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Short communication

Microbiological aspects of processing and storage of edible insects

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ABSTRACT

Growing pressure on the worlds' livestock production sector and enduring protein undernourishment, persuade the search for alternative protein sources. Insects are widely consumed in many parts of the world and are evaluated as food or supplement. Nevertheless, little attention has been given to the food safety and shelf-life of food insects. An exploratory evaluation of the microbiological content of fresh, processed and stored edible insects was carried out, with focus on farmed mealworm larvae (*Tenebrio molitor*) and house crickets (*Acheta domesticus*). A short heating step was sufficient to eliminate Enterobacteriaceae, however some sporeforming bacteria will survive in cooked insects. Simple preservation methods such as drying/acidifying without use of a refrigerator were tested and considered promising. Lactic fermentation of composite flour/water mixtures containing 10, or 20% powdered roasted mealworm larvae resulted in successful acidification and was demonstrated effective in safeguarding shelf-life and safety by the control of Enterobacteria and bacterial spores.

1. Introduction

An ever-increasing world population, combined with a steadily rising economic growth and urbanization results in increasing demand for high-value protein; this will consequently augment the pressure on the worlds' livestock production sector. This development almost certainly has a negative impact on the environment, considering carbon emissions, land and water use, public health risks etc. (Alston, Beddow, & Pardey, 2009; FAO, 2006).

Another pressing problem is the continuous undernourishment which is still manifest in developing countries. For example, recent data indicate that in Lao PDR, 40% of children under the age of 5 are chronically malnourished or stunted (WFP, 2007). The most common type of malnourishment is protein and/or energy malnutrition.

Edible insects are a well-appreciated food consumed in various regions in Asia, Africa and America. It has been estimated that nearly 1800 insect species are used for human consumption globally (Jongema, 2011). In Lao PDR, up to 95% of the population say they eat insects as food, most favoured insect delicacies being ant eggs, crickets and grasshoppers (Barennes, 2011, p. 183).

Insects are comparable to conventional livestock meat in terms of nutritional content. In general, the crude protein content of insects ranges from 40% to 75% on dry weight basis, largely depending on species and stage in the life cycle (Verkerk, Tramper, Van Trijp, & Martens, 2007). Although these values should be corrected (Ramos-Elorduy et al., 1997) for the N-containing chitin which represents approximately 5–20% of insect biomass, these are attractively high concentrations (Paoletti, Norberto, Damini, & Musumeci, 2007).

Apart from nutritional benefits, advantages of insects include their lower emission of greenhouse gases and ammonia per kg mass gain compared with regular livestock such as pigs and cattle (Oonincx et al., 2010). Furthermore, farmed insects reproduce much faster than traditional livestock, have higher feed conversion efficiency, and need far less breeding space than larger animals. An ECI (Efficiency of Conversion of Ingested food) of 53–73% has been reported for *Tenebrio molitor L.*, considerably higher than for chicken (38–43%) and livestock (10–12%) (Ramos-Elorduy, 2008; Wilkinson, 2011).

The majority of the insects consumed is still collected from natural environments, where availability is seasonal and restricted to certain localities (Nonaka, 2009). Only recently, commercial insect farming has evolved as a livelihood in certain countries, in the vicinity of concentrations of customers (suburban farming).

In principle there are three ways insects could be consumed. First as whole insects, recognizable as such; second, whole insects processed in some powder or paste; third, as an extract such as a protein isolate.

Whole, recognizable insects are often consumed as a fried snack or as part of the daily meal with rice in Lao PDR. Ready-to-eat insect

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dishes, as well as live or boiled insects are often sold at local markets. As non-recognizable form, farmed insects could be processed into a dried form, e.g. insect powder, suitable for protein enrichment of a variety of low-nutrient foods or feed. An example of such application is based on the winning concept of 'Developing solutions for developing countries'-competition organized by the Institute of Food Technologists 2009 (IFT, 2011). This proposes the application of enriched sorghum; the nutritionally 'weak' grain, frequently consumed in certain African countries, which is low in proteins and fats and lacks several essential amino acids such as lysine (Kazanas & Fields, 1981). Making use of the nutritious termites (*Macrotermes* spp.), sorghum is enriched with boiled or roasted and ground termite powder. This mixture is fermented and used for porridge preparation, which can be consumed as part of the daily diet. Both raw materials are easily obtained locally. Finally, proteins could be extracted from the insects and applied in food and/or feed as an alternative to soy or meat proteins.

Despite the relatively widespread consumption of insects as food, the information concerning their cross-border trade, economic importance, or food safety aspects of their processed products remains limited. Insects are, similar to other meat products, rich in nutrients and moisture, providing favourable conditions for microbial survival and growth. There are a number of culturally different ways to cook insects, but usually they are consumed whole, containing their gut microflora which may affect the microbiological quality of food. Due to increasing commercialization of insect farming and trade in Laos and other countries, an exploratory evaluation of microbial content of fresh, processed and stored insects seemed timely.

For this study we took a two-pronged approach: (a) analysis of the microbiological content of whole edible insects as fresh, processed and stored; (b) the use of insects for protein enrichment of food products. In the latter part, we will evaluate the effect of lactic acid fermentation of sorghum–insect mixtures on their microbiological composition, based on the initiative described above.

2. Materials and methods

2.1. Sample preparation

Live mealworm larvae (*T. molitor*) were kindly provided by the insect farm 'Van de Ven' in Deurne, The Netherlands and live house crickets (*Acheta domesticus*) were purchased from a local producer in Vientiane, Lao PDR and stored at −18 °C in laboratory conditions until further use. Large crickets (*Brachytrupus* sp.) were traditionally collected on a tobacco field in Nam Lo, 132 km south of Vientiane, Lao PDR and frozen alive until further use.

To prepare dry, ground cricket flour, the portion of 25 g of crickets was rinsed and boiled in distilled water for 5 min followed by drying in a hot air oven for approximately 24 h at 55 °C until dry. Finally, dry insects were ground with mortar or coffee grinder.

Mealworm larvae were boiled and dried as described above or roasted in a tumbling roasting machine. The composite meal was prepared with sorghum (*Sorghum bicolor* (*L.*) Moench) washed with hot and cold water. After drying, the sorghum kernels and roasted larvae were mixed in the desired ratio. This mixture was carefully ground using a grinding machine (Retsh, zm 1 34962, with a sieve of 0.5 mm). A homogenous composite flour of sorghum and insects was obtained and stored at −20 °C.

2.2. Dry matter content

The dry matter content of mealworm larvae was determined by weighing (0.001 g) approx 1–2 g fresh larvae in an aluminium sample cup. This was dried in an oven at 105 °C and weighed until a constant weight.

2.3. Microbiological analysis

A 5 g sample was mixed with 45 ml sterile PPS (Peptone Physiological Salt, 1 g/L bacteriological peptone (OXOID LP0034) and 8.5 g/L NaCl) in a sterile filter stomacher bag and homogenized (Seward, 400 circulator) at normal speed for 1 min. The homogenate was then diluted with PPS and desired decimal dilutions poured to agar plates.

Aerobic mesophilic micro-organisms were enumerated by a Total Viable Count (TVC) using plate count agar (PCA, Merck VM105363), incubated at 30 °C for 48 h.

Enterobacteriaceae were enumerated in pour-plates of violet red bile glucose (VRBG) medium (OXOID CM0485) with an overlay of the same medium and incubated at 37 °C for 24 h.

For the enumeration of *Lactic Acid Bacteria* (LAB), de Man, Rogosa and Sharpe (MRS) broth (Merck VM986641) and 15 g/L Bacteriological agar (BA) (Oxoid LP0011) were mixed and supplemented with 2 g/L Delvocid (50% Natamycin, DSM). The pour plates with overlay were incubated under microaerophilic conditions (air evacuated to 300 mbar with an Anoxomat WS9000, Mart, and filled with 80% N_2, 10% CO_2, and 10% H_2 gas mixture leaving a final concentration of 6% O_2) at 30 °C for 48 h.

Bacterial endospores were enumerated by heat shocking 5 ml of the 10^{-1} dilution for 5 min at 80 °C in a sterile tube, after which pour plates of PCA with an overlay of BA were incubated at 30 °C for 48 h.

2.4. Lactic acid fermentation

A natural starter for lactic acid fermentation was created by enrichment by making repetitive use of a previously natural fermented batch as a starter, in order to accelerate the acidification, also called 'backslopping' (Nout, Rombouts, & Havelaar, 1989). The first batch ("fermentation cycle") was prepared by mixing the composite flour and tap water in the ratio of 40:60% (w/w) and incubated at 30 °C for 24 h. Further fermentation cycles were prepared by inoculating the diluted flour with the previous fermentation cycle using an inoculation rate of 10% (w/w). After 5 fermentation cycles, the batch was used as a natural starter for the actual fermentation. The pH was measured after each cycle, and at the end of the fermentation experiment.

Composite flour samples were collected before the addition of the natural starter ($t = -1$), and at the point of inoculation with the starter ($t = 0$) followed by sampling during fermentation at 24 h ($t = 2$) and 48 h ($t = 3$). The composite flour:water mixture was inoculated with 10^8 CFU g^{-1} at $t = 0$, enumerated with MRS-agar.

2.5. DNA extraction and sequencing

Randomly picked colonies of sporeforming bacteria were streaked and purified on PCA and incubated for 2 days at 30 °C. Pure sporeforming strains were grown in BHI overnight in a shaking water bath at 30 °C. The DNA was extracted using a DNA Purification kit (Promega, wizard Genomic, Promega A1125) according to manufacturer's instruction. Amplification of the 16S rRNA gene of the isolated DNA, was done with polymerase chain reaction (PCR), using forward primer 5′-AGA GTT TGA TCC TGG CTC AG-3′ and reverse primer 5′-AAG GAG GTG ATC CAG CCG CA-3′ (Oomes et al., 2007). The PCR was done using a GeneAmp PCR system 9700 (Applied Biosystems) with PCR conditions as follows: initial denaturation of double-stranded DNA for 5 min at 94 °C; 35 cycles each consisting of 30 s at 94 °C, 20 s at 56 °C, and 1 min at 72 °C; and

extension of incomplete products for 7 min at 72 °C followed by cooling at 4 °C. The PCR products were sent to GATC Biotech (http://www.gatc-biotech.com) in Germany for purification and sequencing. Obtained sequences were viewed for errors using Chromas Lite V2.01 software (http://www.technelysium.com.au/) and if necessary corrected manually with the IUB/IUPAC nucleic acid code and exported to FASTA format. These files were used to obtain strain identities by using the nucleotide BLAST program from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). The most probable match with a 16s rDNA sequence was selected based on the percentage of identification (%ID).

3. Results and discussion

3.1. Microbiological analysis of edible insects

The dry matter content of mealworm larvae was 35.3% which is similar to the value (37.1%) found in literature (Barker, 1998). The results of three microbiological parameters of three edible insect species analyzed both fresh and after a heat treatment are shown in Table 1. Overall levels of 10^7 cfu/g Total Viable Count, 10^4–10^6 cfu/g Enterobacteriaceae and 10^2–10^4 cfu/g sporeforming bacteria were found in the fresh edible insects, which are typical levels for fresh food harvested from soil or having been in contact with soil materials. Crushing of the mealworm larvae resulted in higher counts of viable bacteria, which may have resulted from the release of microbiota from the insect's intestines which are then distributed throughout the product. Enterobacteriaceae were killed during boiling of all the insect species, but not completely during the roasting; thus roasting may not fully kill the microbiota from the insects' intestines. Blanching the insects in boiling water for a few minutes previous to the roasting should eliminate most of the present Enterobacteriaceae. The prolongation of boiling time from 5 to 10 min of the large crickets did not significantly influence the level of remaining bacteria, however all Enterobacteriaceae were eliminated already after 5 min of boiling.

Lower counts of sporeforming bacteria were determined in mealworm larvae than in house- and large crickets, which is most likely related to farming practices. Mealworm larvae are grown in wheat flour, whereas large crickets are caught from the soil or farmed in containers containing soil (particularly house crickets). These findings are comparable to the data of Mpuchane et al. (2000). Bacterial species, such as *Bacillus spp.* are often introduced from soil and are not completely inactivated despite the heat treatment applied, and thus could cause spoilage or health risks if favourable conditions return for their germination and growth (Ter Beek, Brul, 2010).

3.2. Storage test (house crickets)

Three microbiological hygiene parameters were analyzed during the storage of house crickets in the refrigerator at (4–6 °C) and at room temperature (28–30 °C in Lao PDR), both fresh and after heat treatments. Results are presented in Table 2. Levels of micro-organisms in fresh insects were rather high and the sensory quality of insects deteriorated during the storage in refrigerator. We thus recommend applying a heating step (boiling) before storing insects in the refrigerator; this would also preclude enzymatic spoilage such as manifested by black discolouration. The levels of bacteria on boiled house crickets stored in the refrigerator remained fairly stable during more than two weeks of storage. On the other hand, boiled insects (1 min) stored at room temperature spoiled rapidly. The high water content, high temperature and nutrient-rich environment creates a favourable environment for growth of surviving spoilage bacteria. Since the aim of the experiment was to identify preservation methods for insects as food without use of a refrigerator, we evaluated drying as an option. Microbial levels in dried and ground insects were higher than expected (TVC) but remained stable during storage at room temperature. Higher TVC levels may have been caused by sub-optimum drying and grinding conditions. Also acidifying insects with vinegar to pH 4.5 prevented rapid spoilage during storage at room temperature. These simple interventions might be promising and still need to be investigated further, taking into account other aspects than safety, such as sensory aspects.

Table 2
Microbiota outcome of preservation and storage scenarios of house crickets (*Acheta domesticus*).

Storage conditions		Log cfu/g	Storage time (days)				
			0	2	6	10	16
Refrigerator (5–7 °C)	Fresh	Total Viable Count	7.2	8.3	8.1	8.0	8.2
		Enterobacteriaceae	4.2	6.5	6.6	4.5	6.1
		Bacterial spores	3.6	4.4	4.4	4.2	2.6
	Boiled (1 min)	Total Viable Count	3.1	3.3	3.8	n.d.	4.1
		Enterobacteriaceae	<1	<1	1.3	1.3	3.2
		Bacterial spores	2.0	2.8	2.4	2.0	2.0
Ambient (28–30 °C)	Boiled (1 min)	Total Viable Count	3.1	10.1	sp	sp	sp
		Enterobacteriaceae	<1	>9			
		Bacterial spores	2.0	7.8			
	Boiled (5 min) dried* and ground	Total Viable Count	5.4	5.6	5.5	n.d.	n.d.
		Enterobacteriaceae	<1	<1	1.3		
		Bacterial spores	1.9	1.7	2.0		
	Boiled in acid (product pH 4.5)**	Total Viable Count	3.0	2.6	n.d.	n.d.	n.d.
		Bacterial spores	2.2	2.0			

sp: spoiled; n.d.: not determined; * dried at 55 °C for 24 h; **: Vinegar with 5% acetic acid. Single experiments, analysed in duplicate. Data represent mean values, with cv < 15%.

3.3. Insects as ingredient for fermented food (mealworm larvae)

Ground insects have potential use in the (protein) enrichment of food products, including fermented food products. As shown in Table 3, no clear differences in microbiological composition during lactic acid fermentation could be noticed between the composite meal consisting of sorghum mixed with 10 or 20% mealworm

Table 1
Microbiota of fresh and heat-treated insects.

Log cfu/g	Mealworm larvae (*Tenebrio molitor*)					Small cricket (*Acheta domesticus*)			Large cricket (*Brachytrupus* sp.)		
	Fresh	Boiled (10 min)		Roasted (10 min)		Fresh	Boiled (5 min)	Stir-fried (5 min)	Fresh	Boiled	
		Whole	Crushed	Whole	Crushed					(5 min)	(10 min)
Total Viable Count	7.7	<1.7	2.5	<1.7	4.8	7.2	1.7	2.7	6.7	2.5	2.8
Enterobacteriaceae	6.8	<1	<1	2.2	2.6	4.2	<1	<1	4.4	<1	<1
Bacterial spores	2.1	<1	2.5	1.6	<1	3.6	1.5	1.5	4.4	2.5	2.7

Single experiments, analysed in duplicate. Data represent mean values, with cv < 15%.

Table 3
Microbiological composition during lactic acid fermentation of composite meals of sorghum and mealworm larvae (*Tenebrio molitor*).

Log cfu/g	Fermentation period (h)	Roasted larvae (*Tenebrio molitor*) (% d.m. of composite meal)		
		0	10	20
Total Viable Count	−1	4.4	3.9	5.4
	0	8.0	8.0	8.0
	24	8.6	8.6	8.7
	48	9.1	8.6	8.7
Lactic acid bacteria	−1	<1	3.2	4.7
	0	7.9	8.1	8.2
	24	8.6	8.7	8.9
	48	8.7	8.8	8.8
Enterobacteriaceae	−1	3.1	3.1	3.3
	0	3.5	2.8	3.2
	24	<1	<1	<1
	48	<1	<1	<1
Bacterial spores	−1	3.1	2.9	3.0
	0	3.1	3.0	3.0
	24	3.3	2.5	<2
	48	2.9	2.7	2.5
pH	−1	6.4	6.4	6.5
	0	5.4	5.4	5
	24	3.6	3.6	3.7
	48	3.7	3.7	3.8

Single experiments, analysed in duplicate. Data represent mean values, with cv < 15%. The composite flour : water mixture was inoculated with a natural enrichment of lactic acid bacteria at 10^8 CFU g^{-1} at $t = 0$.

larvae, and the control (100%) sorghum meal. Furthermore the pH decreased equally in all meals during fermentation. These results indicate that it is possible to apply a fermentation process on composite flour containing 20% ground insects.

From Table 3 it can also be concluded that Enterobacteriaceae are inactivated during the fermentation in all meals, likely as a result of the antimicrobial effect on Enterobacteriaceae during an acidic fermentation. The levels of sporeforming bacteria remained stable at acceptable levels ($<10^3$ cfu/g), during fermentation. Spore forming bacteria were not inactivated, but were unable to germinate and grow in these conditions, indicating that they should not be expected to pose an elevated risk in porridge made from the fermented flour. A potential risk with edible insects is the presence of sporeforming bacteria. From both the composite flour of sorghum and mealworm larvae as well as pure mealworm larvae most often *Bacillus licheniformis* was isolated. Also *Bacillus subtilis* and *Bacillus megaterium* were occasionally identified. These *Bacillus* species are often found in soil and are not considered as pathogenic, however they could cause spoilage when able to proliferate (EFSA, 2005).

4. Conclusion

Similar to other animal derived products, insects are rich in nutrients, moisture and contain their gut microflora providing a medium for growth of unwanted micro-organisms in certain conditions. This makes insects susceptible for microbiological hazards if proper heat treatment or storage conditions are not applied. Therefore edible insects need to be processed and stored with care. A heating step is sufficient for inactivation of Enterobacteriaceae, however a remaining potential risk with edible insects is the presence of sporeforming bacteria which are most probably introduced through soil contact and cannot be fully eliminated by boiling. Alternative preservation techniques without the use of a refrigerator were explored of which drying and acidifying seemed practical and promising, however this requires some further consumer research.

The use of insects for protein enrichment of fermented food products is possible and has mutual benefits as the decreased pH in lactic acid fermented products prevents the growth of potentially harmful microorganisms. Cultural preferences and organoleptic aspects need to be considered in further studies.

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