

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

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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 (Bailey *et al.*, 1999; Cahagnier, 1998; Schnurrer, 1993; Schwarzenbach 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 (Kadalkal and Artik, 2004; Sashdhar *et al.*, 1989). Moreover, significant correlations were found between ergosterol and the major mycotoxins notably Fumonisin B₁, 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 (Kadalkal 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 mycotoxin 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, ergosterol 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.

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Determination of fungal counts. Surface sterilized cocoa beans were plated on malt extract agar after serial dilution. Incubation was at $28 \pm 2^\circ\text{C}$ 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 $28 \pm 2^\circ\text{C}$ 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 Discussion

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; Czaczuk *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 CB₁ with fungi counts of 62.10×10^2 cfu/g had 6.33 $\mu\text{g}/\text{kg}$ of ochratoxin A and 24.23 mg/kg of ergosterol. High fungal counts of 33.02×10^2 cfu/g in CB₄ and ergosterol content of 20.41 mg/kg corresponded with the OTA observed (3.12 $\mu\text{g}/\text{kg}$). Sample CB₆ 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 $\mu\text{g}/\text{kg}$). 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 fungal counts of 11.10×10^2 cfu/g, ergosterol of 10.47 mg/kg but a correspondingly lower OTA of 0.09 $\mu\text{g}/\text{kg}$ 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 (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 $\mu\text{g}/\text{kg}$ and 18.25% fell in the FEQ grade with the ergosterol of 50~10 mg/kg and OTA > 1 $\mu\text{g}/\text{kg}$. 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 $\mu\text{g}/\text{kg}$ while 31.25% also fell in the last grade Fuel quality grade (FuQ) with ergosterol > 20 mg/kg and OTA from 3.12 $\mu\text{g}/\text{kg}$ and above. Based on the grading system, the cocoa beans obtained from 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. Those in the fuel quality grade (FuQ) category were CB₁ and CB₄ 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 1. Fungal contamination, Ergosterol and OTA of cocoa bean samples

Cocoa Sample	Fungal counts cfu/g $\times 10^2$	*Ergosterol mg/kg	*OTA mg/kg
CB ₁	62.10	24.23 \pm 0.01FUQ	6.33 \pm 1.00
CB ₂	18.00	12.48 \pm 0.02SFM	1.68 \pm 0.14
CB ₃	28.00	11.23 \pm 0.03SFM	1.86 \pm 0.33
CB ₄	33.02	20.41 \pm 0.12FUQ	3.12 \pm 1.01
CB ₅	7.61	6.38 \pm 0.22FEQ	1.00 \pm 0.01
CB ₆	30.00	17.21 \pm 0.11SFM	2.31 \pm 0.32
CB ₇	16.40	8.23 \pm 1.12FEQ	1.54 \pm 0.21
CB ₈	45.00	22.5 \pm 0.22FUQ	3.80 \pm 0.07
CB ₉	8.81	7.64 \pm 0.03FEQ	1.04 \pm 0.03
CB ₁₀	11.10	3.47 \pm 0.21FOQ	0.09 \pm 0.01
CB ₁₁	6.30	5.48 \pm 0.02FEQ	0.73 \pm 0.12
CB ₁₂	2.42	4.12 \pm 0.01FOQ	0.42 \pm 0.31
CB ₁₃	3.66	3.00 \pm 0.22FOQ	0.11 \pm 0.11
CB ₁₄	5.30	5.84 \pm 1.13FEQ	1.26 \pm 0.16
CB ₁₅	21.0	14.4 \pm 1.11SFM	1.71 \pm 0.21
CB ₁₆	23.90	15.3 \pm 0.31SFM	2.02 \pm 0.13

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

Table 2. Cocoa Bean grading based on ergosterol values

Grade/ergosterol content (mg/kg)	Cocoa samples
Food Quality < 5	CB ₁₀
	CB ₁₂
	CB ₁₃
Feed Quality (5-10)	CB ₅
	CB ₇
	CB ₉
	CB ₁₁
	CB ₁₄
Screen for mycotoxin (10-20)	CB ₂
	CB ₃
	CB ₆
	CB ₁₅
	CB ₁₆
Fuel Quality (> 20)	CB ₁
	CB ₄
	CB ₈

meant for export.

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