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Full Length Research Paper

Microbiological quality of an edible caterpillar of an emperor moth, *Bunaea alcinoe*

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An investigation into the microbiological status of processed caterpillar of a lepidopteran, *Bunaea alcinoe* revealed the presence of six genera of bacteria and three genera of moulds including one species of yeast. The microbial population of 4.49 × 10^7 (bacteria) and 9.5 × 10^6 (fungi) indicates contamination of the product. *Pseudomonas aeruginosa* and *Proteus mirabilis* are food contaminants with high protein contents. *P. aeruginosa* produce protease and lipase that catalysis reaction causing degradation of proteins and lipids respectively, resulting in an undesirable flavours in food products. *P. mirabilis* rarely give rise to food borne infections, but lowers the nutritional quality of contaminated foods. *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli* produce various toxins associated with food infection and intoxication. *Streptococcus mitis* is involved in dental caries and periodontal disease when ingested in food. *Aspergillus, Penicillium* and *Fusarium* species elaborate lethal mycotoxins associated with carcinogenicity and nephrotoxicity in humans and animals. Majority of the isolates are soil borne and may have contaminated the product during harvest. Recontamination of the product could arise from poor handling, inadequate temperature of processing and exposure during sun drying. The use of modified atmospheric packaging system is strongly recommended to reduce moisture, microbial contamination and enhanced the shelf life of the nutritious product.

Key word: Edible, caterpillar, microbiological quality.

INTRODUCTION

The caterpillar of *Bunaea alcinoe* is popularly called *Egu* by the Igbo speaking tribe of eastern and southern part of Nigeria. The caterpillar is edible and highly nutritious (Amadi et al., 2005; Braide et al., 2010a), and feeds on the leaves of some economic and ornamental plants such as *Gmelina arborea, Spondias mombim, Pentaclethra macrophylla, Terminlia cattapa, Cananga odorata, Harungana madagascariensis, Anthocleista* species, and cause enormous defoliation of the leaves. The leaves are rich in nutrients (Braide et al., 2010b) and provide food for the highly voracious larvae.

The matured larvae (5^{th} to 6^{th}) are usually harvested directly from the leaves or hand picked when they fall on the ground. It is also a common practice by some harvester to dig out larvae that burrow into the ground to pupate. In all these methods of harvest, larvae are exposed to contamination caused by microorganisms, especially the larvae that burrow into the soil. Braide et al. (2008) had reported that some microorganisms are associated with the metamorphoses of the larvae of *B. alcinoe*.

The method of processing, packaging, storage and marketing of the products are primitive and unhygienic and may further expose the final products to recontamination. The microbiological characteristics of some edible insects larvae had been reported (Gashe et al., 1996, 1997; Mpuchane et al., 1996, 2000). Amadi et al. (2005) and Braide et al. (2009) reported that bacterial isolates belonging to the genera, *Staphylococcus*, *Bacillus, Micrococcus* and *Acinetobacter* occurred on the skin and intestinal tract of *B. alcinoe*. Some of these isolates are known to produce enterotoxins (Eisenberg et

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al., 1975; Jawetz et al., 1980; Pelczar et al., 1986; Pelczar et al., 1986; Bryant et al., 1988; Nester et al., 1998; Prescott et al., 2002). Inadequate cooking temperature may encourage the sporulation of *Bacillus cereus* even though *Staphylococcus aureus* may be destroyed.

This study reported on the microbiological status of processed and ready to eat caterpillar of an emperor moth, *B. alcinoe*.

MATERIALS AND METHODS

Collection of sample

Twenty (20) live 5th instar larvae of the caterpillar were collected directly from the leaves of *Cananga odorata* plant into a perforated basket and transported to the laboratory for processing.

Preparation of sample

The samples were degutted by pushing a blunt stick through the anus, washed, spiced with pepper and onion, salted and finally wrapped in the leaves of *Gmelina arborea*. Roasting was done over red hot charcoal for 5 min, and sun dried by spacing on a transparent polyethylene material for 8 h.

Microbiological analysis of sample

Sun-dried samples were transferred into a sterile aluminum foil and transported to the laboratory. Samples were stored in a refrigerator at 4°C until analyzed.

Determination of microbial load

Twenty grams of the sun dried sample was blended in 180 ml of sterile distilled water to obtain 10^{-1} dilution. Further dilutions up to 10^{-6} were made. One-tenth milliliter (0.1 ml) of the 4th and 6th dilutions were aseptically inoculated in duplicate onto freshly prepared surface-dried Sabouraud dextrose agar (SDA) and nutrient agar (NA) media, respectively (ICMSF, 1978; Pelczar and Chan, 1977). Similarly, 0.1 ml of 10^{6} dilutions was inoculated onto MacConkey agar. Inoculate were spread evenly over the surface of the media with a sterile spreader. Culture plates were incubated at the recommended temperature and time according to Cheesbrough (2000).

Colony counts of isolates

Colony count was done using the Gallenkamp electronic colony counter for bacteria and magnifying lens for moulds. Mean colony counts were calculated and expressed as colony forming units per gram (cfu/g) of the sample analyzed (ICMSF, 1978; Harrigan and McCance, 1990).

Characterization and identification of isolates

The methods described in Cheesbrough (2000) and Pelczar et al. (1993) were adopted in characterization of isolates. Isolates were identified by standard method (Buchanan and Gibbon, 1974; Barnett and Hunter, 1987; Abbey, 2007).

RESULTS

Table 1 show the total heterotrophic colony counts and colonial characteristics of bacteria isolated from the processed larvae of *B. alcinoe*. Total bacterial population of 4.49×10^{7} cfu/g was obtained on both nutrient and MacConkey agar.

The microscopic and biochemical characteristics of the bacterial isolates are shown in Table 2. Six genera of bacteria, namely, *Staphylococcus, Bacillus, Proteus, Pseudomonas, Streptococcus* and *Escherichia* were identified (Table 2) with reference to Buchanan and Gibbon (1974), Duguid et al. (1978), Harrigan and McCance (1990) and Cheesbrough (2000).

A total of 9.5×10^{6} Cfu/g of fungi was isolated on SDA medium. Three genera of moulds, namely, *Aspergillus, Penicillium* and *Fusarium,* and one species of yeast, *Saccharomyces cerevisiae,* were isolated from the processed larvae on the basis of their colonial morphology and microscopic characteristics (Table 3). The identities of the fungal isolates were *cross*-matched with those described in Barnett and Hunter (1987) and Harrigan and McCance (1990).

DISCUSSION

Egu is nutritious, especially in proteins (Amadi et al., 2005; Braide et al., 2010a) and therefore may support the growth and proliferation of a large population, especially proteolytic bacteria and fungi. The large population of bacteria (4.49×10^{7} cfu/g) and fungi (9.5×10^{6} cfu/g) indicates contamination.

Altogether, six genera of bacteria, three genera of moulds and one species of yeast were isolated from the sample (Tables 2 and 3). The bacteria were *S. aureus, B. cereus. Pseudomonas aeruginosa, Escherichia coli*, *Proteus mirabilis* and *Streptococcus mitis*, while the fungi were *Aspergillus niger, Penicillium caseioculum, Fusarium moniliforme,* and *Saccharomyces cerevisiae. Pseudomonas aeruginosa* and *Proteus mirabilis* are proteolytic bacteria implicated in food deterioration and spoilage. *Pseudomonas* is a contaminant in food with high moisture content. It produces protease and lipase that catalyse reactions causing degradation of proteins and lipids resulting in undesirable flavours in the food products (Pelczar et al., 1986; Harrigan and McCance, 1990; Nester et al., 1998).

Proteus spp. are associated with food with high protein contents, but rarely give rise to food borne infections, though they lower the nutritional quality of contaminated foods (Prescott et al., 2002). *B. cereus,* an aerobic spore former, produces exotoxins which cause mainly diarrhoea and vomiting during ingestion. Some strains of *S. aureus*

Table 1. Total counts and colonial characteristics of bacteria isolated from processed larvae of *B. alcinoe*

Colony count (cfu/g)	Cultural morphology	Microscopy
2.0×10^5	Cream moist colonies on MCA, discrete colonies rarely seen, growth spread over surface on NA	Gram negative short rods
7.0×10^6	Golden-yellow colonies on NA, pink colonies on MCA	Gram positive cocci predominantly in clusters
1.32×10^7	Cream moist pin-point colonies on NA	Gram positive cocci in chains
1.77×10^7	Rose-pink, pin-point colonies on MCA	Gram negative rods predominantly singles
3.0×10^6	Cream dull and dry wavy, flat colonies on NA.	Gram positive bead like rods in short chains
3.8×10^6 (4.49×10^7)	Green-blue colonies on NA, cream irregular surface on MCA	Gram negative rods in singles, few in short chains

No. in parentheses indicates total colony counts; NA, nutrient agar; MCA, MacConkey agar.

Table 2. Biochemical characteristics of bacteria isolated from processed larvae

Cell morphology			Sugar utilization					Biochemical test									Cit	Most probable identity
Mot	Spore	Cap	Glu	Suc	Mal	Lac	Mann	Cat	Oxi	Coag	In	MR	VP	No_3	H_2S	Urease		
+	-	-	+	-	-	-	-	+	-	-	+	+	-	+	-	+	+	*Proteus* sp.
+	-	-	+	-	-	-	+	+	+	-	+	+	-	+	nd	+	+	*Pseudomonas aeruginosa*
-	-	-	+	+	+	+	+	+	-	+	+	+	-	+	nd	+	+	*Staphylococcus aureus*
-	+	-	+	-	nd	-	-	+	-	nd	-	-	+	nd	nd	-	+	*Bacillus* sp.
-	-	-	+	+	+	-	-	+	-	-	-	+	-	nd	nd	nd	-	*Streptococcus* sp.
+	-	-	+	+	+	+	+	+	-	-	+	+	-	+	-	-	-	*Escherichia coli*

Mot, motility; Cap, capsule, Glu, glucose; Suc, sucrose; Mal, maltose; Mann, mannitol; Cat, catalase; Oxi, oxidase; Coag, coagulase; In, indole; MR, methyl red; VP, Voges Proskaeur; No_3, nitrate reduction; H_2S, hydrogen sulphide; Cit, citrate; nd, not done; +, positive; -, negative.

produce enterotoxins that have been found to cause staphylococcal food poisoning. *E. coli* also produce toxins which cause food infections like diarrhoea and severe abdominal pains (Nester et al., 1998). *S. mitis* is an alpha heamolytic bacterium less frequently involved in food spoilage and are commonly found in large numbers in dental plaque, carious teeth and root abscesses and probably play an important part in initiating caries and periodontal disease (Duguid et al., 1978). Fungi are widely distributed in soil and air. *Aspergillus* is frequently isolated from food (Frazier and Westhoff, 1978). Fungi may have contaminated the product through the deposits of their spores during sun-drying or from soil during harvesting. *A. Penicillium* and *Fusarium* species are known to produce various mycotoxins in foods and feeds in fields or under storage (Frazier and Westhoff, 1978). Aflatoxins produced by *Aspergillus* species may induce hepato-cellular carcinoma. Toxins produced by *Penicillium* may be nephrotoxic and carcinogenic. *Fusarium* sp. produces a variety of mycotoxins with widely divergent biological and toxicological effects in humans and animals consuming the contaminated commodities (Siame et al., 1998). *Fusarium* toxins when ingested from food gives rise to allergic symptoms or may be carcinogenic in long term consumption (Pitt, 2000; Pitt and Hocking, 1985). The source of the yeast could not be ascertained. The microbiology of edible insects and their products have been discussed (Gashe et al., 1996, 1997; Amadi et al., 2005; Wachukwu

Table 3. Total colony counts and morphological characteristics of fungi isolated from processed larvae.

Cultural morphology	Microscopy	Colony counts (Cfu/g)	Probable identity
Short slender cotton-like mycelia with slight pigmentation	Curved sickle shaped irregular branching with thick conidial cell wall	7.4×10^6	*Fusarium moniliforme*
White filamentous mycelia with visible dark brown spores above mycelia	Hyphal cells are septate and bears non-septate conidiophore with terminal enlarged spherical conidial head (vesicles) which bears phialides with a mop-head	6.0×10^5	*Aspergillus niger*
Colonies are white velvety-like dot. Colonies remain white even on prolonged incubation	Hyphate is septate. Conidiophore branched to form brush-like conidial head with whorl of phialides	$1.2 \times 1\ 0^6$	*Penicillium caseicolum*
Dull cream mucoid and butyrous large colonies on NA, pink on MCA	Gram positive large spherical/oval budding cells	3.0×10^5 (9.5×10^6)	*Saccharomyces cerevisiae*

No. in parentheses indicates total colony counts; NA, nutrient agar; MCA, MacConkey agar.

et al., 2002). As the use of insects and their products in food and feeds in Africa, Asia and other parts of the world becomes more prominent (Oliveira et al., 1976; DeFoliart, 1989; Fasoranti and Ajiboye, 1993; Allotey and Mpuchane, 2003), efforts in improving on the quality of the finished products should be intensified. To achieve this, adequate precautions should be taken during processing and handling of the sample. For example, during processing caterpillar may come in contact with soil, faecal matters, gut contents and consequently becomes recontaminated with microorganisms that cause spoilage during drying and storage. This if adhered strictly will go a long way in reducing the health risk associated with microbial contamination. Storage and packaging are also important factors to be considered in ensuring the safety of the product. In storage, caterpillar may lose quality characterized by increased infestation by fungi. During fungal infestation, there is loss of dry matter through utilization of proteins and lipids leading to reduction in nutritional value. Microbial load of contaminants in processed caterpillar can be reduced by vinegar treatment during processing and post-processing stages. The use of modified atmospheric packaging (MAP) system (Prescott et al., 2002) should be advocated.

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