ochratoxin A, and patulin. Significant relationship has also been found between fungal development and ergosterol production in maize (Peitri *et al.*, 2004), rice (Saxena *et al.*, 2001), tomato (Kadakal and Ekincir, 2005) and wheat (Abramson *et al.*, 2005). Therefore, ergosterol determination can be considered as a good index of fungal development on cereals and could be an early indicator of potential mycotoxin production. Its determination can be used in industry to screen productions, prior to mycotoxin analysis. On cereals, according to (Cahagnier, 1998), 3 μg of ergosterol per gram is considered as the maximum acceptable level for maize while for wheat, 8 μg of ergosterol per gram is the retained value for certifying correct quality of the grains. On the other hand, when the amounts of ergosterol are upper than 8 μg/g on maize and 12 μg/g on wheat, a quality of grains is suspected (Cahagnier, 1998).

Cocoa beans, a produce of commerce of *Theobroma cacao* is a principal raw material for the chocolate industry. Cocoa of commercial grade should conform to some criteria among which the absence of molds and mycotoxin production is one (Aroyeun *et al.*, 2007). Grading of cocoa beans into acidity, slatiness, mouldiness are among the most prominent quality parameters for grading cocoa beans. All these methods are time-consuming and laborious. Using ergosterol index as to measure the possibility of mycotxin production in cocoa beans has not been reported. Since the method of ergosterol determination can be a faster and relatively precise method, this study was designed with the aim of grading contaminated cocoa beans using fungal determinations, erogsterol index and ochratoxin A formation.

Materials and Methods

Raw materials. Sixty four samples of cocoa beans replicated in quadruplicates were collected from five warehouses from southwest Nigeria.

*Corresponding author <E-mail : aroyeun2000@yahoo.co.uk>

Determination of fungal counts. Surface sterilized cocoa beans were plated on malt extract agar after serial dilution. Incubation was at 28 ± 2°C for seven days. Thereafter, colonies growing on the plates were counted using a colony counter.

Extraction of ochratoxin A and quantification. After 7 days, isolates were grown in yeast extract sucrose broth containing (2% yeast extract, 15% sucrose). Incubation was done at 28 ± 2°C for 7 days. The culture broths were later extracted using the method of (Varga *et al.*, 2002).

Quantification of ochratoxin A by high performance liquid chromatography (HPLC). This was done in accordance with (Teren *et al.*, 1996).

Determination of ergosterol content in cocoa beans. Extraction, quantification of ergosterol was done using HPLC apparatus in accordance with (Lamper *et al.*, 2000).

Results and Discusssion

In Table 1, the higher the fungal counts and the ergosterol values are, in most cases the higher the ochratoxin A values are. This finding was in agreement with previous reports on the direct relationship between ergosterol, fungal counts and mycotoxin production (Lamper *et al.*, 1999; Varga *et al.*, 2002; Czaczyk *et al.*, 2002). (Reid *et al.*, 1999) observed an interaction between *Fusarium graminearum* and *Fusarium moniliforme* and disease progress, fungal biomass, mycotoxin accumulation and ergosterol formation. (Ng *et al.*, 2008) estimated fungal growth using ergosterol assay as a rapid tool in assessing the microbiological status of grains and feeds. Ergosterol has been determined in grains with different levels of fungal contamination by (Maria *et al.*, 2001). Samples CB_1 with fungi counts of 62.10×10^2 cfu/g had 6.33 μg/kg of ochratoxin A and 24.23 mg/kg of ergosterol. High fungal counts of 33.02×10^2 cfu/g in CB_4 and ergosterol content of 20.41 mg/kg corresponded with the OTA observed (3.12 μg/kg). Sample CB_6 with fungal counts of 30×10^2 cfu/g, had a corresponding ergosterol content of 17.21 mg/kg and a low content of OTA (1.86 μg/kg). In this case, there is a possibility that OTA producing fungi might be present in the cocoa samples but maybe the OTA production capacity of the fungi was low. Samples CB_1, CB_6, CB_8 had high fungal counts, high ergosterol and high OTA values. In sample C_{14}, fungal counts, ergosterol and OTA did not correlate. The high OTA production, which does not correlate with either ergosterol or OTA, might indicate presence of high OTA producing fungi even though it might be present in low counts. Samples CB_{12}, and CB_{13} had low fungal counts; low ergosterol and low OTA in agreement with the hypothesis of (Schnurrer, 1995) on the direct relationship of fungal counts, ergosterol and deoxynivalenol. CB_{10} had fungal counts of 11.10×10^2 cfu/g, ergosterol of 10.47 mg/kg but a correspondingly lower OTA of 0.09 μg/kg indicating the presence of OTA producing fungi with low production capacity. Samples CB_{15}, CB_{16} fell in the category where the ergosterol, fungal counts and OTA have a direct relationship.

Table 1. Fungal contamination, Ergosterol and OTA of cocoa bean samples

Cocoa Sample	Fungal counts cfu/g × 10^2	*Ergosterol mg/kg	*OTA mg/kg
CB_1	62.10	24.23 ± 0.01FUQ	6.33 ± 1.00
CB_2	18.00	12.48 ± 0.02SFM	1.68 ± 0.14
CB_3	28.00	11.23 ± 0.03SFM	1.86 ± 0.33
CB_4	33.02	20.41 ± 0.12FUQ	3.12 ± 1.01
CB_5	7.61	6.38 ± 0.22FEQ	1.00 ± 0.01
CB_6	30.00	17.21 ± 0.11SFM	2.31 ± 0.32
CB_7	16.40	8.23 ± 1.12FEQ	1.54 ± 0.21
CB_8	45.00	22.5 ± 0.22FUQ	3.80 ± 0.07
CB_9	8.81	7.64 ± 0.03FEQ	1.04 ± 0.03
CB_{10}	11.10	3.47 ± 0.21FOQ	0.09 ± 0.01
CB_{11}	6.30	5.48 ± 0.02FEQ	0.73 ± 0.12
CB_{12}	2.42	4.12 ± 0.01FOQ	0.42 ± 0.31
CB_{13}	3.66	3.00 ± 0.22FOQ	0.11 ± 0.11
CB_{14}	5.30	5.84 ± 1.13FEQ	1.26 ± 0.16
CB_{15}	21.0	14.4 ± 1.11SFM	1.71 ± 0.21
CB_{16}	23.90	15.3 ± 0.31SFM	2.02 ± 0.13

*Values recorded were averages of separate experiments; ±- standard deviations; FOQ- Food Quality; FUQ- Fuel Quality; SFM- Screen for Mycotoxin- FEQ- Feed Quality

The results obtained in this study supported the usefulness of the quality grading system described by (Schnurrer, 1995). Based upon ergosterol and OTA content of the samples, only 18.25% of the samples reached FOQ grade (< 5 mg/kg ergosterol), OTA of this class is < 1 μg/kg and 18.25% fell in the FEQ grade with the ergosterol of 50~10 mg/kg and OTA > 1 μg/kg. 31.25% fell in the category of Screen for mycotoxin (SFM) grade having ergosterol of 10~20 mg/kg and OTA in the range of 1.68~2.31 μg/kg while 31.25% also fell in the last grade Fuel quality grade (FuQ) with ergosterol > 20 mg/kg and OTA from 3.12 μg/kg and above. Based on the grading system, the cocoa beans obtained form the warehouses in the food quality grade (FOQ) included CB_{10}, CB_{12}, CB_{13}. Samples in the feed quality grade (FeQ) were CB_5, CB_7, CB_9, CB_{11} and CB_{14}. Those for screen for mycotoxin (SFM) included CB_2, CB_3, CB_6, CB_{15} and CB_{16}. Those in the fuel quality grade (FuQ) category were CB1 and CB_4 respectively (Table 2).

In conclusion, the use of ergosterol can be used as an indicator of good cocoa bean quality and to predict the possibility of ochratoxin A formation by mycotoxigenic fungi in cocoa beans. In conclusion, this index can be relevant as a rapid test for screening cocoa beans samples

Table 2. Cocoa Bean grading based on ergosterol values

Grade/ergosterol content (mg/kg)	Cocoa samples
Food Quality	CB_{10}
< 5	CB_{12}
	CB_{13}
	CB_5
Feed Quality	CB_7
(5-10)	CB_9
	CB_{11}
	CB_{14}
	CB_2
Screen for mycotoxin	CB_3
(10-20)	CB_6
	CB_{15}
	CB_{16}
	CB_1
Fuel Quality	CB_4
(> 20)	CB_8

meant for export.

Acknowledgments

Authors wish to appreciate the Management of the University of Szeged, Hungary, Faculty of Science, Department of Microbiology for permission to use their facilities. Professor, G. O. Iremiren, the Executive Director, Cocoa Research Institute of Nigeria is also acknowledged for permission to publish this paper. Dr. O. Olubamiwa, Director, Research and Dr. Fademi, Director, P, B and T of the Cocoa Research Institute of Nigeria, Ibadan are also acknowledged their for useful suggestions in making this paper publishable.

References

Abramson, D., Hulasarer, R., York, R. K., White, N. D. G. and Jayas, D. S. 2005. Mycotoxins, ergosterol and odours volatiles in durum wheat during granary storage at 16% and 20% moisture content. *J. Stored Products Res*. 41:67-76.

Aroyeun, S. O., Adegoke, G. O., Varga, J., Kocsube, S., Pal, K. and Vagvolgyi, C. 2007. Effect of fermentation and storage on mycotoxigenic fungi, ochratoxin A and aflatoxin B_1 in cocoa beans from southwestern Nigeria. *Malaysian Cocoa J.* 3:35-46.

Bailly, J. D., Bars, L. E., Pietria, P., Bernard, A. and Lebarsj, G. 1999. Evaluation of fluorodensitometric method for analysis of ergosterol as a fungal marker in compound feeds. *J. Food Prot.* 62:686-690.

Cahagnier, B. 1998. Moisssures des aliments peu hydrate, 225 pages, Lavoissier, Paris.

Czaczyk, K., Trojanowska, K. and Stachowiak, B. 2002. Inhibition of ergosterol biosynthesis in fungal plant pathogens by *Bacillus* sp. *Polish J. Environ. Studies* 11, 5:593-597.

Hippelein, M. and Rugamer, M. 2004. Ergosterol as an indicator of mould growths on building materials. *Int. J. Hyg. Environ. Health* 207:379-385.

Kadakal, C. and Artik, N. 2004. A new quality parameter in tomato and tomato products: Ergosterol. *Crit. Rev. Food Sci. Nutr*. 44:349-351.

Kadakal, C. and Ekincir, N. 2005. Ergosterol as a new quality parameter together with patulin in raw apple juice produced from decayed apples. *Food Chem*. 90:95-100.

Lamper, C. S., Teren, J., Bartok, T., Komoroczy, R., Mesterherzy, A. and Sagi, F. 2000. Predicting DON contamination in Fusarium-infected wheat grains via determination of ergosterol content. *Cereal Res. Commun*. 28, 3:337-344.

Ng, H. E., Raj, S. S. A., Wong, S. H., Tey, D. and Tan, H. M. 2008. Estimation of fungal growth using the ergosterol assay: a rapid tool in assessing the microbiological status of grains and feeds. *Lett. Appl. Microbiol*. 46:113-118.

Pietri, A. B. and Pallaroni, L. P. 2004. Occurrence of mycotoxin and ergosterol in maize harvested over 5 years in Northern Italy. *Food Additive Contam*. 21:479-487.

Pitt, J. L. and Hocking, A. D. 1985. Fungi and food spoilage. Academic press, New York.

Reid, L. M., Nicoi, R. W., Ouellet, T., Savard, M., Miller J. D., Young, J. C., Stewart, D. W. and Schaafsma, A. W. 1999. Interaction of *Fusarium graminearum* and *F. moniliforme* in maize Ears: Disease progress, Fungal biomass and mycotoxin accumulation *Phytopathology* 89, 11:1028-1037.

Sashidhar, R. B., Sudershan, R. V., Ramakrishna, Y. and Bhat, R. V. 1989. Rapid and specific method for screening ergosterol as index of fungal contamination in cereal grains. *Food Chem*. 31:51-56.

Saxena, J., Munimbazi, C. and Bullerman, L. B. 2001. Relationship of mould counts, ergosterol and ochratoxin A production *Int. J. Food Microbiol*. 71:29-34.

Schnurrer, J. 1993. Comparison of methods for estimating the biomass of three food-borne fungi with different growth patterns. *Appl. Environ. Microbiol*. 59:552-555.

Schnurrer, J. 1995. Detection and quantification of fungi in foods. Proceedings from the workshop on fungal identification Techniques. Barcelona, 5-8, April, 1995. 153-159.

Schwardorfk, K. and Muller, H. M. 1980. Determination of ergosterol in cereals mixed food components and mixed feeds by liquid chromatography. *J. Assoc. Off. Anal. Chem*. 72:457-462.

Seitz, L. M., Mohr, H. E., Burroughs, R. and Salier, D. B. 1977. Ergosterol as an indicator of fungal invasion in grains. *Cereal Chem*. 54:1207-1217.

Seitz, L. M., Salier, D. B., Burroughs, R., Mohr, H. E. and Hubard, J. D. 1979. Ergosterol as a measure of fungi growth. *Phytopathology* 69:1202-1203.

Teren, J., Varga, J., Hamari, Z., Rinyu, E. and Kevei, F. 1996. Immunochemical detection of Ochratoxin A in black *Aspergillus strains. Mycopathologia* 134:171-176.

Varga, J., Kriszina R., Csila, L., Teren, J. and Szabo, G. 2002. Kinetics of ochratoxin A production in different *Aspergillus* species. *Acta Biologica Hungarica* 53, 3:381-388.