

Fungal Contamination and Nutritional Status of Dried Hide Skin (“Eha”) During Storage

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Abstract

This study was carried out to assess the fungal contamination and nutritional status of dried hide skin (“Eha”) during twenty four weeks of storage. Dried hide skin were purchased randomly from selected selling points at Oja-Bisi market located situated at Ado Ekiti, Ekiti State, Nigeria. They were stored for the period of twenty four weeks (six months). The minerals and proximate analysis of the samples were carried out at four weeks intervals while the mycobiota was isolated using direct plating, washing and dilution method. The associated fungal species were identified using standard methods. The aflatoxin extraction, quantitative and qualitative determination was carried out as previously described while the results were analysed using Duncan multiple range test. The proximate analyses showed that the ash, fat, fibre and carbohydrate content decreased while crude protein and moisture content increased during the period of storage. The mineral composition of the samples during the twenty four weeks of storage showed that all the minerals decreased except for Pb and Ca which were not detected. Four fungal species belonging to one genus was found to be associated with the stored dried hide skin from the markets sites. The fungal species were *Aspergillus flavus*, *A. niger*, *A. versicolor* and *A. tamari*. *A. flavus* was found to produce Aflatoxin B1 and B2 of 200 µg/kg. This study showed that dried hide skin (“Eha”) sold at Oja-Bisi market were contaminated with species of *Aspergillus* and were able to producing aflatoxins that poses great risk on the health of the consumers. Strict hygiene, constant microbial contamination investigation, good storage conditions and enlightenment programmes for meat sellers and consumers will reduce the environmental contamination and the risk to human health.

Keywords

Aflatoxins, “Eha”, Minerals, Proximate, Mycobiota

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1. Introduction

Eha, an edible extract of ponmo (dried hide skin) is consumed in major parts of Southwestern Nigeria as a food supplement and is now a delicacy in several parts of Africa [1, 2]. It is seen as a roughage source, with little reported nutritional value although Lehninger [3] and ACS.JELLO [4] considers it as a protein source because hide is majorly collagen. The process of Eha production is described by [1]

involving skinning, of cow meat and removal of the subcutaneous fat attached to the skin which is dried, salted or ashed and stored.

During storage process, Eha is known to be infected with fungi which are known to cause deterioration to stored products like grains, seeds, meat and meat products [5, 6]. Spores of spoilage fungi may be present on the Eha going for storage and improper storage conditions results in germination of spores and subsequent deterioration [7].

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Moisture level, pH and temperature are important factors influencing the deterioration and subsequent nutrient reduction, discoloration of stored meat products [8]. Another risk of fungi contamination of Eha is mycotoxin production with its resulting carcinogenic and mortality effects on consumption [9]. However, little information is available on the effect of fungal contamination on the nutritional status of dried meat, hides and skin in Nigeria; hence this study is aimed at evaluating the proximate and mineral composition as well as mycoflora and aflatoxin evaluation of Eha over a six month storage period.

2. Materials and Methods

2.1. Collection of Samples

Sixty pieces of ‘Eha’ were purchased from different selling points at Oja-Bisi market located in Ado-Ekiti, Nigeria. The samples were stored for the period of six months in a sterile airtight container at room temperature of 28-34°C and relative humidity of 65-72.

2.2. Isolation of Fungi from the Stored ‘Eha’

The fungi associated with the stored Eha was isolated using three methods as reported and described by Fagbohun and Lawal [10].

2.2.1. Direct Plating Method

Mouldy dried hide skin extract (Eha) was selected from the stored pieces and washed by two changes of sterile distilled water. Adopting the method of Arotupin and Akinyosoye [11], a sterile dissecting forceps was used to scrap gently a small portion and aseptically plated on prepared potato dextrose agar plated incubated at 28°C for 5 days. Further sub-culturing was done until pure colonies were obtained by successive hypha tip transfer method of Fagbohun *et al.* [12]. The cultures were examined under the microscope for fruiting bodies, hyphae to determine the common fungi present. The procedure above was carried out in duplicates.

2.2.2. Washing Method

One gram of mouldy Eha pieces was weighed into 10ml of sterile distilled water followed by vigorous shaking with a vortex laboratory shaker at 200 rpm for 5mins before drops of suspension of the contaminated water was introduced into potato dextrose agar plates. The drops were spread evenly on the plates using a sterile glass spreader. The inoculated plates were incubated for 5 days at 28°C and visible growths were observed.

2.3. Identification of Mycobiota

The pure cultures obtained were identified according to Alexopoulos [13] and Dugan [14], using morphological

characteristics, spore formation, the production of fruiting body and biochemical reactions. They were also compared with already identified fungal species obtained from the Plant Pathology Laboratory of the Institute of Agricultural Research and Training, Obafemi Awolowo University, Moor Plantation, Ibadan, Nigeria.

2.4. Nutrient Analysis

2.4.1. Proximate Analysis

The proximate analysis of the samples for moisture, ash, fibre and fat were done using the method described by AOAC [15]. The nitrogen was determined by micro-Kjeldahl method of Pearson [16], while the percentage nitrogen was converted to crude protein by multiplying with 6.25. All determinations were performed in triplicates.

2.4.2. Mineral Analysis

The minerals were analyzed by dry ashing the samples at 550°C to constant weight and dissolving the ash in volumetric flask using distilled water, deionized water with a few drop of concentrated HCl. Sodium and potassium content were determined by using a flame photometer (Model 405 Corning, UK) employing NaCl and KCl standards. Phosphorus was determined colorimetrically using Spectronic 20 (Gallenkamp, London, UK) as described by Pearson [16] with KH₂PO₄ as standard. All other metals were determined by using an atomic absorption spectrophotometer (Pekin-Elmer Model 403, Norwalk CT, USA). The detection limits had previously been determined using the methods of Techtron (17) as Mn 0.01, Cu 0.005, Co 0.05, Zn 0.005, Fe 0.02, Mg 0.002, Ca 0.004, Na 0.001ppm (all for aqueous solution). The optimum analytical range was 0.5 to 10 absorbance units with coefficient of variation of 0.05-0.04% phosphovanadomolybdate method using a Spectronic 20 colorimeter (Gallenkamp, London, UK) [15]. All the proximate values were reported in g/100g while the minerals were reported as mg/100 g. All determinations were done in triplicates. All chemicals used were analytical grade (BDH, London).

2.5. Aflatoxin Assay

2.5.1. Extraction of Aflatoxins

The fungal isolates belonging to the genus *Aspergillus* (*A. flavus*, *A. niger*, *A. versicolor* and *A. tamari*) were cultivated on Potato dextrose broth at 28°C for 7 days under stationary conditions. For each fungal isolate, the mycelium suspension was homogenized using liquid nitrogen and remixed with the broth medium in the flask. Mycotoxin extraction was done using chloroform: water (10:1 v/v) mixture. Column chromatography containing anhydrous sodium sulphate (15g) and silica gel (10g) was used to purify the crude extracts

obtained. Extracts were air dried and kept in dark vials until chromatographic analysis as described by Coomes *et al.* [18]; Lawal and Fagbohun [19] and Faleye and Fagbohun [20].

2.5.2. Qualitative Estimation of Aflatoxins

Precoated silica gel plates 60F254 (Merck, Germany) were used. Rectangular glass jar was used for developing chromatographic plates. A suitable volume of solvent mixture (chloroform: methanol, 97:3 v/v) was placed in the bottom of the jar so that the starting spots on the plates would be 1 cm above the upper surface of the solvent mixture. The chromatographic plates were activated by heating 1 hour at 120°C in a hot air oven, and removed immediately to a desiccator to cool [21]. Parallel starting spots, 2 cm from each side of the plates and 1.5 cm apart, were made with micropipettes from chloroform extracts with reference aflatoxins. Spots were left to air dry. Prepared plates were then transferred to the chromatographic jar, developed to a suitable distance (10 cm), and removed. The solvent front was marked and the plates were air dry. Spots were viewed under UV light (366 nm) and the outline of each fluorescent

spots was marked by sharp pin. Retention factor (R_f) values, colors, and intensities of the spots were compared with reference mycotoxins [18, 19].

2.5.3. Quantitative Determination of Aflatoxins

The dilution-to-extinction and comparison of standards techniques were used for estimation of aflatoxins concentrations [18, 19].

2.6. Statistical Analysis

The data obtained were analysed for mean, standard deviation, standard error and analysis of variance with SPSS version 17 software. Duncan multiple range test (DMRT) was used to determine the P values at $p = 0.05$.

3. Results and Discussion

The proximate content content of dried stored “Eha” during twenty four weeks of storage is shown in Table 1 below.

Table 1. Results of proximate analysis of dried stored “Eha” during twenty four weeks of storage (g/100g).

Weeks of storage	Ash	MC	CP	Fat	Fibre	CHO
4	12.04±0.01 ^f	24.22±0.01 ^a	36.23±0.01 ^a	2.42±0.01 ^c	3.52±0.01 ^f	21.58±0.01 ^d
8	11.95±0.01 ^e	24.50±0.01 ^b	36.49±0.01 ^b	2.38±0.01 ^d	3.47±0.01 ^e	21.23±0.01 ^b
12	11.88±0.01 ^d	24.70±0.01 ^d	36.51±0.01 ^b	2.36±0.01 ^d	3.42±0.01 ^d	21.14±0.01 ^a
16	11.76±0.01 ^c	24.55±0.01 ^c	36.52±0.01 ^{bc}	2.31±0.01 ^c	3.38±0.01 ^c	21.55±0.01 ^d
20	11.55±0.01 ^b	24.98±0.01 ^e	36.54±0.01 ^c	2.27±0.01 ^b	3.34±0.01 ^b	21.34±0.01 ^c
24	11.38±0.01 ^a	26.11±0.01 ^f	36.85±0.01 ^d	2.23±0.01 ^a	3.21±0.01 ^a	21.23±0.01 ^b

CHO: Carbohydrate, C.P: Crude protein, MC: Moisture content; Values represent mean of three replicates. Means with the same letter are not significantly different by Duncan’s multiple tests

The mineral content of ‘Eha’ during the twenty four weeks of storage is shown in table 2 below

Table 2. Results of mineral analysis of ‘Eha’ during twenty four weeks of storage (mg/100g).

Weeks of storage	Na	K	Ca	Mg	Zn	Fe	Cu	Pb	Mn	P
4	1.05±0.01 ^b	2.01±0.04 ^d	1.38±0.01 ^f	8.39±0.01 ^f	3.02±0.01 ^d	1.00±0.12 ^d	ND	ND	0.01	0.05
8	1.00±0.12 ^b	1.98±0.01 ^d	1.35±0.01 ^e	8.35±0.01 ^e	3.00±0.12 ^d	0.96±0.01 ^{cd}	ND	ND	ND	0.03
12	0.96±0.01 ^b	1.75±0.01 ^c	1.33±0.01 ^d	8.30±0.01 ^d	2.77±0.01 ^c	0.86±0.01 ^{bcd}	ND	ND	ND	ND
16	0.93±0.01 ^b	0.54±0.01 ^b	1.30±0.01 ^c	7.56±0.01 ^c	2.70±0.01 ^c	0.83±0.01 ^{bc}	ND	ND	ND	ND
20	0.50±0.01 ^a	0.47±0.01 ^a	1.25±0.01 ^b	6.25±0.01 ^b	2.50±0.01 ^b	0.77±0.01 ^b	ND	ND	ND	ND
24	0.42±0.01 ^a	0.45±0.01 ^a	1.03±0.01 ^a	5.60±0.01 ^a	2.31±0.01 ^a	0.53±0.01 ^a	ND	ND	ND	ND

ND: Not Detected; Values represent mean of three replicates. Means with the same letter are not significantly different by Duncan’s multiple tests

Table 3 shows the fungi species isolated from the stored ‘Eha’ during the storage period using various methods

Table 3. Fungi species isolated from stored dried “Eha” using various methods.

Fungal species	Number of weeks													
	0		4		8		12		16		20		24	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
<i>Aspergillus flavus</i>	-	-	+	+	+	+	+	+	+	+	+	+	+	+
<i>A. niger</i>	-	-	+	+	+	+	+	+	+	+	+	+	+	+
<i>A. versicolor</i>	-	-	+	-	+	-	-	-	+	-	+	-	+	-
<i>A. tamari</i>	-	-	-	+	-	+	-	+	-	+	-	+	-	+

Key: A: washing method, B: direct plating method, (+): isolated, (-): not isolated

Table 4 reveals the fungi species recovered from the stored ‘Eha’ samples as well as the isolates that produced aflatoxins

Table 4. Fungi species isolated from stored dried “Eha” using various methods.

Fungal species	Aflatoxin detected	Approximate concentration ($\mu\text{g}/\text{kg}$) medium
<i>A. flavus</i>	Aflatoxin B1	200
	Aflatoxin B2	200
<i>A. niger</i>	ND	ND
<i>A. tamari</i>	ND	ND
<i>A. versicolor</i>	ND	ND

3.1. Proximate Analysis

There was a reduction in ash content (12.04 ± 0.01 - 11.38 ± 0.01), fat (2.42 ± 0.01 - 2.23 ± 0.01), fibre content (3.52 ± 0.01 - 3.21 ± 0.01) and carbohydrate content (21.58 ± 0.01 - 21.23 ± 0.01) while it was observed that the crude protein (36.23 ± 0.01 - 36.85 ± 0.01) and moisture (24.22 ± 0.01 - 26.11 ± 0.01) increased over the period of storage and similar to the findings of Lawal *et al.* [22]. However, Faleye and Fagbohun [23] in their investigation on the proximate composition of “Tinco” dried meat observed that ash content (2.05 ± 0.01 to 3.07 ± 0.40), crude protein 69.98 ± 0.15 to 74.56 ± 0.80 and carbohydrate 4.08 ± 0.05 to 4.48 ± 0.15 increased which was in contrast to the result of this report. Rodolfo *et al.* [24] and Bilgrami and Dube [25] reported that fungi increased the protein content of the samples on which they grow. Protein increase could as well be from slight protein synthesis by proliferation of microorganisms and synthesis of enzyme protein [26].

3.2. Mineral Analysis

The summary of the mineral composition of “Eha” during 24 weeks of storage in mg/100g is shown in table 2. It was observed that there was a decrease during storage of Sodium (1.05 ± 0.01 - 0.42 ± 0.01), potassium (2.01 ± 0.04 - 0.45 ± 0.01), calcium (1.38 ± 0.01 - 1.03 ± 0.01), magnesium (8.39 ± 0.01 - 5.60 ± 0.01), zinc (3.02 ± 0.01 - 2.31 ± 0.01), iron (1.00 ± 0.12 - 0.54 ± 0.01), manganese (0.01 - 0.00) and phosphorus (0.05 - 0.00) while lead and copper were not detected. Oladejo and Adebayo-Tayo [6], observed in their study, an increase in sodium content of “Banda” during storage in contrast to the observation in this study. A high sodium content in food is unhealthy and is implicated in cases of high blood pressure, hence eating dried hide (Eha) could be beneficial for hypertensive and renal disease patients [27]. The manganese content of stored cattle hide was reduced during storage in this study which was in contrast to the observation of Bilgrami and Dube [25] who reported increased Mn^{2+} levels in unsinged hides (0.27 - 2.01) mg/100g and (0.18 - 3.07) mg/100g in singed hides. The contrast observed in this study

may be due to variations in mineral composition of soils, pasture and water sources on which the animals were raised [28]. The minute value of manganese detected in this study is well below the RDA value hence other micronutrient sources must be consumed by humans [29].

Potassium is of high importance in humans [30] and it was observed in this study that there was a decrease in its levels in “Eha” during storage (2.01 - 0.45) mg/100g. Mensah *et al.* [31] however found out high potassium levels in stored “Kale” (7.03) mg/100g suggesting that “Eha” is not a good source of K^+ neither is it beneficial for diuretic patients who requires replenishment of K^+ lost in urine [29]. The magnesium (8.39 ± 0.01 - 5.60 ± 0.01), calcium (1.38 ± 0.01 - 1.03 ± 0.01), iron (1.00 ± 0.12 - 0.54 ± 0.01), phosphorus (0.05 - 0.00) values were reduced during storage as observed in this current study hence indicating that stored “Eha” doesn’t meet the RDA value of these micronutrients for humans [29, 30, 31]. However, the zinc values, though reduced during storage, as also reported by Obiri-Danso *et al.* [32] on singed cattle hides, was in range with the RDA values for infants and adults and is a good source of zinc [33].

3.3. Mycobiota of Eha

The mycoflora of stored “Eha” were identified as *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus versicolor* and *Aspergillus tamari*. This agreed with the findings of Oladejo and Adebayo-Tayo [6] that isolated *Aspergillus* species from “Banda” dried meat. *Aspergillus flavus* and *Aspergillus niger* are commonly isolated from dried meat and meat products [34, 35, 36, 37]. Also, Mahmoud *et al.* [35] isolated toxigenic species of *Aspergillus* from dried meat and Fakolade and Omojola [38] established that fungi occurrence on stored products depends on growth conditions and method of isolation employed.

3.4. Aflatoxin Detection

The results of the aflatoxin production by some species of *Aspergillus* isolated from “Eha” are shown in Table 4. It was observed that only *Aspergillus flavus* produce aflatoxins B1 and aflatoxin B2 after the 16th week of storage. The result of this study is in agreement with Alhussaini [20] and Lawal and Fagbohun [19] who studied the mycobiota and mycotoxins of stored marketed product and reported the detection of Aflatoxin B1 and B2 produced by *Aspergillus flavus*. The isolation of associated fungi especially *Aspergillus* spp. from dried hide skin might be as a result of the appreciable nutrient present in the skin which the fungi species utilize. However, the production of aflatoxin by fungi species result in contamination of the hides and rendering it unfit for human consumption because of their health implications.

4. Conclusion

This present work showed that the examined stored “Eha” were contaminated with *Aspergillus* species capable of producing mycotoxins hence indicating risk of intoxication on consumption of improperly processed “Eha”. The non-detection of heavy metals Pb and Cu could be attributed to the animal rearing or environmental factors. However various levels of Fe, Zn, Mn and Ca were detected. Strict hygiene, constant microbial investigation, good storage conditions and enlightenment of dried meat sellers on appropriate storage methods which can reduce contamination and deterioration of stored meat products should be implemented to address the concerns and expectations of consumers of dried stored meats and meat products.

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