Grading of fermented and dried cocoa beans using fungal contamination, ergosterol index and ochratoxin A production

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Citation

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Abstract

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

INTRODUCTION

Molds are common contaminants of agricultural commodities. Foods and feeds. Fungal development on 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 molds development is of interest to estimate global quality of raw materials and may be useful to take decision on their possible 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, Scnurrer, 1993, Schwardorfk and Muller, 1980, Seitz et al, 1979, Seitz et al., 1977). Ergosterol levels are commonly used as quality parameters in ecological (Sashdhar et al., 1989,), industrial, (Hippelein and Rugermer, 2004), and agronomics environments (Kadakal and Artik, 2004, Sashdhar et al., 1989). Moreover, significant correlations were found between ergosterol and the major mycotoxins (fumonisin B1, Zearalenone, Deoxynivalenol, ochratoxin A, patulin) 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□g of ergosterol per gram is considered as the maximum acceptable level for maize while for wheat, 8□g 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□g/g on maize and 12□g/g on wheat, a doubtful 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 absence from mouldiness and mycotoxin production is one (Aroyeun et al, 2007). Grading of cocoa beans into Acidity, slatiness, mou;ldiness 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 posssibilit6y 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 consisting of sixteen sub samples replicated in quadruplicates were collected from five warehouses from southwest Nigeria.

DETERMINATION OF FUNGAL COUNTS

Surface sterilized cocoa beans were plated on malt extract agar after serial dilution. Incubtion was at 28° 2OC for seven days. Thereafter, colonies growing on thee plate were counted using a colony counter.

EXTRACTION OF OCHRATOXIN A AND QUANTIFICATION

After 7days, isolates were grown in yeast extract sucrose broth containing 2% yeast extract, 15% sucrose). Incubation was done at 28° 2OC for 7 days. The culture broths were later extrcated 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 4.38, the higher the fungal counts, the higher the ergosterol values and in most cases the higher the ochratoxin A values. 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 graminearun and Fusarium moniliforme and disease progress, fungal biomass and 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 fungal grains with different levels of fungal contamination by Maria et al., (2001). Samples CB1 with fungi counts of 62.10 x 102 cfu/g had 6.33ug/kg of ochratoxin A and 24.23mg/kg of ergosterol. High fungal counts of 33.02 x 102 cfu/g in CB4 and ergosterol content of 20.41mg/kg corresponded with the OTA observed (3.12ug/kg). .Sample CB6 with fungal counts of 30 x 102 cfu/g, had a corresponding ergpsterol content of 17.21mg/kg and a low content of OTA (1.86ug/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 CB1, CB6, CB8 had high fungal counts, high ergosterol and high OTA values. In sample C14, 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 CB12, and CB13 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. CB10 had fungi counts of 11.10 x 102 cfu/g, ergosterol of 10.47mg/kg but a correspondingly lower OTA of 0.09ug/kg indicating the presence of OTA producing fungi with low production capacity. Samples CB15, CB16 fell in the category where the ergosterol, fungal counts and OTA have a direct relationship..

The results obtained in this study supported the usefulness of the quality grading system described by Scnurrer, (1995). Based upon ergosterol and OTA content of the samples, only 18.25% of the samples reached FOQ grade (<5mg/kg ergosterol), OTA of this class is <1ug/kg and 18.25% fell in the FUQ grade with the ergosterol of 5010mg/kg and OTA >1ug/kg. 31.25% fell in the category of Screen for mycotoxin (SFM) grade having ergosterol of 10-20mg/kg and OTA in the range of 1.68-2.31ug/kg while 31.25% also fell in the last grade Fuel quality grade (FuQ) with ergosterol > 20mg/kg and OTA > . 3.12ug/kg. Based on the grading system, the cocoa beans obtained form the warehouses in the food quality grade (FOQ) included CB10, CB12, CB13. Samples in the feed quality grade (FeQ) were CB5, CB7, CB9, CB11 and CB14. Those for screen for mycotoxin (SFM) included CB2, CB3, CB6, CB15 and CB16. The fuel quality grade (FuQ) category were CB1 and CB4 respectively (table 4.39)

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 meant for export.

Figure 1

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

Cocoa Sample	Fungal counts Cfu/g x 10^2	Ergosterol mg/kg	OTA µg/kg
CB1	62.10	24.23FUQ	6.33
CB2	18.00	12.48SFM	1.68
CB3	28.00	11.23SFM	1.86
CB4	33.02	20.41FUQ	3.12
CB5	7.61	6.38FEQ	1.00
CB6	30.00	17.21SFM	2.31
CB7	16.40	8.23FEQ	1.54
CB8	45.00	22.5FUQ	3.80
CB9	8.81	7.64FEQ	1.04
CB10	11.10	3.47FOQ	0.09
CB11	6.30	5.48FEQ	0.73
CB12	2.42	4.12FOQ	0.42
CB13	3.66	3.00FOQ	0.11
CB14	5.30	5.84FEQ	1.26
CB15	21.0	14.4SFM	1.71
CB16	23.90	15.3SFM	2.02

FOQ- food quality; FUQ- fuel quality; SFM- screen for mycotoxin- FEQ- feed quality grade

Figure 2

Table 2: Cocoa Bean grading based on ergosterol value

Grade/ergosterol content (mg/kg)	Cocoa samples
Food Quality	CB10
< 5	CB12
	CB13
Feed Quality	CB5
(5-10)	CB7
	CB9
	CB11
	CB14
Screen for mycotoxin	CB2
(10-20)	CB3
	CB6
	CB15
	CB16
Fuel Quality	CB1
(> 20)	CB4
	CB8

References

r-0. Abramson, D., Hulasarer, R., York, R.K., White, N.D.G. , Jayas, D.S. (2005) Mycotoxins, ergosterol and odours volatiles in durum wheat during granary storage at 16% and 20% moisture content. Journal of stored products research, 41, 67-76

r-1. 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 B1 in cocoa beans from southwestern Nigeria Malaysian Cocoa Journal, 3:35-46.

r-2. Bailly, J.D., Bars, L.E., Pietria, P., Bernard, A., Lebarsj G., (1999) Evaluation of fluorodensitometric method for analysis of ergosterol as a fungal marker in compound feeds. Journal of Food protection, 62, 686-690

r-3. Cahagnier, B., (1998) Moisssures des aliments peu hydrate, 225 pages, Lavoissier, Paris

r-4. Czaczyk, K. Trojanowska, K., Stachowiak, B., (2002), Inhibition of Ergosterol Biosynthesis in Fungal Plant Pathogens by Bacillus sp. Polish Journal of Environmental Studies Vol. 11, No. 5 593-597

r-5. Hippelein, M.Rugamer, M.,(2004) Ergosterol as an indicator of mould growths on building materials. International journal of hygiene and environmental health, 207, 379-385

r-6. Kadakal, C.,Artik, N.,(2004) A new quality parameter in tomato and tomato products: ergosterol. Criritical Review in food science and nutrition 44, 349-351

r-7. Kadakal, C., Ekincir, N.(2005) Ergosterol as a new quality parameter together with patulin in raw apple juice produced from decayed apples. Food Chemistry, 90,95-100

r-8. Lamper, C.S. Teren J., Bartok, T., Komoroczy, R., Mesterherzy A., Sagi., F. (2000) Predicting DON contamination in Fusarium-infected wheat grains via determination of ergosterol content Cereal Research Communication 28, 3, 337-344

r-9. Ng, H.E., Raj, S.S.A., Wong , S.H., Tey, D. and Tan H. M. (2008) Estimation of fungl growth using the ergosterol assay: a rapid tool in assessing the microbiological status of grains and feeds. Lett. Appl. Microbiol. (46) : 113=118

r-10. Pietri, A., B., Pallaroni. L.P (2004) Occurrence of mycotoxin aaand ergosterol in maize harvested over 5 years in Northern Italy. Food Additives and Contaminats 21, 479-487

r-11. Pitt, J.L., Hocking A.D. (1985) Fungi and food spoilage. Academic press, New York

r-12. Sashidhar , R.B., Sudershan R.V., Ramakrishna Y., Bhat, R.V. (1989) Rapid and specific method for screening ergosterol as index of fungal contamination in cereal grains. Food Chemistry 31, 51-56

r-13. 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

r-14. Saxena J., Munimbazi C., Bullerman L.B. (2001) Relationship of mould counts, ergosterol and ochratoxin A production .International journal of food microbiology 71, 29-34

r-15. Schnurrer J., (1993) Comparison of methods for estimating the biomass of three food-borne fungi with different growth patterns. Applied Enviromental Microbiology, 59, 552-555

r-16. Schnurrer, J. (1995) Detection and quantification of fungi in foods. Proceedings from the worksop fungal identification ntechniques. Barcelona, 5-8, April, 1995. 153-159

r-17. Schwardorfk K., Muller, H.M (1980) Determination of ergosterol in cereals mixed food components and mixed feeds by liquid chromatography. Journal of Association of Analytical Chemists 72, 457-462

r-18. Teren, J., Varga, J., Hamari, Z., Rinyu, E., Kevei, F. (1996). Immunochemical detection of Ochratoxin A in black Aspergillus strains. Mycopathologia 134:171-176.

r-19. Seitz L. M., Mohr, H.E., Burroughs R., Salier D.B., (1977) Ergosterol as an indicator of fungal invasion in grains. Cerela Chemistry, 54, 1207-1217

r-20. Seitz L.M., Salier D.B., Burroughs R., Mohr, H.E. Hubard, J.D. (1979) Ergosterol as a measure of fungi

growth. Phytopathology, 69, 1202-1203
r-21. 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

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