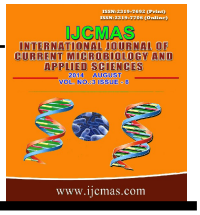
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 9 (2014) pp. 1085-1094**
http://www.ijcmas.com



Original Research Article

Microbiological quality of *Gonimbrasia belina* processed under different traditional practices in Gwanda, Zimbabwe

Felix M. Mujuru*, Raphael Kwiri, Clarice Nyambi, Clive Winini and Dinga N. Moyo

Department of Food Processing Technology, School of Industrial Science & Technology, Harare Institute of Technology, Ganges Rd, Box BE 277, Belvedere, Harare, Zimbabwe.
**Corresponding author*

Keywords

Contamination, Microbiological quality, Mopani worm, drying, indicator microorganism pathogens, spoilage organisms.

A B S T R A C T

The aim of this study was to investigate the microbiological quality of Mopani Worms (MW) (*Gonimbrasia belina)* processed under different traditional practices in Gwanda, Zimbabwe.Freshly harvested Mopane worms (MW) were degutted using bare hands or gloved hands. The degutted worms were sub-divided into 4 equal portions and subjected to different traditional drying methods namely, boiling in salted water (5% w/w salt) for 30 minutes and solar drying, open pan roasting, drum roasting and hot-ash drying to approximately 15% moisture content. Thereafter, dried MWs were microbiologically analysed against indicator micro-organisms (total bacteria counts, coliforms and *Escherichia coli)* and pathogens (*Salmonella* spp. and *Staphylococcus aureus*) as well as spoilage organisms (yeast and molds).Microbiological tests conducted showed that, total bacteria counts (TBC) were relatively low with values ranging from 10-2500 $CFUg^{-1}$. Significantly, degutting MWs using bare hands followed by drum roasting and boiling in salt water coupled with open pan roasting, had the least TBC (10 $CFUg^{-1}$and 30 $CFUg^{-1}$) respectively. Coliforms and *E. coli* were also detected in MWs with values ranging from 0-43 CFU g^{-1} and 0-30 CFU g^{-1} respectively (bare hands degutted) whilst in gloves degutted MWs coliforms counts were 0-45 CFU g^{-1} and *E. coli* counts were 0-35 CFU g^{-1}. Furthermore, some MWs especially ash in dried samples that contained varying levels and types of yeasts and molds that included *Fusarium and Penicillium*. No *Salmonella* specie were detected in all sample tested. The study showed that, MW processing method and subsequent handlingare important parameters in determining the type and levels of their contamination. As such, it is imperative for harvesters and processors to observe good harvesting and manufacturing practices and follow protocols that do not result in re-contamination of produce as this might present a danger to the public consumers.

Introduction

In Africa, most people living in rural areas are dependent on non-timber forest products for nutrition and income generation (FAO, 2012a). It has been reported that insects offer diet diversity and opportunities for income generation amongst the rural poor in developing countries (Illgner and Nel, 2000; Sunderland *et al*., 2004; Bukkens, 2005).

Insects are a highly nutritious and healthy food source with high fat, protein, vitamin, fibre and mineral content (DeFoliart, 1989, 1990; Barker *et al*., 1998; Ayieko and Oriaro, 2008). Dufour (1987) noted that insects provide valuable buffers against seasonal shortages of food. For instance, as well as being food, insects do provide for disposable incomes which can be used on food, farming inputs etc (Agea*et al*., 2008; Hope *et al*., 2009; Yen, 2009a).

Given their rich nutritional profiles, insects do provide favourable environments for microbial growth and survival (Klunder *et al*., 2012). However, microbial growth and survival is also influenced by processing and storage conditions afforded to them along the value chain (Belluco *et al*., 2013). In most instances, the insects are subjected to traditional processing methods, such as boiling, roasting and sun drying (Glew, 1998). These are done to improve the taste, storability and palatability of edible insects with an implied assumption of ensuring production of safe food product (van Huis *et al*., 2013).

Gonimbrasia belina, commonly referred to as Mopane Worms or *amacimbi* or *madora* are an important source of nutrition and income for most rural households in Zimbabwe (Stack *et al*., 2003; Gardiner, 2005). MWs are an excellent source of protein and minerals with a reported crude protein value of approximately 50% (Headings, 2002). MWs are the caterpillar stage of the emperor moth and feeds exclusively on *Colophospermum* mopane (Timberlake, 1996). As noted by Gardiner (2005), MWs are a key resource for poor farmers and landless locals, which they exploit for improvement of their livelihoods (Styles and Skinner, 1996).

Given the economic importance and food security value, it is of paramount importance that the MW value chain should ensure production of good quality and safe products (FAO, 2010a). In practice, MW processing, packaging and storage are generally considered as basic and primarily poor leading to spoilage by pests and microorganisms (Gardiner, 2005). Traditionally, MWs are processed by hand degutting followed by sun or hot-ash or sand drying (Nonaka, 1996; Mulhane*et al*., 2001; Allotey and Mpuchane, 2003). Although in dry environments, the drying operation can be effective in limiting microbial contamination and spoilage, in humid areas outgrowth of microbes can be stimulated. In addition, contamination through the air, soil and packaging material used could result in poor quality and unsafe products for human consumption (Taylor, 2003; Banjo *et al*., 2006b).

As noted by Mulhane*et al* (2001), edible insects, including MWs could be contaminated by bacteria, fungi and pesticide residues. As such, regulatory guidelines, which are based on scientific data should be formulated to protect consumers along the MW value chain (Zhou, 2004). However, lack of specific scientific information on microbiological content hinders the development of appropriate legislative framework to consider insects as food at various stages of the value chain (Belluco *et al*., 2013).

To this effect a study was conducted to evaluate the effect of various processing and hygiene regimes on the microbial quality of mopane worms harvested and processed by local communities in Gwanda, Zimbabwe.

Materials and Methods

Site and sample selection

This study was conducted in Samlodi ward

of Gwanda district located in the South Western part of Zimbabwe, which is one of the biggest sources of the MW. A total of 15 women were selected to conduct the harvesting and processing of MWs. The selection was done following community consultations for people involved in MWharvesting, processing and trade.Harvesting and processing of MWs was done on 5 separate days during the April-May season in they will be abundant. Approximately 28kg of fresh MW were harvested at various points confined to an area of $3km^2$. The harvested MWs were divided into 2 equal portions and degutted at theharvesting site either using a) bare hands and b) by personnel wearing vinyl reusable hand gloves. The MWswas put in different plastic buckets and degutted within 4 hours of harvesting before drying.

Drying methods employed

The two degutted worm samples wereinstantly sub-divided into 4 equal portions and subjected to different drying methods namely, boiling in salted water (5% w/w salt) for 30 minutes and solar drying, open pan roasting, drum roasting and hot-ash drying to acceptable moisture levels of approximately 15%. Of the 14kg of MWs that had been degutted using gloves and boiled in salted water, 3.5kg were dried in an open pan roaster, with the other 3.5kg dried in a solar drier.

The open pan roaster was basically a flat metal pan with a diameter of 60 cm with raised edges of about 15 cm, whilst the cabinet type solar drier was made up of a (2m x1m x1m) metal and wooden frame with green house plastic cover and wire mesh trays. In addition another 3.5kg was dried in hot ash while the remaining 3.5kg was dried in a drum roaster. The roaster comprise of a black, non-perforated drum with diameter of about 0.5m and length of 1.0m, fitted with rotating handle and placed over burning charcoal. The same treatments were applied for the other 14kg batch degutted using bare hands.

The dried mopane worms were then vacuum packaged in air tight high density polyethylene bags and transported to Harare Institute of Technology Food Analysis laboratory for microbiological tests where they were refrigerated prior to analyses.

Microbial reagents, equipment and sample preparation

Chemicals, reagents and media used were all of analytical grade. Ancillary equipment, namely refrigerators, thermometers, water baths, incubators, analytical balances and were all well calibrated, blenders used were sourced by the Food Laboratory from Capri, Limited, Zimbabwe and Incotherm Limited United Kingdom.Before analysis, each sample individually underwent particle size reduction by milling in a sterile blender (Hamilton Beach HBF500S, China) after which, 10g of the sample powder was homogenised in 90ml sterile peptone water (Oxoid Ltd, Basingstoke Hampshire England). The sample homogenates underwent serial dilutions i.e. 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} depending on the tests that were conducted. Thereafter 1ml aliquots from the resultant dilutions were inoculated in triplicate plates using the pour plate and spread plate techniques.

Microbial Analysis

TBC*, E.coli, Staphylococcus. aureus and Salmonella spp* Coliforms, determination

Plate Count Agar (PCA) (Oxoid Ltd, Basingstoke Hampshire England) was used for Total Bacterial Count (TBC) and was done in conformity with Association of

Official Analytical Chemists (AOAC) protocols, 1990; Maturin and Peeler, 2001. Enumeration of total coliforms was done using Violet Red Bile Agar (VRBA) (Scharlau Ltd, Spain) of which *E. coli* colonies were distinguished among all the coliform colonies on the VRBA by their increased bluish fluorescence around colonies under long wave UV light 366nm on adding 100 µg of 4-methyl-umbelliferyl-β-D-glucuronide (MUG) (Oxoid Ltd, Basingstoke Hampshire England) in the VRBA overlay. Enumeration of *S. aureus* was done by transferring 1ml of the sample homogenate onto 3 plates of Baird-Parker Agar, (Oxoid Ltd, Basingstoke Hampshire England) as follows; 0.4ml, 0.3ml and 0.3ml and incubated at 35^{o}C for 45-48 hours in which colonies of *S. aureus* were distinguished by their circular, smooth, convex, moist, 2-3 mm appearance in conformity with Bacteriological Analytical Manual, 2001, Bennett and Lancette, 2001.

Yeasts and molds determination

Dichloran 18% Glycerol (DG18) Agar (Oxoid Ltd, Basingstoke Hampshire England) was used for enumeration of yeast and moulds and was done in conformity with Bacteriological Analytical Manual (BAM) protocols, (Tournas et al.,2001). The plates were incubated in the dark at 25^{o}C for 5 days and thereafter colonies were counted, all in accordance with BAM (Tournas et al., 2001) general methods for enumeration of yeast and moulds.

Results and Discussion

Specific studies on the microbiological quality and safety of insects as food are rare in the scientific literature. As such, microbiological guidelines of similar products derived from animals sources that include cured or salted meat, miscellaneous dried foods and foods requiring further cooking (> 70°C)(Stannard, 1997)were used for comparison.

Klunder et al. (2012) noted that, insects like many meat products, are rich in nutrients and moisture, providing a favourable environment for microbial survival and growth.This was supported by Vega and Kaya (2012), who highlighted that insects collected in nature and insects raised on farms may be infected with pathogenic micro-organisms, including bacteria, virus, fungi and protozoa. Practically, most food stuffs including processed MWs can be contaminated through the environment, from human contact, processing and post handling practices which could potentially cause food borne diseases (Belluco *et al*., 2013).

Table 1show relatively low counts of total bacteria count (TBC) in MWs degutted using either bare hands or gloves compared to products such as cured and salted meat where TBCs of up to 10^6 CFU g^{-1} are within acceptable range or even in food stuff that require further cooking (> 70°C) (Stannard, 1997, *foodsafety.govt.nz).* TBC rangeswere10-2500 CFU$g^{-1}$with higher values observed inMWs degutted using gloves compared to those which were degutted using bare hands. TBCs in MWs degutted using gloves were ranging from 300-2500 CFU g^{-1} whereas those degutted using bare hands ranged from 10-300 CFU g^{-1}. Although the use of gloves was effective in preventing personnel hand damages during the degutting process (Kozanayi and Frost, 2002), but use of gloves increased microbiological counts on the product. This could be attributed to the fact that the gloves used were not hygienically designed and were considered difficult to clean and sanitise. In addition, they did provide a moisture rich environment which could be favourable for bacterial growth and multiplication (Klunder *et al.*, 2012). In fact Kozanayi and Frost (2002) stated that,

overall function of using gloves during *G. belina* degutting is for hand protection rather than hygienic use, hence reason why the gloves might not hygienically designed.

It was also observed that degutting using bare hands followed by drum roasting and boiling in salt water coupled with open pan roasting, had the least TBCs (10 CFU $g^{-1}$and 30 CFU g^{-1}) respectively. This is attributable to exposure of the product to high temperatures (during roasting) as noted by Klunder *et al* (2012), and high osmotic stress due to salt addition in boiling water, which could kill microbes.

According to Gardiner (2005), insects such as MWs can be contaminated with both pathogenic and spoilage microorganisms during harvesting, processing, packaging and/ or storage. This is mainly attributed to the fact that such practices are often considered crude and poor (DeFoliart, 1989).Microbiologically, insects can harbour different kinds of food pathogenic bacteria (Belluco *et al*., 2013) as demonstrated by the presence of *S. aureus*, *E. coli* and coliforms in the MW samples. In addition, from the studies by Braide (2012), microorganisms found in the diet could be found on the skin and gut contents of the caterpillar. As such, the MWs could be contaminated with the microorganisms well before human handling and manipulation although at low levels (Belluco *et al*., 2013).

Indicator microorganisms such as coliforms and *E. coli* were both present in MWs degutted using both bare hands and gloves and followed by subsequent processing (ash drying, drum roasting and boiling in salted water and solar drying) except in those that were boiled in salted water and roasted on an open pan. From Table 1,MWsdegutted using bare hands,coliforms and *E. coli* ranged from 0-43 CFU $g^{-1}$and 0-30 CFU g^{-1} respectively. On the other hand in those degutted using gloves coliforms ranged from 0-45 CFU g^{-1} while *E. coli* were from 0-35 CFU g^{-1}.Coliforms counts were within expected limits of less than 1000 CFU g^{-1} in food stuff that require further cooking (> 70°C) or in cured and salted meat (Stannard, 1997; Lim et al.2012) for instance MWs, while their presence including *E.coli* signifies dangerto public health(Oranusi and Braide, 2012).

Relatively high levels of *S. aureus* were observed in hand degutted and ash dried MWs compared to other drying methods. The presence of *S. aureus* in food is a good indication of poor personal hygiene since its main sources are food handlers (via their hands` direct contact with food or through respiratory secretions (Argudin *et al*., 2010), hence justifying higher counts in MWs degutted using bare hands than when gloves are used. Contrary to this, Taylor (2003) noted that degutting by hand is quite common and a faster practice in MWs processing, hence the most preferred method (Kozanayi and Frost, 2002). However, despite high temperatures being achieved during ash drying and the presence in other samples, the subsequent handling of the product could be a source of recontamination (Reij*et al*.,2004).

Although high levels of *S. aureus* and *E.coli* were observed in samples that were ash dried, drum roasted and boiled and solar dried, it should be noted that in general, insects themselves have been shown to harbour intrinsically dangerous pathogens for humans (Veldkamp*et al*., 2012). However, Giaccone (2005) could not exclude the possibility of insects becoming carriers of pathogenic microorganisms to humans hence recommended cooking or pasteurisation as risk minimizing steps. This was supported by Amadi*et al* (2005) who

noted that boiling insects for at least 5 minutes was an efficient process for eliminating Enterobacteriaceae but not sporeforming bacteria, which could pose spoilage and safety risks in cooked insects (Klunder *et al*., 2012). This explains the presence of significant levels of *E.coli* in ash dried compared to the other 3 methods where boiling was done prior to drying of the MWs.

Significantly, in all processing practices pathogenic *Salmonella spp.*was not detected, indicating that the product was safe in as far as this pathogenic species is concerned. In research done on microbiological content of fresh, processed, and stored edible insects (MWs excluded) by Klunder *et al*(2012) and Giaccone (2005) pathogenic species such as *Salmonella spp*and *Listeria monocytogenes* were never isolated in the tested samples. This led to the conclusion that the insects concerned were unlikely to attract microbial flora that could pose risks to human health (van Huis *et al*., 2013). It is also important to note that even *S.aureus* was present, but it was within expected limits of less than 1000 CFU g^{-1} in food stuff that require further cooking (> 70°C) or in cured and salted meat for instance MWs (Lim et al. 2012).

According to Stannard (1997) yeasts and molds are a common cause of food spoilage, particularly foods of reduced water activity (a_w) such as dried MWs. Yeast have not been implicated in food poisoning whilst molds and some of its strains are able are able to produce mycotoxins which can cause serious chronic illness if consumed (Mpuchane *et al*., 2000). Table 1, also shows the presence of various yeasts and molds in MWs processedunder different practices. The presence of yeasts and molds was limited in MWs samples which were degutted using bare hands followed by boiling in salted water and roasted in an open pan or in a drum roaster. From Table 1, it can be noted that in ash dried MWs, although at low levels, fungi were present, which were confirmed to be *Fusarium* and *Penicillium* types. This supported the assertion made by Taligoola and Gashe, (1996) who noted that frequent fungal isolates in dried MWs were species of *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*and*Phycomycetes*, of which some may produce mycotoxins (Mpuchane *et al*., 2000). For example, (Frazier and Westhoff, 1978) highlighted that *Aspergillus, Penicillium*and *Rhizopus*species are known to produce various mycotoxins in food and feeds infield and under storage. From table 1, it can be observed that yeasts and molds were most abundant in ash and solar dried MWs, with higher value in boiled and solar dried samples. The relatively high presence in solar dried samples could be attributable to dust and air as the solar driers used had a mesh cloth which allowed for air and dust circulation. This was supported by Frazier and Westhoff (1978) who stated that fungi was ubiquitously distributed in soil and air and further isolated *Aspergillus* in food products that are sun-dried owing to spores deposition.

Conclusion and recommendations

The microbiological profiles of the Mopane worms are determined to a large extent by the processing method and subsequent handling of the product along the production and supply chain. From the studies it can be concluded that the processing method and subsequent handling are important parameters in determining the type and levels of contamination of the MWs. For it was shown that regardless of degutting method employed, boiling MWs in salted water followed by open pan drying produced a microbiologically safe product. In

addition, it can also be concluded that use of gloves does not reduce microbial load effectively and that wider sanitary conditions are of key consideration particularly post processing. As such, it is imperative for harvesters and processors to observe good harvesting and manufacturing practices and follow protocols that do not result in re-contamination of produce.

Table.1 Mean microbial examination (CFU g^{-1}) of *G. belina* processed under different traditional methods

	Process	TBC	Coliforms	*E.coli*	*S.aureus*	*Salmonella spp*	YM*	*types
Degutted using bare hands	Ash drying	60	43	30	25	0	35	*Fusarium & Penicillium*
	Drum roaster	10	28	8	5	0	0	-
	Boiled in salted water and solar drying	300	13	3	10	0	200	Yeasts & soil slime moulds
	Boiled in salted water and open pan roasting	30	0	0	0	0	0	-
Degutted using gloves	Ash drying	2500	45	35	6	0	40	yeasts
	Drum roasting	660	3	5	2	0	0	-
	Boiled in salted water and solar drying	450	14	2	2	0	150	Yeasts, rhizopus, soil slime moulds
	Boiled in salted water and open pan roasting	300	0	0	0	0	0	

YM* yeast and mould, *types yeasts and molds types, the values shown in Table 1 are mean values

Acknowledgements

We acknowledge the entire team of researchers involved in this project for their commitment and diligence in executing their roles thus making it successful. We would also like to appreciate the support from Harare Institute of Technology through the Department of Food Processing Technology.

References

Agea, J.G., D.Biryomumaisho, M. Buyinza, and Nabanoga, G.N. 2008. Commercialization of *Ruspolianitidula* (Nsenene grass hoppers) in Central Uganda. *African Journal of Food Agriculture and Development*, 8(3): 319–332.

Allotey., J. and Mpuchane, S.F. 2003.Utilization of useful insects as food source.*African Journal of Food Agriculture,Nutrition and Development*, **3**: 1 - 8.

Amadi, E. N., O. K. Ogbalu., I.S. Barimalaa and Pius, M. 2005.Microbiology and nutritional composition of an edible larva (*Bunaeaalcinoe*.Stoll) of the Niger Delta. *Journal of Food Safety*, **25**: 193-197

Argudin, M.A., M.C. Mendoza and Rodicio, M.R. 2010. Food poisoning and *Staphylococcus aureus*

Association Official Analytical Chemists. 1990." Official Method of Analysis, 15 Edition, AOAC, Alrington, Viginia.

Atlas, R.M., 1997. Handbook of Microbiological Media. Second edition.L.C.Parks(editor). CRC Press Inc.

Ayieko, M.A., and Oriaro, V. 2008. Consumption, indigeneous knowledge and cultural values of the lake fly species within the Lake Victoria region.*African Journal of Environmental Science and Technology*, 2(10): 282–286.

Banjo, A.D., O.A. Lawal and Songonuga, E.A. 2006.The nutritional value of fourteen species of edible insects in southwesternNigeria.*African Journal of Biotechnology*, 5(3): 298–301.

Barker, D., M. P. Fitzpatrick, E. S. Dierenfeld. 1998. Nutrient composition of selected whole invertebrates. Zoo Biology 17:123-134.

Belluco, S., C. Losasso, M. Maggioletti, M. C. Alonzi, M. G. Paoletti and Ricci, A.2013. Edible Insects in a Food Safety and Nutritional Perspective: A Critical Review, Comprehensive Reviews in Food Science and Food Safety, Vol.12

Bennett, R. W., and Lancette, G. A. 2001."Staphylococcus aureus, Chapter 12, rev. Jan. 2001.In FDA Bacteriological analytical manual, 8th ed., Rev. A. AOAC International, Gaithersburg, MD.

Braide, W., 2012.Perspectives in the Microbiology of the Leaves of Three Plant species as Food for an Edible Caterpillar of an Emperor Moth. International Journal of Research in Pure and Applied Microbiology 2 (1): 1-6

Bukkens, S.G.F., 2005. Insects in the human diet. In Ecological Implications of Minilivestock: Potential of Insects, Rodents, Frogs and Snails, ed. MG Paoletti, pp. 545–77. Enfield, NH: Science Publ.

DeFoliart, G.R., 1999. Insects as food: Why the western attitude is important. Ann. Rev. Entomol., 44: 21-50.

Dufour, D.L., 1987. Insects as food-A case-study from the northwest Amazon.*Am. Anthropol*. 89:383–97

FAO.2010a. *Development of regional*

standard for edible crickets and their products. Paper presented at the Joint FAO/WHO meeting Food Standards Programme: FAO/ WHO Coordinating Committee for Asia, Bali, Indonesia.

Frazier, W.F., and Westhoff, D.C. 1978. Food Microbiology,3rdedn. Tata McGraw-Hill Publishing Co. Limited, New- Delhi, India, pp. l7-64, 454-456.

Gardiner, A., 2005. Farming Mopane Worms - a household guide.English version. Unpublished manuscript, Harare

Gashe, B.A., S.F. Mpuchane and Taligoola, H.K.1996. Fungi associated with *Imbresiabelina*, an edible caterpillar. Botswana Notes Records, 28: 193-198

Giaccone, V., 2005. Hygiene and health features of "minilivestock".In M.G. Paoletti, ed. Ecological implications of minilivestock; role of rodents, frogs, snails, and insects for sustainable development, pp. 579–598.New Hampshire, Science Publishers.

Glew, R. H., D. Jackson, L. Sena, D. J. Vanderjagt, A. Pastuszyn, and M. Millson. 1999. *Gonimbrasiabelina*(Lepidoptera: Saturniidae): A nutritional food source rich in protein, fatty acids and minerals. *American Entomologist* 45:250–253.

Headings, M.E., and Rahnema, S. 2002. The nutritional value of mopane worms, *Gonimbrasiabelina* (Lepidoptera: Saturniidae) for human consumption. Presentation at the Ten-Minute Papers: Section B. Physiology, Biochemistry, Toxicology and Molecular Biology Series, 20 November 2002. Ohio, USA, Ohio State University.

Illgner, P., and Nel, E. 2000. The geography of edible insects in Sub-Saharan Africa: A study of the mopane caterpillar. Department of Geography, Rhodes University, South Africa.

International Commission on Microbiological Specifications for Foods. 1986. Microorganisms in Foods. 2. Sampling for Microbiological Analysis:Principles and Specific Applications, 2nd ed. University of Toronto Press, Toronto, Ontario, Canada.

Kiiyukia, C., 2003. in Laboratory Manual Of Food Microbiology For Ethiopian Health And Nutrition Research Institute (Food Microbiology Laboratory)

Klunder, H.C., J. Wolkers-Rooijackers, , J.M. Korpela, and Nout, M.J.R. 2012.Microbiological aspects of processing and storage of edible insects.*Food Control*, 26: 628–631.

Kozanayi, W., and Frost, P. 2002. *Marketing of mopane worm in Southern Zimbabwe*. Harare, Institute of Environmental Studies.

Lim, E., L. Lopez, A.Borman, P.Cressey and Pirie, R. 2012.Annual report concerning foodbornedisease in New Zealand 2011.Ministry for Primary Industry, New Zealand.

Maturin, L., and Peeler, J. T. 2001. "Aerobic Plate Count, Chapter 3, rev. Jan. 2001. In FDA Bacteriological analytical manual, 8th ed., Rev. A. AOAC International, Gaithersburg, MD.

Montville, T.J., and Matthews, K.R. 2008. Food microbiology: An introduction. 2nd ed, ASM Press,

Mpuchane, S.F., B.A Gashe, J. Allotey, A.B. Siame, G. Teferra and Dilthogo, M. 2000. Quality deterioration of Phane, the edible caterpillar of an emperor moth, *Imbresiabelina*.Food Control, 11: 453- 458.

Mulhane, S., B. AGashe, J. Allotey, A. B.Siame, G.Teferra and Ditlhogo, M. 2001. Quality determination of Phane,

the edible caterpillar of an Emperor moth, *Imbrasiabelina*.Food Control11, 453–458

Nonaka, K., 1996. Ethnoentomology of the Central Kalahari San.*African Study Monographs*, 22: 29–46.

Reij, M.W., and DenAantrekker, E.D. 2004. Recontamination as a source of pathogens in processed foods. International Journal of Food Microbiology 91: 1– 11

Simone, B., L. Carmen, M. Michela, C. A. Cristiana, G. P. Maurizio and Antonia, R. 2013. Edible Insects in a Food Safety and Nutritional Perspective: A Critical Review Comprehensive Reviews in Food Science and Food Safety Vol.12

Stack, J., A. Dorward, T. Gondo, P. Frost, F. Taylor and Kurebgaseka, N. 2003. MopaneWorm Utilisation and Rural Livelihoods in Southern Africa. Paper presented at International Conference on Rural Livelihoods, Forests and Biodiversity, 19-23 May, 2003, Bonn, Germany.

Stannard, C., 1997. Development and use of microbiological criteria for foods. *Food Science and Technology Today* 11, 137-177.

Styles, C. V., and Skinner, J. D. 1996. Possible factors contributing to the exclusion of saturniid caterpillars (mopane worms) from a protected area in Botswana. *African Journal of Zoology*, 34, 276-283.

Sunderland, T.C.H., A.B. Cunningham, Z. Tchoundjeu, M.-L.Ngo-Mpeck and Laird S.A. 2004. Yohimbe. (Pausinystalia ohimbe). In: L.E. Clark & T.C.H.Sunderland (Eds.) The Key Non-Timber Forest Products of Central Africa: State of the Knowledge. Technical Paper No. 122, USAID. Pp. 121-140

Taylor, F., 2003.Mopane Worm (MW) Utilisation and Rural Livelihoods in Botswana Preliminary Field Survey Report. Unpublished report, Veld products Institute, Botswana, Jabuary 2003 pp. 8

Timberlake, J., 1996. Colophosper mummopane - a tree for all seasons. In: Mushove, P.T., Shumba, E.M. and Matose, F. (eds.), Sustainable Management of Indigenous Forests in the dry tropics. Zimbabwe Forestry Commission.

Tournas, V., Michael, E. , B. Philip, Mislivec, A. Koch Herbert, and Bandler, R. 2001. "Yeasts, Molds and Mycotoxins, Chapter 18, rev. April. 2001. *In* FDA Bacteriological Analytical Manual, 8th ed., Rev. A/1998. AOAC International, Gaithersburg, MD.

Vanhuis, A., 2013. Potential of insects as food and feed in assuring food security. Annual Review of Entomology, 58(1): 563–583.

Vega, F., and Kaya, H. (2012) ,.*Insect Pathology*. London, Academic Press.

Veldkamp, T., G. van Duinkerken, A. van Huis, E. Lakemond, E. Ottevanger, and van Boekel, M.A.J.S. 2012. *Insects as a sustainable feed ingredient in pig and poultry diets.A feasibility study*.Wageningen UR Livestock Research, Report 638. Washington D.C.

Yen, A.L. 2009.Entomophagy and insect conservation: some thoughts for digestion. *Journal of Insect Conservation*, 13: 667–670.

Zhou, J. X. 2004. Health food derived from silkworm pupae approved. Food Information and Technology4, 64.