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The effect of biodeterioration on the nutritional composition and microbiology of an edible long- winged reproductive termite, *Macroterms bellicosus.* Smeathman

[1]W. Braide*., [2]R.N. Nwaoguikpe ., [1]S .E Oranusi., [1]L.I.Udegbunam., [1]C. Akobondu and [1]S.I. Okorondu

1.Department of Microbiology, Federal University of Technology, P.M.B 1526, Owerri, Imo State, Nigeria.
2. Department of Biochemistry, Federal University of Technology, P.M.B 1526, Owerri, Imo State, Nigeria.

Abstract

Macroterms bellicoccus, an edible long-winged reproductive termite is an important food source and its harvesting is becoming a major activity in some rural areas in Nigeria. The microbiology and proximate characteristics of the fresh products had been reported. Proximate analysis and gross energy determined for the deteriorated sample proved the effect of microorganisms on the nutritional composition of the sample. There was a drastic reduction in the protein and fat content as well as the gross energy value of the deteriorated sample as a result of disintegration of valuable food components by microorganisms. Moisture content was high and may encourage deterioration and spoilage. Seven genera of bacteria and six genera of moulds and one species of yeast were isolated from the deteriorated sample. The colony count of bacteria are *Staphylococcus* (3.0×10^5) , *Bacillus* (1.2×10^4), *Pseudomonas* (6.0×10^3), *Lactobacillus* (2.0×10^2), *Acinetobacter* (2.0×10^4), *Proteus* (1.4×10^3) and *Enterococcus* (3.0×10^4), while the fungal counts were *Mucor* (3.0×10^2), *Rhizopus* (1.0×10^2), *Aspergillus* (3.0×10^2), *Penicillium* (5.0×10^2), *Fusarium* (4.0×10^2), *Geotrichum* (3.0×10^3) and *Sacchoromyces* (2.0×10^4), *Mucor* sp (1.5×102), *Aspergillus flavus* (3.0×10^1). *Staphylococcus* and *Bacillus* species produce potent toxins and their presence portends health risk to the consumers. *Aspergillus flavus* and *Penicillium verrucosum* produce mycotoxins that cause cancer and liver disfunctioning amongst others. *Mucor* sp are involved in food deterioration and spoilage. Deterioration appeared to be a concerted effort of microorganisms, moisture and high nutrient composition of the sample.

Keywords: biodeterioration,termite,microbiology,nutrition

Introduction

There has been increased curiosity and interest in the use of insects as food after an international conference held outside Africa. Allotey and Mpuchane (2003) reported that insects are and have been traditionally and nutritionally important food for Africans, Asians, Australians and Latin Americans where they have been utilized as food.

As the population of the world grows, greater demands are being placed on food security and production, and since insects are so abundant and contained many useful nutrients, including protein (Amadi *et al*., 2005; Braide *et al*., 2009; Braide *et al*., 2010), it seems reasonable to eat them.

In Nigeria, numerous edible insects and caterpillar are known, but relevant literature on their proximate composition and microbiological qualities are few (Wachukwu *et al*., 2002; Amadi *et al*., 2005; Braide *et al*., 2009; Braide *et al*., 2010).

The long-winged reproductive termite, *Macroterm bellicosus* for instance is a delicay in some parts of Nigeria where they are harvested and eaten raw or roasted or is dried and added to foods (Professor Anayo Adibo per comm.).

The poor sanitary harvest, preparation, storage and marketing of the finished product may contaminate and reduce its shelf life. An internationally acceptable standard in food quality emphasized that food (processed or raw)

*Corresponding author. mailing address: wesleybraide2005@yahoo.com. +234 08037100964

should be wholesome and free of contaminants (FAO, 1992).

Food borne illnesses have been associated with several microorganisms including bacteria and fungi (Eisenberg *et al*., 1975; Frazier and Westhoff, 1978; Duguid *et al*., 1978; Bryant *et al*., 1988; Jawetz *et al.*, 1980; Pelczar *et al*., 1986; Perry and Staley, 1997; Nester *et al*., 1998; Prescott *et al*., 1998; Greenhood *et al.*, 2002; Willey *et al*., 2008). Fungi are the major cause of food deterioration and spoilage world wide, ranking second to insects (Jarvis *et al*., 1983). The nutritional value of stored products had been affected by fungal infestation resulting in loss of dry matter through utilization of proteins and lipids (Christensen and Kaufmann, 1965).

Although insects may have supplied a substantial amount of animal proteins in the diets of Africans (Allotey and Mpuchane, 2003), lack of adequate storage facilities may have profound effects on the keeping quality of this insect product in Africa and developing countries.

However, research which considers the major agents of deterioration as well as the assessment of quality and safety of the edible long-winged reproductive termite has not been fully documented.

In this paper, the effect of deterioration on the nutritional values of the edible termite was determined. The microbiology of the product was also studied.

Materials and Methods

Sample collection

Fifty grams (50g) of the termite preserved by frying were purchased at the popular *Ekeonuwa* daily market in Owerri, the capital city of Imo State in Nigeria between the months of June and July. Ten samples were collected at random and composited into a sterile cellophane bag.

Deterioration and preparation of sample for analysis

The composite sample was transferred into a tray and left on the laboratory bench for one week to allow for natural deterioration.

Twenty grams of the composite sample was weighed with sartorious electrical balance and sorted to remove dirt's and other particulate matters before drying.

Drying of sample for analysis

Timely drying with reduced heat was employed to maintain the nutrient contents. Cabolite moisture extraction oven controlled at 60^0C-65^0C was used to dry the sample for 12hours. Dried sample was ground in a laboratory mill (Arthur Thomas brand) for 10mins at maximum speed, and the powder sieved through a 212µm sieve (A.O.A.C, 1995).

Chemical analysis of sample

Sample was analysed in triplicates and the mean values obtained. Sample was determined on dry matter basis and the results interpreted in percentage. Crude fibre and ash were determined by the Weenden gravimetric method (Kirk and Sawyer, 1998). Moisture content of dried powder was determined by gravimetric method (A.O.A.C, 2000). Crude protein was determined by the Kjedahl method (James, 1995; Teferra *et al*., 1997; Chang, 2003) in which nitrogen content was determined and multiplied by 6.25 to obtain the protein content. Soxhlet solvent extraction method described by James (1995) was used in the determination of fat content. Nitrogen free extracts (Total carbohydrate) was determined by calculation.

Determination of microbial load

Determination of the microbial population of the sample was done by standard method adopted from I.C.M.S.F (1978) and Harrigan and McCance (1990). Twenty grams of the sample was macerated in a sterile stomacher blender containing 180ml of sterile peptone water and homogenized by shaking vigorously, and diluted decimally until 10^4 was obtained.

An aliquot portion (0.1ml) of the 10^4 dilution was inoculated in duplicate onto a surface dried freshly prepared nutrient and MacConkey agar. The same volume of the 10^2 was transferred in duplicate onto potato dextrose agar. The plates were spread evenly with a sterile spreader and incubated for 5 days at ambient temperature for heterotrophic fungi, and at 37^0C for heterotrophic bacteria and coliforms (Pelczar and Chan, 1977; Beishir, 1987; Cheesbrough, 2000).

Identification of isolates

Cultures growing on PDA were identified based on macro and micromorphology, reverse and surface colouration of colonies and slide culture technique (Ellis, 1971; Ellis, 1976; Samson and van Reenen- Hoekstra, 1988; Domsch *et al*., 1993; Pitt and Hocking, 1994; Abbey, 2007;). *Penicillium* species were identified using colony diameter, macro and micromorphology according to standardized conditions of Pitt (1979) and Pitt and Hocking (1985). Colony counts of isolates were done using the Gallenkamp electronic counter for bacteria and magnifying lens for fungi. Bacterial isolates were identified on the basis of their colonial, microscopic and biochemical characteristics (Pelczar *et al*., 1993; Cheesbrough, 2000). Confirmation of the organisms was done with reference to standard manuals (Buchanan and Gibbon, 1974; Barnett and Hunter, 1987).

Results

Tables 1 and 2 shows the values of proximate composition and gross energy of the deteriorated sample of *Macroterms bellicosus* respectively. Total heterotrophic counts and the characteristics (morphological and microscopic) of fungi isolated from the sample are shown in Table 3. Table 4 shows the total heterotrophic counts, colonial, microscopic and biochemical characteristics of bacteria isolated from the sample.

Seven species of bacteria and fungi were isolated from the deteriorated sample.

Table 1
Proximate composition of deteriorated sample of *M. bellicosus* (MEAN ± SD)

moisture content	crude protein	fat	crude fibre	carbohydrate	ash
20.13	34.20	1.10	6.19	33.83	4.55
21.20	36.10	1.19	6.20	30.70	4.60
21.02	34.90	1.21	6.17	32.17	4.53
20.78±0.35	35.07±0.95	1.17±0.07	6.19±0.07	32.23±1.57	4.56±0.03

Table 2
Gross energy (Kcal/100g) 0f deteriorated sample of *M. bellicosus* (MEAN ± SD)

Sample code	P × 4.0 (a)	CHO × 4.0 (b)	F × 9.0 (c)	Total energy (Kcal/100g) (a+b+c)
A	136.80	135.32	9.90	282.02
B	144.40	122.80	10.71	277. 91
C	139.60	128.68	10.89	279.17
				279.70±2.11

Table 3
Total heterotrophic counts and characteristics of fungi isolated on deteriorated sample of *M. bellicosus*

Colonial characteristics	colony counts	microscopic appearance	identity of isolate
Colonies short and velvety	3.0×10^2	hyphae non septate, sporangium globosed, grey sporangiophores branched	*Mucor* sp
White filamentous aerial mycelia thick and cotton wool-like	1.0×10^2	hyphae of mycelium non septate. sporangiophore bear terminal black sporangium with spores	*Rhizopus stolonifer*
Colonies appear as thick yellow dots, turns dark brown to black on prolonged Incubation.	3.0×10^2	hyphal cells are septate and bears conidiophores.Vesicles bears phialides with mop- head conidia.	*Aspergilus flavus*
White velvety dot- like colonies on culture plate. Turns green on prolonged incubation	5.0×10^2	hyphae are septate. Conidiophores branched to form brush-like conidial head. Phialides bears conidiophores	*Penicillium caseicolum*
Short slender cotton-like mycelia with slight pigmentation	4.0×10^2	curved sickle shaped irregular branching with thick conidial cell wall	*Fusarium poae*
Colonies appeared as light yellow dots and cotton wool-like	3.0×10^3	hyphae septate and dichotomously branched.Arthrospores appeared rectangular or cylindrical	*Geotrichum candidum*
Dull cream mucoid and butyrous large colonies	2.0×10^4	gram positive large spherical and oval budding cells	*Saccharomyces cerevisiae*

Table 4
Total heterotrophic counts and characteristics of bacteria isolated on deteriorated sample of *M. bellicosus*

Pigmentation identity of isolates	Gram stain	Cat	Oxi	Coag	In	MR	VP	Cit	Mot	G	S	L	Mn	colony count
Golden yellow *Staphylococcus aureus*	+S	+	-	+	-	-	+	+	-	+	+	+	+	3.0×10^4
Green *Pseudomonas aeruginosa*	-R	+	+	-	-	+	-	+	+	+	-	-	+	6.0×10^3
- *Bacillus cereus*	+R	+	-	-	-	-	+	+	+	+	+	+	+	1.2 * 104
- *Proteus mirabilis*	-R	+	-	-	-	+	-	-	-	+	+	-	-	1.4×10^3
- *Acinetobacter* sp	-S/R	+	-	-	-	+	-	+	-	+	+	+	+	2.0×10^4
- *Enterococcus faecalis*	+S	+	-	-	-	+	-	+	-	+	+	+	+	3.0×10^4
- *Lactobacillus* sp	+R	-	-	-	-	+	-	+	-	+	-	+	+	2.0×10^4

R,rod shaped; S, spherical shaped; Cat, catalase; Oxi, oxidase; Coag, coagulase; In, indole; MR, methyl red; VP, Voges Proskaeur; Cit, citrate; Mot, motility;
G, glucose; S, sucrose; L, lactose; Mn, mannitol

Discussion

Macroterms bellicosus is a highly perishable product. It differs from stored commodities because it absorbs moisture even during storage (FAO, 1992). Braide *et al.* (2009) reported that the crude protein content of freshly processed termite is high (68.02% – 69.07%). Tables 1 and 2 shows the nutritional status and gross energy values of the deteriorated sample of *Macroterms bellicosus.* Crude protein (35.07%) value is low whereas the moisture content is high. The high moisture contents may have encouraged the growth and proliferation of spoilage and pathogenic microorganisms, especially bacteria and fungi shown in Tables 3 and 4 respectively. The activities of the microorganisms growing on the sample may have lowered the protein and lipid quality as was reported by Christensen and Kaufmann (1965). Gross energy content was low as a result of the microbial attack on the protein and lipids.

Some of the microbial isolates are important medically. *Bacillus cereus* and *Staphylococcus aureus* produce potent

toxins associated with food borne illnesses (Eisenberg *et al*., 1975; Willey *et al*., 2008; Greenhood *et al*., 2002). *Escherichia coli* produce toxin which cause food infection with symptom like diarrhea and severe abdominal pains. *Pseudomonas aeruginosa* contaminate food with high water activity and produce protease and lipase that catalyse reaction causing degradation of proteins and fats resulting in an undesirable flavour of the food (Pelczar *et al.*, 1986; Perry and Staley, 1997; Bryant *et al*., 2005; Frazier and Westhoff, 1978; Talaro and Talaro, 2002; Willey *et al*., 2008). *Proteus* spp contaminate highly proteinous food and lowers the nutritional quality, but rarely cause food borne infections (Prescott *et al*., 1998; Willey *et al*., 2008).

Fungi are widely distributed in air and in the soil (Frazier and Westhoff, 1978; Braide *et al*., 2008). *Aspergillus* and *Penicillium* species are frequently isolated from food (Frazier and Westhoff, 1978; Pitt and Hocking, 1985; Abbey, 2007; Mpuchane *et al*., 1996) and may have contaminated the products through their spores or from soil during harvest and storage. *Aspergillus flavus, Penicillium verrucosum, Fusarium poae* and *Geotrichum candidum* produce various mycotoxins in food and feeds in the field or under storage (Abbey, 2007, Efiuvwevwere, 2000; Frazier and Westhoff, 1978; Pitt, 2000; Pitt and Hocking, 1985). *A. flavus* elaborate aflatoxins that may induce hepatocellular carcinoma. Toxins produced by *P.verrucosum* may be nephrotoxic and carcinogenic, *F. poae* toxins give rise to allergic symptoms or are carcinogenic in long term consumption (Abbey, 2007; Efiuvwevwere, 2000; Pitt, 2000). *Rh. Stolonifer* and *Mucor* spp are less fastidious and are frequently involved in the deterioration and spoilage of food with low moisture content. They also produce mycotoxins associated with various mycotoxicoses (Efiuvwevwere, 2000; Abbey, 2007).

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