Microbiological quality of dried larvae of *Tenebrio molitor* under various processing methods as a food and feed in Iran

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Summary

Edible insects are considered as an alternative protein source for consumption in many countries and special attention has been given to their microbiological safety. In this study, larvae of dried yellow mealworm *Tenebrio molitor* were collected and subjected to various drying techniques, namely, sun-drying (T1), boiling for 30 s with subsequent 24 h drying at 60 °C (T2) and boiling for 1 min with subsequent 48 h drying at 70 °C (T3). A variety of bacteria were isolated from the dried samples and were analysed based on biochemical, pathogenicity characteristics and molecular analysis. Results showed that the bacteria were *Escherichia coli, Enterococcus gallinarum, Staphylococcus succinus, Bacillus cereus, Bacillus atrophaeus* and *Cronobacter* sp. Two important pathogenic bacteria were also identified and assigned as *E. coli* and *B. cereus*. Total bacterial populations in T1, T2 and in the ready product were 2.19×10^5 CFU·g^{-1}, 2.16×10^5 CFU·g^{-1} and 2.04×10^5 CFU·g^{-1}, respectively. T3 was the most effective drying method with no bacterial growth. These results indicate that a short heating treatment is an effective approach to eliminating bacteria, except for Gram-positive endospore-forming bacteria. Otherwise, some hygienic practices and proper processing methods are necessary to treat the larvae before consumption.

Keywords

dried insects; edible insects; food safety; microbiological quality; antibiotic test; molecular analysis

Currently, due to an increase in population, animal resources are not enough to feed humans and alternative sources need to compensate for food shortage [1]. In this respect, insects have been suggested as an alternative protein source by the Food and Agriculture Organization (FAO) [2]. Edible insects have long been used as food and feed in various countries [3]. Insects have the most biodiversity in the world and are rich sources of nutrients for humans and animals. Moreover, insect rearing requires a smaller space, less feed and less time compared to animal husbandry, making insects a favourable alternative to animal products [4].

Some of the important groups edible insects include grasshoppers, caterpillars, beetles, grubs, termites, bees and wasps. Their use is affected by region, cultural and religious differences. Various technologies have been developed for collection, preparation and processing of edible insect [5]. Some of these include roasting, smoking, stewing, frying and boiling [6]. Insects provide a rich source of nutrients such as proteins, carbohydrates, lipids, minerals and certain vitamins [7].

Despite all these benefits, edible insects may harbour harmful microorganisms, which could potentially cause disease upon consumption. Attention to food safety issues of edible insects such as microorganisms, allergens and toxins are the most important factors that have to be respected at development of edible insect products for human consumption. For these reasons, attention to safety and potential hazards of raw insects is emphasized [8–10].

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The natural and rearing insects may be infected with pathogenic microorganisms, including bacteria, viruses, fungi and protozoa [11]. The traditional processing techniques do not devitalize all of them [12]. Certain factors may play a role in the primary contamination including collection methods, rearing, containers utilized, preservation and storage [13]. Therefore, hygiene condition during rearing and processing of insects is very important to reduce contamination. Unfortunately, information concerning consumer health and food safety aspects of insect products remains limited and need widespread research [8, 12, 13].

One of these insects called yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) is a common pest of stored grain in Iran, and is currently found worldwide, namely in Asia, Africa, USA and United Kingdom) [14]. Mealworm larvae are processed industrially in some companies such as Hatortempt in China or Chubby Mealworms in USA. The processed larvae are consumed as food or feed by humans or animals. These insects are rich in nutrients, are reared easily and have a short life cycle [14, 15].

Since information on the beneficial or deleterious microbial load of insects is scarce, the present study was conducted on yellow mealworm larvae to clarify the dimensions of this issue.

MATERIALS AND METHODS

Insect culture

The mealworm (*T. molitor*) larvae were reared on wheat bran and pieces of carrot as a source of food and humidity in plastic containers under controlled laboratory conditions (temperature at 27 ± 1 °C, relative humidity 55 ± 5 %, in darkness) in Razi Laboratory, Tehran, Iran. Mealworm larvae were obtained from a local producer in Sari-Iran and reared in the laboratory by the following methods:

- Treatment 1 (T1): 50 g of larvae were washed with distilled water for a minute and then dried in the sun for 1 day;
- Treatment 2 (T2): 50 g of larvae were washed with distilled water for a minute and then boiled in beaker for 30 s and then were dried for 24 h at 60 °C in an oven;
- Treatment 3 (T3): 50 g of larvae were washed with distilled water for a minute and then boiled for 1 min and finally dried for 48 h at 70 °C in an oven;
- Treatment 4 (T4): The ready-made dried mealworm larvae were purchased from the market and stored in the refrigerator at 4 °C for use in a subsequent step. They were used as a control for comparison with other methods used in this study.

Samples were stored at 4 °C for 1–2 weeks for subsequent analyses.

Preparation of samples for analysis

One gram of each sample was blended with 10 ml sterile distilled water in a sterile condition. Three dilutions were prepared for each sample (10^{-1}, 10^{-3}, and 10^{-6}). Each 1 ml portion was transferred into 9 ml distilled water and diluted to yield a solution with a concentration of 10^{-1}, 10^{-3}, and 10^{-6}. A portion of 1 ml of the 1th, 3th and 6th dilutions obtained from all samples was inoculated on Nutrient agar (Merck, Darmstadt, Germany) plates, using a sterile wire loop under a laminar airflow hood. The plates were then incubated at 27 °C for 24–72 h. The experiment was done in triplicate for each sample dilution.

Microbial counts determination

The number of bacterial colonies formed was counted using a manual colony counter. The mean counts were obtained and multiplied with the appropriate dilution factor to obtain the total viable cells per unit weight of the sample expressed as colony-forming units per gram of the sample [16].

Bicochemical and pathogencity tests

The morphological, physiological and biochemical tests were performed for each isolate. These included the Gram and spore staining, testing of motility, fluorescent pigmentation on King's B medium, NaCl tolerance, oxidative/fermentation glucose, catalase, methyl red Voges Proskauer (MRVP), nitrate reduction, oxidase, arabinose fermentation, starch, lignin, and cellulose hydrolysis tests, citrate utilization and levan production. The pathogenicity test was carried out on geranium plants [16, 17].

Antibiogram tests

Antibiotic sensitivity tests were done by using the disk diffusion method. A colony from each isolation was suspended in 5 ml of distilled water and was uniformly distributed on agar plates in triplicates. The antibiotic disks used were chloramphenicol (30 μg), penicillins (10 μg), amoxicillin (25 μg), tetracycline (30 μg) and azithromycin (15 μg; all from Padtan Teb, Tehran, Iran). An antibiotic disk was placed on the surface of the culture medium plate lawned by bacteria. The agar plates were allowed to soak up and incubated at

27 °C for 24–48 h. The diameter of the inhibition zone was measured and expressed in millimetres. The results were recorded as resistant (R) or sensitive (S) [18].

Extraction of bacterial DNA

Bacterial DNA of all target strains was extracted by the boiling method. The bacteria were grown on Nutrient agar at 27 °C for 48 h. A few colonies of each bacterium were put in a test tube containing lysis buffer containing 500 μl of 1% NaOH and 10 μl of 0.5% SDS. The mixture was boiled in a water bath (ZenithLab, Changzhou, China) for 15 min and then subjected to centrifugation for 15 min at 16 089 ×*g*. A volume of 100 μl of the supernatant containing DNA was transferred to a tube and stored at –20 °C for a week [19].

16S rRNA gene amplification and sequencing

The polymerase chain reactions (PCR) for amplification of the bacterial 16S rRNA genes were performed in a total volume of 25 μl using master mixture (12.5 μl; Amplicon, Odense, Denmark), distilled water (9.5 μl), primers (2 μl) and extracted DNA solution (1 μl). The primer pair, forward (Eubak3): 5'-ATA TAT AAG CGG CCG CAG AAA GGA GGT GAT CC-3') and reverse (Eubak3): 5'-ATA TAT AAG CGG CCG CAG AGT TTG ATC ATG CCT C-3' was used. The PCR amplification was carried out using the PCR thermal cycler Mycycler (Bio-Rad, Hercules, California, USA) using a hot-start procedure. The PCR protocol used was 4 min at 94 °C, followed by 35 cycles of 60 s at 94 °C, 45 s at 60 °C, 60 s at 72 °C, and a final extension step was performed for 10 min at 72 °C. PCR products were separated by using 0.8 % agarose gel electrophoresis in Tris-borate-EDTA (TBE) buffer.

DNA sequencing and phylogenetic analysis

Purified PCR products were sent to Microsynth (Balgach, Switzerland) for sequencing. The sequences were then trimmed with Chromas V 2.6.6 (Technelysium, South Brisbane, Australia) and assembled with DNA Baser Assembler V 5.15.0 (Heracle BioSoft, Arges, Romania). The sequences of approximately 1.5 kb were evaluated using BLAST (Basic Local Alignment Search Tool) in GenBank database (National Center for Biotechnology Information, Bethesda, Maryland, USA). The phylogenetic tree was constructed after trimming the unaligned regions by the maximum likelihood using MEGA 6.0 software (Pennsylvania State University, Pennsylvania, USA). The tree topologies were evaluated using bootstrap analysis based on 1 000 replicates and using the method based on the Tamura-Nei model [20].

RESULTS AND DISCUSSION

The total viable counts of dried mealworm larvae for treatments T1, T2 and T4 were 2.19×10^5 CFU·g^{-1}, 2.16×10^5 CFU·g^{-1}, and 2.04×10^5 CFU·g^{-1}, respectively. No microbial growth was observed for T3 samples after 72 h. The six species, which were isolated and identified from dried larvae samples, included *Staphylococcus succinus, Escherichia coli, Enterococcus gallinarum, Bacillus cereus, B. atrophaeus* and *Cronobacter* sp. The predominant bacteria included *E. coli, S. succinus, E. gallinarum* and *B. cereus* (Tab. 1).

According to the results, the presence of bacteria was detected in all samples and found to be remarkably different in samples treated by different drying methods. The most microbial load was observed at sun-drying conditions. According to the results, sun-dried samples (T1) had a considerable microbial load in all three dilutions (10^{-1}, 10^{-3}, and 10^{-6}), 2.51×10^5 CFU·g^{-1}, 1.49×10^5 CFU·g^{-1}, and 1.07×10^5 CFU·g^{-1}, respectively. Meanwhile, in T2 and T4 samples, the microbial load was observed only in 10^{-1} dilution, and no bacteria grew on Nutrient agar plates in other dilutions. It is worth mentioning that in both T2 and T4 samples, only *B. cereus* was detected.

Tab. 1. Analysis of bacteria in dried *Tenebrio molitor* larvae.

Treatments		Total bacterial counts [CFU·g^{-1}]	Bacteria isolated
T1	Sun-dried	2.19×10^5	*Enterococcus gallinarum, Staphylococcus succinus, Escherichia coli, Bacillus atrophaeus, Cronobacter* sp.
T2	Boiling and drying for 24 h	2.16×10^5	*Bacillus cereus*
T3	Boiling and drying for 48 h	< *LOD*	< *LOD*
T4	Market products	2.04×10^5	*Bacillus cereus*

LOD – limit of detection.

The morphological and biochemical characterization of the bacteria isolated from different samples of dried mealworm larvae is shown in Tab. 2 and Tab. 3. The tolerance of *Staph. succinus* to high concentrations of NaCl (10 %) in medium is shown in Tab. 3. According to the pathogenicity test on the healthy geranium plants, symptoms appeared as necrotic lesions in some samples and with bacteria *E. coli* (most hypersensitivity reactions shown), *B. cereus, E. gallinarum, Staph. succinus* and *B. atrophaeus*.

The isolates were sensitive to amoxicillin, azithromycin, chloramphenicol and tetracycline (Tab. 4). *E. coli* K4 was the only one that showed resistance to all antibiotics. On the other hand, *Staph. succinus* (K2), *B. atrophaeus* (K5, K9) and *Cronobacter* sp. (K6, K10) were sensitive to all antibiotics. *E. gallinarum* (K1, K7, K11) and *B. cereus*

Tab. 2. Characteristics of the bacteria isolated from *Tenebrio molitor* larvae.

	Species identity						
	Enterococcus gallinarum	*Staphylococcus succinus*	*Bacillus atrophaeus*	*Escherichia coli*	*Cronobacter* sp.	*Bacillus cereus*	*Bacillus cereus*
Treatment	T1	T1	T1	T1	T1	T2	T4
Gram staining	G^+	G^+	G^+	G^-	G^-	G^+	G^+
Motility	–	–	+	+	+	+	+
Shape	Cocci	Cocci	Rods	Rods	Rods	Rods	Rods
Colony colour	White	White/Yellow	White	Cream	Yellow	White	White
Spore	–	–	+	–	+	+	+
Heat test	+	–	+	–	+	+	+
King's B medium	–	–	–	–	–	–	–

Treatment: T1 – sun-dried, T2 – boiling and drying for 24 h, T3 – boiling and drying for 48 h, T4 – market products.
(+) – positive, (–) – negative.

Tab. 3. Biochemical characteristics of bacteria isolated from *Tenebrio molitor* larvae.

	Species identity						
	Enterococcus gallinarum	*Staphylococcus succinus*	*Bacillus atrophaeus*	*Escherichia coli*	*Cronobacter* sp.	*Bacillus cereus*	*Bacillus cereus*
Treatment	T1	T1	T1	T1	T1	T2	T4
Tests							
Levan production	–	–	–	–	–	–	–
6.5% NaCl	+	+	+	+	+	+	+
10% NaCl	–	+*	–	–	–	–	–
Oxidase	+	–	+	–	–	–	–
Catalase	–	+	+	+	+	+	+
Voges Proskaeur	+	–	+	–	+	+	+
Methyl red	+	–	–	+	–	–	–
Nitrate reduction	+	+	+	+	+	+	+
Arabinose	V	–	+	+	+	–	–
O/F test	F	O	F	F	F	F	F
Citrate utilization	–	–	+	–	–	+	+
Starch hydrolysis	V	–	+	–	+	–	–
Cellulose hydrolysis	–	–	–	–	–	–	–
Lignin hydrolysis	–	–	+	–	–	+	+
Hypersensitivity reaction on geranium	+	+	+	++	–	+	+

Treatment: T1 – sun-dried, T2 – boiling and drying for 24 h, T3 – boiling and drying for 48 h, T4 – market products.
(+) – positive, (–) – negative, * – tolerates 16% NaCl, V – variable, O – oxidative, F – fermentation.
Hypersensitivity reaction: (+) – positive, (++) – moderate, (+++) – severe, (–) – weak.

Tab. 4. Antibiogram results of bacterial isolates.

Identity of isolates		Treatment	Antibiotic				
			Amoxicillin (25 μg)	Chloramphenicol (30 μg)	Penicillins (10 μg)	Azithromycin (15 μg)	Tetracycline (30 μg)
K1	*Enterococcus gallinarum*	T1	S	S	R	S	S
K2	*Staphylococcus succinus*	T1	S	S	S	S	S
K3	*Bacillus cereus*	T2	S	S	R	S	S
K4	*Escherichia coli*	T1	R	R	R	R	R
K5	*Bacillus atrophaeus*	T1	S	S	S	S	S
K6	*Cronobacter* sp.	T1	S	S	S	S	S
K7	*Enterococcus gallinarum*	T1	S	S	R	S	S
K8	*Bacillus cereus*	T4	S	S	R	S	S
K9	*Bacillus atrophaeus*	T1	S	S	S	S	S
K10	*Cronobacter* sp.	T1	S	S	S	S	S
K11	*Enterococcus gallinarum*	T1	S	S	R	S	S

Treatment: T1 – sun-dried, T2 – boiling and drying for 24 h, T3 – boiling and drying for 48 h, T4 – market products.
R – resistant, S – sensitive.

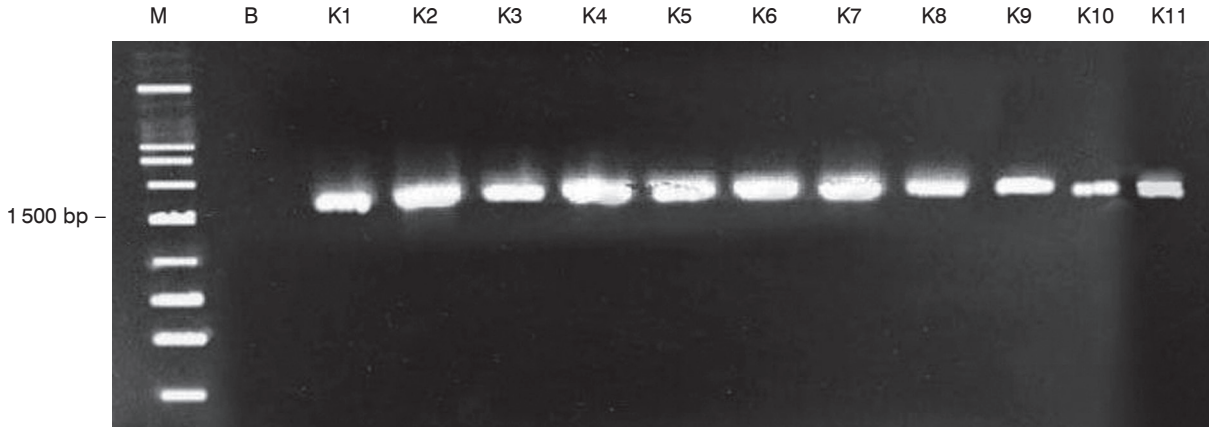

Fig. 1. Gel electrophoretic separation of polymerase chain reaction-amplified six bacterial 16S rDNA genes showing a single band of approximately 1.5 kb.

Lanes: M – molecular size marker (100 bp DNA ladder; Fermentas, Dublin, Ireland), B – negative control, K1 to K11 – bacterial isolates from dried mealworm samples (identification is given in Tab. 4).

(K3, K8) showed resistance to penicillin and to no other antibiotic. As a result, majority of bacteria were found resistant to penicillin (54.5 %).

As expected, a DNA fragment of 1.5 kb was amplified from the 16S rRNA gene by all 11 representative isolates (Fig. 1). Sequence alignment showed that the bacteria isolated from the dried larvae belonged to the genera *Enterococcus, Bacillus, Staphylococcus, Escherichia* and *Cronobacter*. Analysis of the 16S rRNA gene sequences of the strains showed that 72.2 % of bacteria belonged to the *Firmicutes phylum* and others belonged to the *Proteobacteria phylum*. The accession numbers are mentioned in Tab. 5.

A dendrogram with 16S rRNA sequences of 11 representative bacterial isolates from dried *T. molitor* larvae split them into three distinct clusters: bacilli, *Enterococcus* spp. and *Cronobacter* spp. The strains of the same species shared 100% similarity within the group. The analysis revealed a negligible genetic variability among the members of the same species. The most distinct cluster was formed by *Bacillus* spp. which showed a similarity of 34 % with two other species of the same genus. *Acinetobacter pittii* was used as an outgroup (Fig. 2).

The results indicated a diverse bacterial community composition in mealworm larvae. The

Tab. 5. Identification of isolates based on 16S rDNA sequencing

Sample code	Treatment	Strain code	Species	Family	Accession number	Similarity [%]
K4-1	T1	K1	*Enterococcus gallinarum*	Enterococcaceae	MK956183	97
K4-2	T1	K2	*Staphylococcus succinus*	Staphylococcaceae	MK956184	98
K4-3	T2	K3	*Bacillus cereus*	Bacillaceae	MK956185	93
K4-4	T1	K4	*Escherichia coli*	Enterobacteriaceae	MK956186	98
K4-5	T1	K5	*Bacillus atrophaeus*	Bacillaceae	MK956187	97
K4-6	T1	K6	*Cronobacter* sp.	Enterobacteriaceae	MK956188	100
K4-7	T1	K7	*Enterococcus gallinarum*	Enterococcaceae	MK956189	97
K4-8	T4	K8	*Bacillus cereus*	Bacillaceae	MK956190	93
K4-9	T1	K9	*Bacillus atrophaeus*	Bacillaceae	MN339594	99
K4-10	T1	K10	*Cronobacter* sp.	Enterobacteriaceae	MN339595	97
K4-11	T1	K11	*Enterococcus gallinarum*	Enterococcaceae	MN339596	99

Accession number according to the GenBank (National Center for Biotechnology Information, Bethesda, Maryland, USA).
Treatment: T1 – sun-dried, T2 – boiling and drying for 24 h, T3 – boiling and drying for 48 h, T4 – market products.

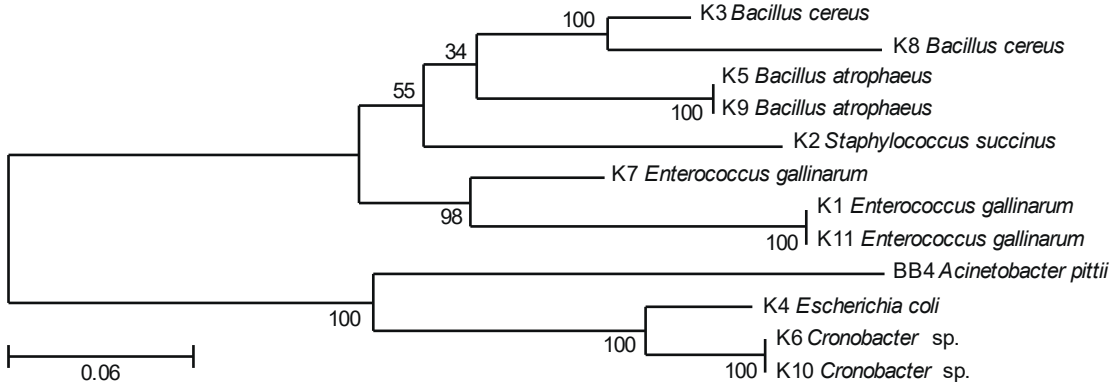

Fig. 2. Maximum likelihood tree based on the 16S rDNA sequences of bacterial isolates.

characterized bacteria belonged to the phyla Proteobacteria and Firmicutes. Phenotypic and PCR analyses confirmed the presence of six bacterial species, namely, *Staph. succinus, E. coli, E. gallinarum, Cronobacter* sp., *B. cereus* and *B. atrophaeus*. According to available reports, two of the genera (*Staphylococcus* and *Bacillus*) are known as the main bacteria in the gut of many insect species and may be beneficiary to their hosts. However, some *Staphylococcus* and *Bacillus* species cause food spoilage or food poisoning that is the consequence of using improper processing and storage techniques [21, 22]. Further, some strains of *E. coli* and *B. cereus* are known as pathogens [23, 24].

According to our observation, the best way to eliminate microbial contaminants is to treat the larvae by one-minute boiling followed by drying them at 70 °C temperature for 48 h (T3). Some bacteria, like the members of Enterobacteriaceae, were eliminated during boiling and heating techniques, but not the endospore-forming bacteria such as *B. cereus*, which were could survive in high-temperature conditions. A comparison of different treatments showed that the number of endospore-forming bacteria decreased in different ways, but none of the treatments could eliminate them. Therefore, the best way to eliminate these bacteria is to boil the larvae for a long time. For such treatments, the quantity, quality and nutritional value of the treated larvae must be taken into account [12, 25, 26]. The results of this study showed that different drying methods, in particular T2 and T3, reduced the microbial load to a desirable extent. Experiments also showed that high temperatures and longer time of boiling caused qualitative changes including decomposition of larvae and possibly reduced their nutritional value.

Several studies on microbial populations of dried insects were published previously. Klunder et al. [12] worked specifically on two species of *Tenebrio molitor* and *Acheta domesticus*. For mealworm larvae, the authors of the study

recorded 7.7 log CFU·g^{-1} for total microbial counts, 6.8 log CFU·g^{-1} for Enterobacteriaceae and 2.1 log CFU·g^{-1} for endospore-forming bacteria. The study showed that a short heating step reduced the bacterial counts significantly, being suitable to eliminate Enterobacteriaceae and some spore-forming bacteria. In the case of processed and dried *Oryctes monocerus* larvae, a variety of pathogenic bacteria were reported, including *Staph. aureus, Pseudomonas aeruginosa* and *B. cereus* together with some non-pathogenic bacteria such as *Bacillus subtilis* or *Bacillus firmus* [27]. The presence of pathogenic microbes was posed to be a threat to consumers and preventive measures were recommended to avoid risk from feeding on these contaminated insects. Similar data were obtained for dried mealworm larvae regarding contamination with *Staphylococcus* and *Bacillus* species. In another study, the microbiological analysis on *Tenebrio molitor* and *Locusta migratoria migratorioides* showed that both insects contained high microbial loads with different bacterial communities. The bacteria in mealworm larvae were found to be from genera *Propionibacterium*, *Haemophilus*, *Staphylococcus* and *Clostridium* [28]. The latter results showed that a processing step is needed to reduce risks involved with the consumption. Mujuru et al. [29] investigated the microbiological quality of processed *Gonimbrasia belina* under various traditional drying methods, namely, boiling in salted water and sun-drying, roasting, drum roasting and hot-ash drying. Microbiological tests indicated relatively low total bacterial counts (10–2 500 CFU·g^{-1}), coliforms, *Staph. aureus* and *E. coli* being the major bacterial contaminants. Also, some *G. belina* samples were contaminated with various yeasts and fungi. Results of that study showed that processing method and handling are the major factors determining the contamination.

In Tanzania, the microbiological quality of wild-harvested and processing methods of *Ruspolia differens* was analysed. The results showed high microbial loads in fresh *R. differens*. Furthermore, high counts after transportation to the market and plucking of wings and legs were observed for total aerobes, endospore-forming bacteria as well as for yeasts and moulds. A significant reduction in all bacteria was observed after processing except for endospore-forming bacteria [30]. In another study, endospore-forming bacteria were found to be able to survive blanching mealworm larvae for 10, 20 and 40 min without any significant changes [26]. Igbabul et al. [25] reported similar findings on dried Cirina forda, which had to be subjected to heat to eliminate pathogens.

Due to high microbial counts in edible insects, the risk of insect consumption is increased regarding pathogens. Therefore, using antibiotics is essential to eliminate bacteria [31]. However, antibiotic resistance in bacteria isolated from infected insects is a potential problem for consumer health. For example, we showed that *E. coli* was 100% resistant to four types of antibiotics. *B. cereus* and *E. gallinarum* strains were resistant to penicillin in all samples. *Staph. succinus, B. atrophaeus* and *Cronobacter* sp. were susceptible to all antibiotics used in the test. Also, *E. coli* isolates possessed resistance to all antibiotics and this somehow coincided with results reported in previous studies [32–34]. Resistance to penicillin was observed in *B. cereus* strains in some previous studies [35, 36]. According to the results of the antibiotic test, the bacteria isolated from the mealworm larvae showed the highest resistance to penicillin in this study.

CONCLUSION

The yellow mealworm (*T. molitor*) larvae, are rich in nutrients, moisture and, therefore, favourable for the growth of microorganisms. In this study, the microbiological quality of dried mealworms was analysed after various processing techniques. Bacteria from genera *Bacillus*, *Staphylococcus*, *Enterococcus*, *Escherichia* and *Cronobacter* were identified in dried mealworm larvae. In T2 and T4 treatments, low microbial loads were determined compared to T3, which showed almost zero contamination. Given the results, the boiling time and high temperatures treatments during drying the insects led to a reduction of most bacterial counts, but not of the endospore-forming bacteria. Elimination of the latter requires more attention and application of more effective devitalization methods.

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REFERENCES

1. Foley, J. A. – Ramankutty, N. – Brauman, K. A. – Cassidy, E. S. – Gerber, J. S. – Johnston, M. – Mueller, N. D. – O'Connell, C. – Ray, D. K. – West, P. C. – Balzer, C. – Bennett, E. M. – Carpenter, S. R. – Hill, J. –Monfreda, C. – Polasky, S. – Rockström, J. – Sheehan, J. – Siebert, S. – Tilman, D. – Zaks, D. P. M.: Solutions for

a cultivated planet. Nature, *478*, 2011, pp. 337–342. DOI: 10.1038/nature10452.
2. Van Huis, A. – Van Itterbeeck, J. – Klunder, H. – Mertens, E. – Halloran, A. – Muir, G. – Vantomme, P.: Edible insects: future prospects for food and feed security. FAO Forestry paper 171. Rome : Food and Agriculture Organization, 2013. ISBN: 978-92-5-107595-1. ISSN: 0258-6150.
3. Allotey, J. – Mpuchane, S.: Utilization of useful insects as food source. African Journal of Food, Agriculture, Nutrition and Development, *3*, 2003, pp. 1–8. ISSN: 1684-5358.
4. Yen, A. L.: Edible insects: traditional knowledge or western phobia? Entomological Research, *39*, 2009, pp. 289–298. DOI: 10.1111/j.1748-5967.2009.00239.x.
5. Nonaka, K.: Feasting on insects. Entomological Research, *39*, 2009, pp. 304–312. DOI: 10.1111/j.1748-5967.2009.00240.x.
6. Ogbalu, O. K. – Williams, J. O.: The edibility, methods of preparation of the raphia palm beetle, *Rhyncophorus phoenicis* (Coleoptera: Curculionidae) in the Niger Delta and associated microorganisms. International Journal of Pharmaceutical and Biological Sciences, *10*, 2015, pp. 125–129. DOI: 10.9790/3008-1012125129.
7. Mlcek, J. – Rop, O. – Borkovcova, M. – Bednarova, M.: A comprehensive look at the possibilities of edible insects as food in Europe – a review. Polish Journal of Food and Nutrition Sciences, *64*, 2014, pp. 147–157. DOI: 10.2478/v10222-012-0099-8.
8. Belluco, S. – Losasso, C. – Maggioletti, M. – Alonzi, M. C. – Paoletti, M. G. – Ricci, A.: Edible insects in a food safety and nutritional perspective: a critical review, comprehensive reviews. Comprehensive Reviews in Food Science and Food Safety, *12*, 2013, pp. 296–313. DOI: 10.1111/1541-4337.12014.
9. Van Huis, A. – Dicke, M. – Van Loon, J. J. A.: Insects to feed the world. Journal of Insects as Food and Feed, *1*, 2015, pp. 3–5. DOI: 10.3920/JIFF2015.x002.
10. Van der Spiegel, M. – Noordam, M. Y. – Van der Fels-Klerx, H. J.: Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production. Comprehensive Reviews in Food Science and Food Safety, *12*, 2013, pp. 662–678. DOI: 10.1111/1541-4337.12032.
11. Vega, F. – Kaya, H. Eds.): Insect Pathology. 2nd ed. London : Academic Press, 2012. ISBN: 9780123849854.
12. Klunder, H. C. – Wolkers-Rooijackers, J. – Korpela, J. M. – Nout, M. J. R.: Microbiological aspects of processing and storage of edible insects. Food Control, *26*, 2012, pp. 628–631. DOI: 10.1016/j.foodcont.2012.02.013.
13. Braide, W. – Nwaoguikpe, R. N.: Assessment of microbiological quality and nutritional values of a processed edible weevil caterpillar (*Rhynchophorus phoenicis*) in Port Harcourt, southern Nigeria. International Journal of Biological and Chemical Sciences, *5*, 2011, pp. 410–418. DOI: 10.4314/ijbcs.v5i2.72059.
14. Ramos-Elorduy, J. – Gonzalez, E. A. – Hernandez, A. R. – Pino, J. M.: Use of *Tenebrio molitor* (Coleoptera: Tenebrionidae) to recycle organic wastes and as feed for broiler chickens. Journal of Economic Entomology, *95*, 2002, pp. 214–220. DOI: 10.1603/0022-0493-95.1.214.
15. Veldkamp, T. – van Duinkerken, G. – van Huis, A. – Lakemond, C. M. M. – Ottevanger, E. – Bosch, G. – Boekel, T. V.: Insects as a sustainable feed ingredient in pig and poultry diets – A feasibility study. Report 638. Wageningen : Wageningen UR Livestock Research, 2012. ISSN: 1570–8616. <https://library.wur.nl/WebQuery/wurpubs/428703>
16. Cheesborough, M.: District laboratory practice in tropical countries, Part 2. 2nd ed. Cambridge : Cambridge University Press, 2000. ISBN: 9780521676311.
17. Schaad, N. W. – Jones, J. B. – Chun, W. (Eds.): Laboratory guide for identification of plant pathogenic bacteria. 3rd ed. St. Paul : APS Press, 2001. ISBN: 0890542635.
18. CLSI document M100-S25. Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplement. Wayne : Clinical and Laboratory Standards Institute, 2015. ISBN: 1-56238-989-0.
19. Elboutahiri, N. – Thami-Alami, I. – Zaďd, E. – Udupa, S. M.: Genotypic characterization of indigenous *Sinorhizobium meliloti* and *Rhizobium sullae* by rep-PCR, RAPD and ARDRA analyses. African Journal of Biotechnology, *8*, 2009, pp. 979–985. DOI: 10.5897/AJB2009.000-9161.
20. Felsenstein, J.: Evolutionary trees from DNA sequences: a maximum likelihood approach. Journal of Molecular Evolution, *17*, 1981, pp. 368–376. DOI: 10.1007/BF01734359.
21. Colman, D. R. – Toolson, E. C. – Takacs-Vesbach, C. D.: Do diet and taxonomy influence insect gut bacterial communities? Molecular Ecology, *21*, 2012, pp. 5124–5137. DOI: 10.1111/j.1365-294X.2012.05752.x.
22. Engels, P. – Moran, N. A.: The gut microbiota of insects – diversity in structure and function. FEMS Microbiology Reviews, *37*, 2013, pp. 699–735. DOI: 10.1111/1574-6976.12025.
23. Kotiranta, A. – Lounatmaa, K. – Haapasalo, M.: Epidemiology and pathogenesis of *Bacillus cereus* infections. Microbes and Infection, *2*, 2000, pp. 189–198. DOI: 10.1016/s1286-4579(00)00269-0.
24. Xia, X. – Meng, J. – McDermott, P. – Ayers, S. – Blickenstaff, K. – Tran, T. T. – Abbott, J. – Jie Zheng, J. – Zhao, S. H.: Presence and characterization of shiga toxin-producing *Escherichia coli* and other potentially diarrheagenic *E. coli* in retail meats. Applied and Environmental Microbiology, *76*, 2010, pp. 1709–1717. DOI: 10.1128/AEM.01968-09.
25. Igbabul, B. D. – Agude, C. H. – Inyang, U.: Nutritional and microbial quality of dried larva of *Cirina forda*. International Journal of Food Sciences and Nutrition, *3*, 2014, pp. 602–606. DOI: 10.11648/j.ijnfs.20140306.28.
26. Vandeweyer, D. – Lenaerts, S. – Callens, A. –

Van Campenhout, L.: Effect of blanching followed by refrigerated storage or industrial microwave drying on the microbial load of yellow mealworm larvae (*Tenebrio molitor*). Food Control, *71*, 2017, pp. 311–314. DOI: 10.1016/j.foodcont.2016.07.011.
27. Banjo, A .D. – Lawal, O. A. – Songonuga, E. A.: The nutritional value of fourteen species of edible insects in southwestern Nigeria. African Journal of Biotechnology, *5*, 2006, pp. 298–301. DOI: 10.5897/AJB05.250.
28. Stoops, J. – Crauwels, S. – Waud, M. – Claes, J. – Lievens, B. – Van Campenhout, L.: Microbial community assessment of mealworm larvae (*Tenebrio molitor*) and grasshoppers (*Locusta migratoria migratorioides*) sold for human consumption. Food Microbiology, *53*, 2016, pp. 122–127. DOI: 10.1016/j.fm.2015.09.010.
29. Mujuru, F. M. – Kwiri, T. – Nyambi, C. – Winini, C. – Moyo, D. N.: Microbiological quality of *Gonimbrasia belina* processed under different traditional practices in Gwanda, Zimbabwe. International Journal of Current Microbiology and Applied Sciences, *3*, 2014, pp. 1085–1094. ISSN: 2319-7706. <https://www.ijcmas.com/vol-3-9/Felix%20M.%20Mujuru,%20et%20al.pdf>
30. Ng'ang'a, J. – Imathiu, S. – Fombong, F. – Ayieko, M. – Broeck, J. V. – Kinyuru, J.: Microbial quality of edible grasshoppers *Ruspolia differens* (Orthoptera: Tettigoniidae): From wild harvesting to fork in the Kagera Region, Tanzania. Journal of Food Safety, *39*, 2019, e12549. DOI: 10.1111/jfs.12549.
31. Eilenberg, J. – Vlak, J. M. – Nielsen-LeRoux, C. – Cappellozza, S. – Jensen, A. B.: Diseases in insects produced for food and feed. Journal of Insects as Food and Feed, *1*, 2015, pp. 87–102. DOI: 10.3920/JIFF2014.0022.
32. Kazemnia, A. – Ahmadi, M. – Dilmaghani, M.: Antibiotic resistance pattern of different *Escherichia coli* phylogenetic groups isolated from human urinary tract infection and avian colibacillosis. Iranian Biomedical Journal, *18*, 2014, pp. 219–224. DOI: 10.6091/ibj.1394.2014.
33. Kibret, M. – Abera, B.: Antimicrobial susceptibility patterns of *E. coli* from clinical sources in northeast Ethiopia. African Health Sciences, *11*, 2011, pp. 40–45. DOI: 10.4314/ahs.v11i3.70069.
34. Olorunmola, F. O. – Kolawole, D. O. – Lamikanra, A.: Antibiotic resistance and virulence properties in *Escherichia coli* strains from cases of urinary tract infections. African Journal of Infectious Diseases, *7*, 2013, pp. 1–7. DOI: 10.4314/ajid.v7i1.1.
35. Abamecha, A. – Wondafrash, B. – Abdissa, A.: Antimicrobial resistance profile of *Enterococcus* species isolated from intestinal tracts of hospitalized patients in Jimma, Ethiopia. BMC Research Notes, *8*, 2015, article 213. DOI: 10.1186/s13104-015-1200-2.
36. Fiedler, G. – Schneider, C. – Igbinosa, E. O. – Kabisch, J. – Brinks, E. – Becker, B. – Stoll, D. A. – Cho, G. S. – Huch, M. – Franz, C. M. A. P.: Antibiotics resistance and toxin profiles of *Bacillus cereus*-group isolates from fresh vegetables from German retail markets. BMC Microbiology, *19*, 2019, article 250. DOI: 10.1186/s12866-019-1632-2.

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