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journal homepage: www.elsevier.com/locate/ifsetComparison of volumetric and surface decontamination techniques for innovative processing of mealworm larvae (*Tenebrio molitor*)Birgit A. Rumpold^a, Antje Fröhling^a, Kai Reineke^a, Dietrich Knorr^b, Stefan Boguslawski^b, Jörg Ehlbeck^c, Oliver Schlüter^{a,*}^a Leibniz Institute for Agricultural Engineering Potsdam-Bornim e.V., Research Program Quality and Safety of Food and Feed, Max-Eyth-Allee 100, D-14469 Potsdam, Germany^b Berlin University of Technology, Department of Food Biotechnology and Food Process Engineering, Königin-Luise-Str. 22, D-14195 Berlin, Germany^c Leibniz Institute for Plasma Science and Technology e.V., Felix-Hausdorff-Str. 2, D-17489 Greifswald, Germany

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ABSTRACT

For food and feed safety of edible insects, effective decontamination methods need to be evaluated and developed. Traditional decontamination and preparation methods were reviewed and thermal and innovative inactivation methods for the decontamination of mealworm larvae were evaluated and compared. The impact of the surface decontamination techniques direct and indirect plasma treatment, and of volumetric methods such as high hydrostatic pressure treatment (400, 500, and 600 MPa) and thermal treatments (45 °C and 90 °C) for up to 15 min on the surface microbial load and on the overall microbial count of mealworm larvae (*Tenebrio molitor*) have been investigated. It was found that the indirect plasma treatment was an effective means for the surface decontamination of mealworm larvae, whereas high hydrostatic pressure at 600 MPa and thermal treatments in a water bath at 90 °C in comparison resulted in the highest reduction of the overall count. It is thus concluded that volumetric methods are favorable for the inactivation of the gut microbiota of insects.

Industrial relevance: Edible insects represent a valuable alternative protein source that could contribute to food and feed security and are industrially mainly unexploited. For a successful marketing of edible insects food and feed safety has to be ensured and effective decontamination methods need to be developed.

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1. Introduction

Edible insects are a traditional food in many parts of the world and eaten by approx. 2 billion people worldwide (van Huis et al., 2013). Since they contain on average 35–61% protein based on dry matter (Rumpold & Schlüter, 2013a), they represent a potential alternative protein source for food and feed. In addition, they largely meet amino acid requirements for humans and have high contents in monounsaturated and polyunsaturated fatty acids and in several micronutrients (Rumpold & Schlüter, 2013a). Therefore, edible insects also represent a valuable food and feed source in general and can contribute to food and feed security especially regarding the increasing world population and increasing demand in animal protein (van Huis, 2013).

For the economic and safe mass production and marketing of edible insects on an industrial scale excessive research is required regarding cost-effective rearing methods and post-harvest processing technologies. Moreover, effective decontamination and storage procedures have to be developed and food and feed safety have to be ensured

(Rumpold & Schlüter, 2013b). Insects are well-known to carry and spread pathogenic and non-pathogenic micro-organisms and have the potential to cause food spoilage or function as vectors transmitting zoonoses. Klunder, Wolkers-Rooijackers, Korpela, and Nout (2012) investigated the microbiological aspects of processing and storage on exemplary edible insects and came to the conclusion that they need to be processed and stored properly. Furthermore, spore-forming bacteria were identified as a potential risk of edible insects that – unlike Enterobacteriaceae – could not be eliminated by boiling.

Consequently it is necessary to develop and employ effective decontamination procedures for edible insects in order to ensure food and feed safety. Numerous methods for the traditional preservation and preparation of edible insects mostly collected in the wild have been published and an overview of traditional ways to preserve and prepare edible insects as stated in literature is given in Table 1. It becomes clear that there is no consistency or rule in how insects are generally prepared, what insect order is preferred as food or at what stage insects are consumed. Lepidopterans are usually consumed in their larval stage (caterpillars), whereas Orthopterans are generally consumed as adults. Preparation methods include post-harvest processes such as removal of head, legs, wings and other appendices and preservation and preparation techniques such as steaming, boiling, baking, deep-frying,

Abbreviations: CPS, casein–peptone–solution.

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Table 1
Traditionally practiced preparation and preservation methods of edible insects.

	Stages consumed	Preparation and preservation methods	Country of origin; wild or reared
Blattodea			
Stinking blattid cockroaches ¹		Removal of elytra, fried and mixed with a porridge of vegetables or fruits	Cameroon; wild
Coleoptera			
<i>Dytiscidae</i> (true water beetle) ²	Adult	Soaked in salty water and dried. Legs and wings removed	China; wild
<i>Allomyrina dichotoma</i> ³	Adult	Appendages discarded, boiled, steamed or roasted	India; wild
<i>Anomala</i> sp. ³	Adult	Roasted or boiled	India; wild
<i>Aristobia</i> sp. ³	Adult	Dewinged, smoked, roasted or boiled	India; wild
<i>Batocera lineolata</i> ⁴	Larvae	Roasted over a flame or in a frying pan	Japan; wild
<i>Batocera roylei</i> ³	Larvae, adult	Dewinged, smoked, roasted or boiled	India; wild
<i>Catharsius</i> sp. ³	Adult	Body cover discarded, wet paste made	India; wild
<i>Chalcosoma atlas</i> L. ⁵	Larvae	Fried	Java, Indonesia; wild
<i>Copris nevinsoni</i> ⁶	Larvae, adult	Deheaded and dewinged, grilled, boiled or streamed	Thailand; wild
<i>Copris nevinsoni</i> Waterhouse (dung beetle) ⁷		Fried	Thailand; wild
<i>Cotilis</i> sp. ⁸		Skewered on a stick and roasted	Papua; Indonesia; wild
<i>Cybister limbatus</i> Fabricius (true water beetle) ⁷		Roasted, lightly curried, fried	Thailand; wild
<i>Dorcus</i> sp. ³	Larvae, adult	Antennae and appendages removed from adults, roasted, boiled or as paste	India; wild
<i>Helicopris bucephalus</i> ⁶	Larvae, adult	Deheaded and dewinged, grilled, boiled or streamed	Thailand; wild
<i>Holotrichia</i> sp. (June beetle) ⁷		Roasted, fried, steamed, lightly curried with vegetables, dipping	Thailand; wild
<i>Hydrous cavistanum</i> Bedel (water scavenger beetle) ⁷		Lightly curried, fried	Thailand; wild
<i>Lepidiota</i> sp. ³	Adult	Boiled or smoked	India; wild
<i>Leucopelaea albescens</i> ⁹	Adult	Fried in pork fat with a local variety of onion, salted and served with 'mote', a soft maize.	Ecuador; wild
<i>Leucopolis irrorata</i> Chevrolat (June beetle) ¹⁰	Larvae	Grilled over charcoal, deep-fried, spicy adobo and sautéed with vegetables	Philippines
<i>Liatongus rhadamitus</i> ⁶	Larvae, adult	Deheaded and dewinged, grilled, boiled or streamed	Thailand; wild
<i>Metamasius</i> spp. ⁹	Larvae, adult	Raw, boiled with salt, cooked with onions	Ecuador; wild
<i>Monochamus versteegi</i> ³	Adult	Dewinged, smoked, roasted or boiled	India; wild
<i>Odontolabis gazella</i> ³	Larvae, adult	Appendages and antennae discarded from adults, larvae fried in oil, then boiled with vegetables, adults are roasted	India; wild
<i>Odontotaenius</i> sp. ³	Larvae, adult	Dewinged, roasted, smoked, boiled or fried	India; wild
<i>Onitis</i> spp. ⁶	Larvae, adult	Deheaded and dewinged, grilled, boiled or streamed	Thailand; wild
<i>Onthophagus mouhoti</i> ⁶	Larvae, adult	Deheaded and dewinged, grilled, boiled or streamed	Thailand; wild
<i>Onthophagus seniculus</i> ⁶	Larvae, adult	Deheaded and dewinged, grilled, boiled or streamed	Thailand; wild
<i>Oplatocera</i> sp. ³	Adult	Wings and appendages discarded, smoked, roasted or boiled	India; wild
<i>Oryctes monocerus</i> ¹¹	Larvae	Thoroughly washed and fried	Nigeria; wild
<i>Oryctes rhinoceros</i> ¹²	Larvae	Raw, boiled, fried or roasted, in stews and soups	Nigeria; wild
<i>Oryctes rhinoceros</i> Linnaeus ¹³	Larvae	Raw, boiled, smoked or fried	Nigeria; wild
<i>Polyphylla</i> sp. ³	Larvae, adult	Antennae and appendages are discarded, roasted	India; wild
<i>Psolidognathus cacticus</i> (long-horned beetle) ⁹	Larvae	Fried	Ecuador; wild
<i>Propomacrus</i> sp. ³	Adult	Dewinged, smoked, roasted or boiled	India; wild
<i>Prosopocoilus</i> sp. ³	Larvae, (adult)	Antennae and appendages are discarded, adults are roasted	India; wild
<i>Protocerus</i> sp. ¹⁴	Larvae, adult	Grubs stir-fried, boiled or cooked with rice, adults roasted	Borneo; wild
<i>Rhynchophorus bilineatus</i> ⁸	Larvae	Raw or roasted	Papua; Indonesia; wild
<i>Rhynchophorus ferrugineus</i> ⁸	Larvae	Raw or roasted	Papua; Indonesia; wild
<i>Rhynchophorus ferrugineus</i> ¹⁴	Larvae	Cleaned, sometimes degutted, raw or made into porridge with ginger or stir-fried with soy sauce and shallots or skewered and toasted	Borneo; wild
<i>Rhynchophorus ferrugineus</i> F. ⁵	Larvae	Fried	Java, Indonesia; wild
<i>Rhynchophorus ferrugineus papuanus</i> ¹⁵	Larvae	Raw, grilled, boiled or roasted over a fire, or incorporated in pancakes	Papua New Guinea; wild
<i>Rhynchophorus palmarum</i> L. ¹⁶	Larvae	Stewed, fried in oil with salt and pepper, as paste or grilled over coals	Africa; wild
<i>Rhynchophorus phoenicis</i> ¹⁷	Larvae	Fried	Nigeria; wild
<i>Rhynchophorus phoenicis</i> ¹²	Larvae	Raw, boiled, fried or roasted, in stews and soups	Nigeria; wild
<i>Rhynchophorus phoenicis</i> ¹	Larvae	Grilled or fried in ashes	Africa; wild
<i>Stenodontes damicornis</i> ¹⁸	Larvae	Broiled over charcoal fire	West Indies; wild
<i>Sternocera</i> sp. ³	Adult	Boiled or smoked	India; wild
<i>Sternicera orissa</i> ¹	Adult	Roasted in hot ash and sand, hind wings and sometimes head removed; pounded and mixed with wild fruits and plants to form a paste	Central Kalahari (Sub-Sahara Africa); wild
<i>Tenebrio molitor</i> (mealworm) ¹⁹	Larvae	Starved for 24 h, frozen, boiled for 6.5 min or baked at 200 °C for 7 min and spiced with vanilla or paprika or dunked in chocolate	Belgium; reared
<i>Tricentotoma</i> sp. ³	Adult	Dewinged, smoked, roasted or boiled	India; wild
<i>Xylorhiza</i> sp. ³	Larvae	Boiled or fried	India; wild
<i>Xylotrupes gideon</i> ³	Adult	Roasted, boiled	India; wild
Diptera			
<i>Muscidae</i> ²	Larvae	Cleaned and made into 'Ba Zhen Cake' with rice powder	China; wild
<i>Chaoborus edulis</i> (aquatic fly) ¹	Adult	Ground and sun-dried, made to cake	Uganda; wild
<i>Ephydra hians</i> ²⁰	Larvae	Cooked with olive oil and garlic	Mexico; wild
<i>Hydropyus hians</i> ²¹	Pupae	Fried in its own grease or sun-dried and then mixed with e.g. berries, acorns, and grass-seeds into a conglomerate	California; wild
Ephemeroptera			
Larvae of <i>Ephemeridae</i> ²	Larvae	Boiled	China; wild
Mayflies ¹⁴	Adult	Stir-fried	Borneo; wild
Hemiptera			
<i>Agonoscelis pubescens</i> (sorghum bug) ²²	Adult	Fried or oil extracted for cooking and medicinal purposes	Sudan; wild
<i>Alcaerhynchus grandis</i> ³	Adult	Fried or boiled with vegetables	India; wild
<i>Antilochus coqueberti</i> ³	Adult	Fried or boiled with vegetables	India; wild

Table 1 (continued)

	Stages consumed	Preparation and preservation methods	Country of origin; wild or reared
Hemiptera			
<i>Aspongopus nepalensis</i> ³	Adult	Part of abdomen is removed, raw or in chutney	India; wild
<i>Aspongopus</i> sp. ³	Adult	Fried or boiled with vegetables	India; wild
<i>Aspongopus viduatus</i> F. (melon bug) ²²	Adult	Oil extracted by hot water as cooking oil or for medicinal purposes	Sudan; wild
<i>A. versicolor</i> ¹	Adult	Roasted; oil extracted	Sudan; wild
<i>Belostoma</i> ²	Adult	Soaked in salty water and dried. Legs and wings removed	China; wild
<i>Cyclochila virens</i> ³	Adult	Dewinged, roasted or as paste	India; wild
<i>Dalader acuticosta</i> ³	Adult	Fried or raw paste	India; wild
<i>Dundubia</i> spp. ¹⁴		Dewinged, roasted over fire, stir-fried with salt and other seasonings but without oil	Borneo; wild
<i>Euschistus</i> sp. ²³	Adult	Boiled in salt water	Mexico; wild
<i>Euterphosia crowfooti</i> ³	Adult	Dewinged, roasted or as paste	India; wild
<i>Halyomorpha picus</i> ³	Adult	Head and abdomen removed, raw in chutney	India; wild
<i>Hoplophorion monogramma</i> ²⁰	Nymphs, adult	Raw, cooked or fried	Mexico; wild
<i>Lethocerus indicus</i> ³	Adult	Boiled or fried	India; wild
<i>Lethocerus indicus</i> Lep.-serv (giant water bug) ⁷		Roasted	Thailand; wild
<i>Meimuna opalifera</i> Walker ⁷	Adult	Roasted, fried, toasted, mixed with chili paste, chopped and cooked	Thailand; wild
<i>Mictis tenebrosa</i> ³	Adult	Fried or raw paste	India; wild
<i>Mormidea notulata</i> ²³	Adult	Boiled in salt water	Mexico; wild
<i>Nezara viridula</i> ³	Adult	Fried or boiled with vegetables	India; wild
<i>Ochrophora montana</i> (Distant) (cinnamon bug) ²⁴	Adult	Fried in oil, as chutney or extraction of oil by traditional processing	Himalaya region, India; wild
<i>Orientopsaltria</i> spp. ¹⁴		Dewinged, roasted over fire, stir-fried with salt and other seasonings but without oil	Borneo; wild
<i>Pomponia merula</i> ¹⁴	Adult	Stir- or deep-fried	Borneo; wild
<i>Pycna repandae</i> ³	Adult	Dewinged, roasted or as paste	India; wild
<i>Tessaratomia papillosa</i> (longan stink bug) ⁷		Roasted, curried	Thailand; wild
<i>Tessaratomia papulosa</i> (litchi bug) ²	Adult	Head, wings, legs and viscera removed, body salted and wrapped into cabbage leaves. then instantly cooked in hot ash	China; wild
<i>Tessaratomia quadrata</i> ³	Adult	Dewinged, raw or turned into chutney	India; wild
<i>Thasus gigas</i> ²³	Nymphs, immature stages	Legs removed, roasted	Mexico; wild
<i>Tibicen pruinosus</i> ³	Adult	Dewinged, boiled or as paste	India; wild
Hymenoptera			
Wasps (e.g. <i>Vespa</i> , <i>Polistes</i>) ²⁵	Larvae, pupae	Deep-fried, fried with chicken eggs, or steamed with vegetables and vinegar, steamed in a soup	China; wild
Bees ¹	Larvae, pupae	Grilled	Congo; wild
Red ant ²⁶		roasted as snack or with rice	India; wild
Red ant ²⁶	Eggs	Fried with salt, chili, spices and mustard oil	India; wild
Ants ¹	Eggs	Raw or fried	Sub-Saharan Africa; wild
<i>Apis cerana</i> ³	Adult, larvae	Wings and antennae discarded, roasted or in a paste	India; wild
<i>Apis cerana</i> ¹⁴	Larvae, pupae	Raw, boiled with porridge or rice, stir-fried or drunk together with honey	Borneo; wild
<i>Apis cerana</i> F. ¹⁰	Extra brood	Fried, sautéed, sautéed with vegetables	Philippines
<i>Apis dorsata</i> ¹⁴	Larvae, pupae	Raw, boiled with porridge or rice, stir-fried or drunk together with honey	Borneo; wild
<i>Apis dorsata</i> F. ¹⁰	Extra brood	Fried, sautéed, sautéed with vegetables	Philippines
<i>Apis</i> sp. ³	Adult	Dewinged, fried and then made into a paste	India; wild
<i>Atta mexicana</i> ²³	Adults	Deheaded and dewinged, roasted, mixed with salsa	Mexico; wild
<i>Atta mexicana</i> ²⁰		Prepared with garlic and parsley or simply roasted	Mexico; wild
<i>Brachygastra azteca</i> ²⁰	Adult	Cooked with chili and onion	Mexico; wild
<i>Brachygastra mellifica</i> ²³	Larvae	Honey comb containing larvae is toasted, cooked larvae are pulled out	Mexico; wild
<i>Brachygastra</i> sp. ²³	Larvae	Honey comb containing larvae is toasted, cooked larvae are pulled out	Mexico; wild
<i>Camponotus</i> spp. (carpenter ant) ¹⁰	Eggs	Cooked with spices or sautéed in garlic and onions with a small amount of pepper	Philippines
<i>Carebara</i> ssp. ¹	Queen caste	Gasters torn off, eaten raw or fried with salt	Sub-Saharan Africa; wild
<i>Eumenes</i> sp. ³	Larvae, pupae	Larvae are eaten directly, pupae are boiled or made in a paste	India; wild
<i>Liometopum apiculatum</i> ²⁰	Eggs, larvae, pupae	Cooked with butter and pepper or in omelet	Mexico; wild
<i>Mischocyttarus</i> sp. ²³	Larvae	Honey comb containing larvae is toasted, cooked larvae are pulled out	Mexico; wild
<i>Oecophylla smaragdina</i> ³	Adult, larvae	Raw	India; wild
<i>Oecophylla smaragdina</i> ¹⁴	Brood, adult	Adults are mixed with chili and salt as condiment, brood is eaten raw or cooked with porridge or rice	Borneo; wild
<i>Oecophylla smaragdina</i> Fabricius ⁷	Egg, pupae, adult	In salad, lightly curried with vegetables	Thailand; wild
<i>Oecophylla smaragdina</i> Fabricius ⁷	Queen caste	Steamed queen caste with curry paste, Thai spicy salad	Thailand; wild
<i>Parachartegus apicalis</i> ²⁰	Adults with hive	Wasps are eaten complete with hive, including immature stages, roasted or cooked	Mexico; wild
<i>Polistes</i> sp. ³	Adults, (larvae, pupae)	Dewinged, fried or fresh; larvae smoked with bee hive	India; wild
<i>Polybia occidentalis bohemani</i> ²³	Larvae	Honey comb containing larvae is toasted, cooked larvae are pulled out	Mexico; wild
<i>Polybia occidentalis nigratella</i> ²³	Larvae	Honey comb containing larvae is toasted, cooked larvae are pulled out	Mexico; wild
<i>Polyrhachis vicina</i> Roger ²⁷	Adult	Put in water or frozen (–20 °C), then sun-dried	China; reared
<i>Ropalidia</i> spp. ¹⁴	Larvae, pupae	Raw, boiled with porridge or rice, stir-fried or drunk together with honey	Borneo; wild
<i>Vespa orientalis</i> ³	Larvae, (adult)	Smoked with nest, adults dewinged	India; wild
<i>Vespa</i> sp. ³	Adults, (larvae, pupae)	Dewinged, fried or fresh	India; wild
<i>Vespa</i> sp. ²		Parched	China; wild

(continued on next page)

Table 1 (continued)

	Stages consumed	Preparation and preservation methods	Country of origin; wild or reared
Hymenoptera			
<i>Vespa</i> ssp. ¹⁴	Larvae, pupae	Raw, boiled with porridge or rice, stir-fried or drunk together with honey	Borneo; wild
<i>Vespula flaviceps</i> ²⁸	Larvae, pupae	Removal of larvae and pupae from nest, boiled with soy sauce or fried with salt; cooked larvae is mixed with rice	Japan; wild
<i>Vespula lewisi</i> ⁴	Larvae, pupae, sometimes adults	Washed, cooked with soy sauce and sugar, sometimes then mixed with cooked rice (wasp-rice)	Japan; wild
<i>Xylocopa latipes</i> Drury ⁵	Larvae, pupae	Fried with butter or with onion and salt	Java, Indonesia; wild
<i>Xylocopa</i> sp. ³	Adult, larvae	Adults dewinged, boiled	India; wild
Isoptera			
Termites ¹⁴		Dewinged, raw or stir-fried without oil or cooked in porridge or rice	Borneo; wild
Termites ²		Parched	China; wild
Termites ¹	Adult	Killed by boiling or roasting for a few minutes, then sun- or smoke-dried; crushed to a mush and eaten with honey; fried in their own oil; steamed or smoked in banana leaves; roasted in hot ash and sand; pounded into a cake; sometimes only the heads are eaten	Sub-Sahara Africa; wild
<i>Macrotermes bellicosus</i> ²⁹	Adult	Dewinged, roasted and salted or ground into flour	Nigeria; wild
<i>Macrotermes nigeriensis</i> ³⁰		Washed, salted, mildly fried or roasted without oil; also eaten raw	Nigeria; wild
<i>Macrotermes subhyllanus</i> ³¹		Dewinged, toasted in their own oil	Kenya; wild
<i>Termes</i> sp. ⁷		Roasted (with salt), fried	Thailand; wild
Lepidoptera			
<i>Lepidoptera</i> ³²	Larvae	Roasted with salt, fried with oil or lard and eaten with salt and pepper in tortillas, boiled and roasted in a “pan”, fried with salt, boiled split into longitudinal axis and mixed with oil, Boiled, drained and stuffed with fresh cheese, cooked with eggs like an omelet, mixed with rice (paella), preserved in brine, or pickled	Mexico; wild
Caterpillars (e.g. <i>Anaphe</i> , <i>Imbrasia</i>) ³³		Purged, washed and usually cooked, preserved by sun-drying or smoking; smoked caterpillars have a shelf life of 3 m	Central Africa; wild
<i>Aegiale hesperiaris</i> ²³	Larvae	Boiled or fried	Mexico; wild
<i>Anaphe infracta</i> ³⁴	Larvae	Roasted, or dry-fried	Nigeria; reared
<i>Anaphe panda</i> ³⁴	Larvae	Roasted, or dry-fried	Congo, Tanzania and Zaire; reared
<i>Anaphe panda</i> ¹	Larvae	Cooked fresh or dried and powdered for storage	Tanzania; wild
<i>Anaphe reticulata</i> ³⁴	Larvae	Roasted, or dry-fried	Nigeria; reared
<i>Anaphe venata</i> ³⁴	Larvae	Roasted, or dry-fried	Nigeria; reared
<i>Arsenura armida</i> ³²	Larvae	Pickled	Mexico; wild
<i>Bombyx mori</i> ³⁴	pupae	Boiled, steamed, baked, fried or roasted	China; reared
<i>Bombyx mori</i> ²	Pupae	Deep-fried in oil, or the dry body stir-fried with chives	China; reared
<i>Bombyx mori</i> ⁴	Pupae, sometimes adults (moths)	Cooked with sugar and soy sauce, canned; or fried and then salted	Japan; wild
<i>Bombyx mori</i> Linnaeus (silkworm) ⁷	Pupae	Fried, steamed, with chili paste, lightly curried with vegetables	Thailand; wild
<i>Cirina forda</i> ³⁵	Larvae	Boiled and dried in the sun	Nigeria; wild
<i>Cirina forda</i> ¹	Larvae	Boiled in water, then fried in karité butter	Mali and Burkina Faso; wild
<i>Comadia redtenbacheri</i> ²³	Larvae	Boiled or fried, eaten whole or ground and mixed with salsa	Mexico; wild
<i>Erionata thrax</i> ¹⁴	Pupae	Raw or boiled until dry	Borneo; wild
<i>Herse convolvuli</i> ¹	Larvae	Intestines squeezed out, roasted in hot ash and sand, sun-dried, then stored in bags and eaten for several months e.g. pounded into powder and mixed with stewed watermelon	central Kalahari (Sub-Sahara Africa); wild
<i>Hyblaea puera</i> Cramer ⁵	Pupae (cocoons)	Fried in oil and seasoned with salt	Java, Indonesia; wild
<i>Latebraria amphipyroides</i> ³²	Larvae	Pickled	Mexico; wild
<i>Lepidoptaria litoralia</i> ³⁶	Larvae	Boiled in water with a pinch of potash-powder for 3 min, strained and sun-dried, then salted and seasoned and roasted in oven for 5 min	Nigeria; wild
<i>Omphisa fuscidentalis</i> (bamboo caterpillar) ⁷	Larvae	Fried, with chili paste, lightly curried with vegetables	Thailand; wild
<i>Paradirphia fumosa</i> ²³	Larvae	Purged in water overnight, boiled with salt, sun dried for 2–3 days	Mexico; wild
<i>Sphinx</i> spp. ²	Larvae	Parched after soaking in salty water and cooked with noodles	China; wild
<i>Odonata</i> ³	Mostly larvae, also adult	Dewinged when adults are consumed, raw with bamboo shoots, seldom boiled or roasted	India; wild
Orthoptera			
<i>Orthoptera</i> ³⁷	Adult, larvae	Fresh, dried, cooked, fried, roasted, or incorporated in a special dish	Mexico; wild
<i>Acrididae</i> ²	Adult	Dried in sun and made into porridge or cake; fried with viscera, head and limbs removed	China; wild
<i>Gryllidae</i> ²	Adult	Chained with steel thread, baked with sauce and sugar	China; wild
Grasshoppers ¹⁴	Adult	Lightly salted, boiled in a little water and then simmered until dry or stir-fried or deep-fried like prawns	Borneo; wild
Grasshoppers ²⁶	Adult	Wings and legs and stomachs removed, washed with water, then roasted or cooked dry with vegetable oil and e.g. chili, salt, onion, ginger, garlic	India; wild
<i>Acheta confirmata</i> Walker (ground cricket) ⁷	Adult	Steamed, curried, fried, roasted	Thailand; wild
<i>Acheta domesticus</i> (L.) (house cricket) ¹⁹		Baked at 200 °C for 15 min or boiled for 8 min	Belgium; reared
<i>Brachytrupes</i> sp. ³	Adult	Antennae and limb appendages discarded, fried, boiled with vegetables or smoked	India; wild
<i>Brachytrupes portentosus</i> L. ⁵	Adult	Intestinal contents removed, cooked	Java, Indonesia; wild
<i>Brachytrupes portentosus</i> Lichtenstein ⁷		Roasted, fried, toasted	Thailand; wild
<i>Chondracris roseapbrunner</i> Uvarov ⁷		Steamed, fried, roasted	Thailand; wild

Table 1 (continued)

	Stages consumed	Preparation and preservation methods	Country of origin; wild or reared
Orthoptera			
<i>Chondacris rosea</i> ³	Adult	Removal of wings, lower abdomen and appendages, boiled, fried, as chutney, or smoked	India; wild
<i>Diabolocanthops innotabilis</i> ³	Adult	Antennae and wings discarded, fried, boiled or smoked	India; wild
<i>Gryllotalpa africana</i> Beauvois (mole cricket) ⁷	Adult	Fried, curried	Thailand; wild
<i>Gryllotalpa longipennis</i> ¹⁴	Adult	Cooked or fried	Borneo; wild
<i>Gryllotalpa</i> sp. ³	Adult	Boiled, roasted, as paste (chutney)	India; wild
<i>Gryllotalpa</i> sp. ¹⁰	Adult	Sautéed in garlic and onions and seasoned with soy sauce, vinegar and hot pepper, sometimes coconut milk or fried	Philippines; wild
<i>Heiroglyphus</i> sp. ³	Adult	Antennae and appendages discarded, fried, boiled with vegetables or as chutney	India; wild
<i>Leptysma</i> sp. ³	Adult	Antennae and anal cirri discarded, boiled, roasted, or as paste	India; wild
<i>Locusta migratoria manilensis</i> Meyen ¹⁰	Adult	Cooked or fried	Philippines; wild
<i>Oxya velox</i> F. ³⁸	Adult	Boiling in soy sauce	Japan; wild
<i>Oxya yezeensis</i> ⁴	Adult	Boiled 3–4 min, dried in the sun 1–2 days, hind legs and wings removed, seasoned with sugar and soy sauce, then heated in a pan until all water evaporates and canned; fried: kept in bag overnight to empty gut, then killed by in boiling water for 3–4 min, sun-dried for 1 day, then put into boiling oil, seasoned with salt; skewered and then roasted	Japan; wild
<i>Patanga succincta</i> L. ⁵	Adult	Wings and legs removed, roasted, seasoned with onion, garlic, chili or soy sauce	Java, Indonesia; wild
<i>Patanga succincta</i> L. ³⁹	Adult	Deep-fried	Thailand; wild
<i>Ruspolia differens</i> ¹	Adult	Antennae, legs and wings are removed, then fried	Uganda; wild
<i>Ruspolia differens</i> (green and brown) ³¹	Adult	Dewinged, toasted in their own oil and/or dried	Kenya; wild
<i>Schistocerca</i> sp. ³	Adult	Wings, lower abdomen, antennae and appendages are discarded, fried or boiled with vegetables	India; wild
<i>Sphenarium purpurascens</i> ²⁰	Larvae, adult	Dried for storage; fried	Mexico; wild
<i>Tarbinskiellus orientalis</i> ³	Adult	Fried or roasted	India; wild
<i>Valanga nigricornis</i> Burmeister ⁵	Adult	Wings and legs removed, roasted, seasoned with onion, garlic, chili or soy sauce	Java, Indonesia; wild
Trichoptera (Caddisflies)			
<i>Hydropsycheodes brevilineata</i> ⁴	Larvae	Cooked with sugar and soy sauce, canned	Japan; wild
<i>Parastenopsyche sauteri</i> ⁴	Larvae	Cooked with sugar and soy sauce, canned	Japan; wild
<i>Stenopsyche griseipennis</i> ⁴	Larvae	Cooked with sugar and soy sauce, canned	Japan; wild

1 – (van Huis, 2003), 2 – (Luo, 1997), 3 – (Chakravorty, Ghosh, & Meyer-Rochow, 2011), 4 – (Mitsuhashi, 1997), 5 – (Lukiwati, 2010), 6 – (Bophimai & Siri, 2010), 7 – (Sirithon & Pornpisanu, 2008, Chap. 16), 8 – (Ramandey & van Mastrigt, 2010), 9 – (Onore, 1997), 10 – (Adalla & Cervancia, 2010), 11 – (Banjo, Lawal, & Adeyemi, 2006), 12 – (Onyeike, Ayalogu, & Okaraonye, 2005), 13 – (Olowu et al., 2012), 14 – (Chung, 2010), 15 – (Mercer, 1997), 16 – (Due, Zabri, Kouadio, & Kouame, 2009), 17 – (Elemo, Elemo, Makinde, & Erukainure, 2011), 18 – (G. DeFoliart, 1999), 19 – (Megido et al., 2014), 20 – (Deguevara, Padilla, Garcia, Pino, & Ramos-Elorduy, 1995), 21 – (G. Defoliart, 1994), 22 – (Mariod, Abdel-Wahab, & Ain, 2011), 23 – (Acuna, Caso, Aliphath, & Vergara, 2011), 24 – (Thakur & Firake, 2012), 25 – (Ying & Long, 2010), 26 – (Srivastava, Babu, & Pandey, 2009), 27 – (Bhulaidok, Sihamala, Shen, & Li, 2010), 28 – (Nonaka, 2010), 29 – (Ekpo & Onigbinde, 2007), 30 – (Igwe, Ujowondo, Nwaogu, & Okwu, 2011), 31 – (Kinyuru, Kenji, Njoroge, & Ayieko, 2010), 32 – (Ramos-Elorduy et al., 2011), 33 – (Johnson, 2010), 34 – (G. R. DeFoliart, 1995), 35 – (Osasona & Olaofe, 2010), 36 – (Solomon & Prisca, 2012), 37 – (Ramos-Elorduy Blasquez, Pino Moreno, & Martinez Camacho, 2012), 38 – (G. DeFoliart, 1992), 39 – (Hanboonsong, 2010).

sun-drying, smoking and processing into a paste or chutney. Some insects are also eaten raw.

For an acceptance study of Belgian consumers, reared mealworms (*Tenebrio molitor*) were starved for 24 h in order to empty their guts prior to inactivation by freezing. The inactivated samples were baked at 200 °C for 7 min or boiled for 6.5 min. Baked mealworms were also spiced with vanilla or paprika or dunked in chocolate (Megido et al., 2014).

In addition to traditional thermal decontamination treatments such as boiling or baking non-thermal treatments such as cold plasma and high hydrostatic pressure treatment should be taken into consideration.

High hydrostatic pressure processing is a well-known volumetric treatment for the effective inactivation of vegetative microorganisms to extend the shelf life of food products (Knorr et al., 2011). Furthermore, it can be used for spore inactivation depending on the treatment time and temperature (Reineke et al., 2012, 2013). Significant spore inactivation is possible at moderate treatment pressures and temperatures for long pressure dwell times. However, for a full spore inactivation in an economical treatment time, high pressures and elevated temperatures are needed (Reineke et al., 2012). This high pressure thermal sterilization (HPTS) process is an emerging technology to produce high quality low acid food products, which are shelf-stable at ambient temperature (Reineke et al., 2013).

High hydrostatic pressure has up to today not been explored for the decontamination of edible insects. A high hydrostatic pressure treatment (220–250 MPa, 50 °C, 10 min) showed to be effective for the

preservation and shelf-life extension of shrimps and clams (Buyukcan, Bozoglu, & Alpas, 2009) belonging to the arthropods just like insects. However, the beneficial effects of the treatment cannot be entirely attributed to the pressure treatment which was comparably low with up to 250 MPa but appear to be temperature-enhanced as well as improved by blending the sample with water.

In addition to high hydrostatic pressure, a general antimicrobial effect of non-thermal plasma was observed (Brandenburg et al., 2009) and the potential of atmospheric pressure non-thermal plasmas for the rapid deactivation of bacterial spores on surfaces has been demonstrated (Birmingham, 2004). It was suggested that oxygen radicals generated by plasma have a sterilizing effect by destroying bacterial cells and endospores (Hong et al., 2009). The potential of cold plasma for the decontamination of food has also been shown. Reduction rates between 0.5 and 5.2 log were achieved by plasma treatment of seeds of *Brassica napus* contaminated with endospores of *Bacillus atrophaeus* (Schnabel et al., 2011). Furthermore, after a cold indirect plasma treatment the aerobic viable count of pork meat remained between 10² and 10³ CFU/g during a storage period of 20 days at 5 °C and showed the applicability of plasma as a gentle surface decontamination method and its potential to prolong the shelf life of food and feed products (Fröhling, Baier, Ehlbeck, Knorr, & Schlüter, 2012; Fröhling, Durek et al., 2012).

The impact of plasma treatment on the microbial inactivation of edible insects has not been investigated or published until today but an insecticidal activity of helium atmospheric pressure plasma

discharge as means of insect control has been shown (Donohue, Bures, Bourham, & Roe, 2008). It was discovered that the insect mortality increased with increasing temperature of plasma and depended on the species as well as the gas composition, such as the oxygen content. This suggests that a surface deactivation and spore inactivation of live insects could possibly be achieved by plasma treatment. Consequently plasma might have a potential as a hygienic surface treatment for rearing and processing of edible insects.

Aim of this study was the assessment of the impact of cold plasma, high hydrostatic pressure and thermal treatments on the microbial surface and overall activity of mealworm larvae with the objective of developing decontamination procedures for edible insects using the example of mealworm larvae and eventually ensuring food and feed security of insect products.

2. Material and methods

2.1. Mealworm larvae

Live mealworm larvae (*T. molitor*) were purchased at Futtertier-Shop.de (Molchow, Germany), packed in sterile bags, and frozen at -18°C in laboratory condition until further use. They were thawed to room temperature before treatments, respectively.

2.2. Thermal treatments

For wet thermal treatments, the mealworm larvae were vacuum-sealed in polyethylene/polyamide-bags and placed in a GFL 1083 water bath (Victor Recker Krankenhausbedarf-Laborbedarf GmbH, Berlin, Germany) at 45°C for 0, 5, 10, and 15 min and at 90°C for 0, 2.5, 5, 10, and 15 min. The heating time needed to reach 45°C (30 s) and 90°C (45 s), respectively, in the inner core of the mealworms was determined via a thermocouple Typ T and the software Personal DaqView Plus and added to the treatment times.

For dry thermal treatments, the mealworm larvae were placed in a drying oven (Heraeus Function Line, Wärme- und Trockenschrank, Thermo Fisher Scientific Inc., USA) at 90°C for 0, 2.5, 5, 10, and 15 min. Analogous to the cooking treatments, 45 s were added to each treatment time.

2.3. High hydrostatic pressurization

High hydrostatic pressure treatments were conducted by the high pressure single vessel apparatus U400 (Unipress Equipment, Warsaw, Poland) with a maximum operating pressure of 1000 MPa, a volume of approx. 0.75 l and a theoretically operable temperature range of -25 to 100°C . This unit is composed of a biphasic pressure build-up with the initial pump for pressures up to 600 MPa and the intensifier pump for the pressure build-up in the second phase from 600 to 1000 MPa in combination with a pressure intensifier (transformation ratio 1:16). Pressure build-up took about 15 s from 0.1 to 400 MPa, 15 s from 0.1 to 500 MPa and 25 s from 0.1 to 600 MPa. Pressure release occurred within about 2 s. The mealworm larvae were vacuum sealed in polyethylene/polyamide-bags and additionally vacuum sealed in polyethylene coated aluminum bags before pressurization at room temperature.

2.4. Indirect plasma treatment

A microwave plasma setup with microwave frequencies of 2.45 GHz and with a power consumption in the range of 1.2 kW was used for indirect plasma treatment. The process gas air had a gas flow of 20 standard liters per min (slm), a gas temperature of approximately 4000 K and relative humidity of 20% (measured with Testo 6651, Testo AG, Lenzkirch, Germany). The torch was connected with the reaction chamber via a metal tube. The discharge was ignited for 5 s

and the reactive gas (plasma processed air) was introduced into the reaction chamber. The gas temperature in the reaction chamber amounted to room temperature and treatment duration was 0, 2.5, 5, 10, and 15 min, respectively.

2.5. Direct plasma treatment

Direct plasma treatment of approximately 1 g of mealworms, respectively, was applied by a rf-driven atmospheric pressure plasma jet (27.12 MHz) with a power consumption in the range of 20 W, a gas composition of argon + 0.10% oxygen and a distance to the sample of 24 mm for 0, 1, 2.5, and 5 min while shaking at 300 rpm on a shaker (Infors HT Labotron, Switzerland). The jet is described in more detail elsewhere (Fröhling, Baier, Ehlbeck, Knorr, & Schlüter, 2012). The maximum temperature of the samples was determined by a thermography camera (SC500, Flir Systems GmbH, Frankfurt a.M., Germany) to be 45°C . The emission spectrum of the plasma is shown in Fig. 1.

2.6. Microbiological analyses

For the determination of the surface contamination, 1 g of mealworm larvae and 9 g of 0.1% casein-peptone-solution (CPS) were shaken for 4 min at 400 rpm in sterilized Erlenmeyer flasks on a shaker (Infors HT Labotron, Switzerland). Subsequently, the shaken solution was serially diluted with CPS in Rotilabo®-microtest plates (96er U-profile, Roth, Germany), and 50 μl of each dilution was spread on plate count agar and incubated at 30°C for 72 h to determine the number of colony forming units per g (CFU/g).

For the determination of the overall microbial count, 3 g of mealworm larvae and 27 g of 0.1% CPS were mixed in a sterile filter stomacher bag and homogenized (Bag Mixer Interscience, St. Nome, France) at speed 8 for 2 min. The homogenate was then serially diluted with CPS in Rotilabo®-microtest plates (96er U-profile, Roth, Germany), and 50 μl of each dilution was spread on plate count agar and incubated at 30°C for 72 h to determine the number of colony forming units per g (CFU/g).

The detection limit of plate count analyses was 200 CFU/g. All analyses were carried out in triplicates.

2.7. Modeling of inactivation kinetics

The inactivation kinetics obtained were modeled with GInaFit (Geeraerd and Van Impe Inactivation Model Fitting Tool) version 1.6, a freeware Add-in for Microsoft® Excel. This tool enables the testing of nine different types of microbial survival models and five statistical measures support the choice of the best fit (Geeraerd, Valdramidis, & Van Impe, 2005). The root mean square error (RMSE) value was chosen

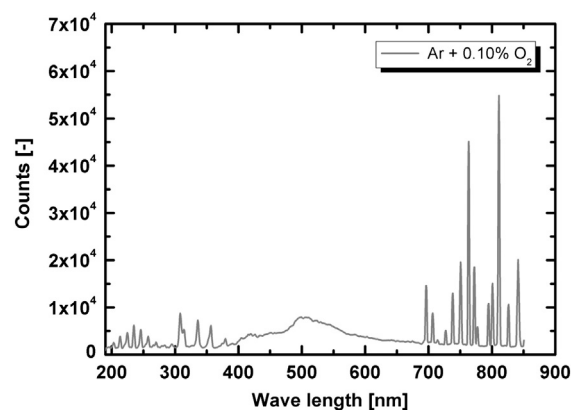


Fig. 1. Emission spectrum of Argon (Ar) + 0.10% oxygen (O_2)-based atmospheric pressure plasma discharge at 20 W (direct plasma treatment).

as indicator for the goodness-of-fit of the tested models according to Ratkowsky (2004).

3. Results and discussion

It was observed that the overall microbial load of the mealworm larvae was generally higher than the surface contamination. This is believed to be due to the internal gut microbiota.

The impact of direct and indirect plasma, high hydrostatic pressure at 400, 500, and 600 MPa and dry and wet thermal treatments at 90 °C of up to 15 min on the surface and overall microbial count of mealworm larvae was investigated. Since a maximum temperature of 45 °C was observed for the direct plasma treatment, a treatment of mealworm larvae in a water bath at 45 °C was also examined in order to rule out thermal effects during the direct plasma treatment.

In general it could be observed that there was an optimum of surface inactivation after 10 min treatment time for all treatments except for the thermal treatment in a drying oven (Fig. 2). In most cases an extension of the treatment time to 15 min even resulted in a reduced surface inactivation in comparison to a 10 min treatment. Thorough inactivation kinetics are required and longer treatment times should be applied to find an explanation for this observation.

Regarding the microbial surface activity of mealworm larvae, treatments with direct plasma, high hydrostatic pressure at 400 and 500 MPa, in a water bath at 45 °C and in a drying oven at 90 °C had little effect on the surface contamination and maximum inactivation of one log cycle was achieved. Concerning the direct plasma treatment this leads to the conclusion that the potentially anti-microbial components emitted by the plasma jet as shown in the emission spectrum in Fig. 1 had little detectable inactivating effect on the surface microflora of mealworm larvae. These components include nitrogen oxides ($\lambda = 200\text{--}300\text{ nm}$), hydroxyl radicals ($\lambda = 280\text{--}310\text{ nm}$), oxygen ($\lambda = 777\text{ nm}$) and the predominant argon ($\lambda = 410\text{--}470\text{ nm}$ and $\lambda = 650\text{--}990\text{ nm}$). In addition shadow effects and overlapping of sample could be the reason for the low effect of direct plasma on the surface decontamination.

A high hydrostatic pressure treatment at 600 MPa for 10 min resulted in an inactivation of 3 log cycles and a treatment in a water bath at 90 °C for 10 min resulted in an inactivation of 4 log cycles. And the indirect plasma treatment resulted in the best surface inactivation of mealworm larvae of all five treatments compared. After 10 min, an inactivation to the detection limit was observed (reduction of 7 log cycles). However, after a 15 min treatment a reduced inactivation of 4 log cycles was found. Consequently, indirect plasma represents a high potential non-thermal treatment for surface sterilization of edible insects. Using FT-IR measurements, emissions of 0.6% NO, 1.8% NO₂, 0.07% CO₂, 0.04% HNO₃, 0.08% HNO₂, and 0.03% H₂O were detected in the indirect plasma (Fröhling, Durek, et al., 2012). Comparing with

the emission spectrum of the direct plasma treatment, especially the presence of nitrogen oxides seems to enhance the inactivation of the surface microflora of mealworm larvae.

The use of non-thermal plasma for insect control resulting in increased insect mortality with increasing temperature of plasma and depending on the species as well as the gas composition (Donohue et al., 2008) suggests that an inactivation of surface microorganisms of live insects could possibly be achieved. Consequently more research is required and more data are needed to assess the potential of indirect cold plasma as a hygienic surface treatment for rearing and processing of edible insects.

The overall microbial count of mealworm larvae after direct and indirect plasma, high hydrostatic pressure at 400, 500, and 600 MPa and dry and wet thermal treatments at 90 °C and different treatment times is shown in Fig. 3. Both the indirect and direct plasma treatment had no noticeable effect on the overall microbial count of the mealworm larvae. This was expected and due to the limited depth of penetration of plasma. Treatment in a water bath at 45 °C for up to 15 min also had no noticeable effect on the overall microbial count.

Applying high hydrostatic pressure treatments, the inactivation of the overall microbial count approximately increased with increasing pressure. A high hydrostatic pressure treatment at 400 MPa for 10 and 15 min resulted in a deactivation of one log cycle of the overall microbial count of mealworm larvae, respectively, at 500 MPa for 10 and 15 min resulted in a reduction of two log cycles, respectively. A high hydrostatic pressure treatment at 600 MPa for 10 min resulted in an inactivation of three log cycles, for 15 min only in a reduction of one log cycle. The low effect of high hydrostatic pressure, taking effect across the entire volume of samples, on the overall microbial count of mealworm larvae was unexpected and might be due to the low availability of water since the mealworms have been pressure treated in vacuum sealed bags without added water. It is also possible that the parameters chosen (up to 600 MPa, room temperature, up to 15 min treatment time) were not sufficient for the inactivation especially of spore forming bacteria and higher pressures, higher temperatures and/or longer treatment times are necessary. More data are necessary for a profound statement of the potential of high hydrostatic pressure for the decontamination of edible insects.

The best inactivation of the overall microbial count was achieved by thermal treatments at 90 °C. The treatment in a drying oven reduced the overall microbial count after 10 or 15 min by two log cycles, respectively. And the treatment in a water bath for 10 or 15 min resulted in a reduction of three log cycles, respectively. This reduction was already observed after a short treatment time of 2.5 min but the results obtained cannot confirm that the preparation procedure performed in the context of acceptance studies of Belgian consumers where mealworms were boiled for 6.5 min (Megido et al., 2014) were sufficient for a safe consumption.

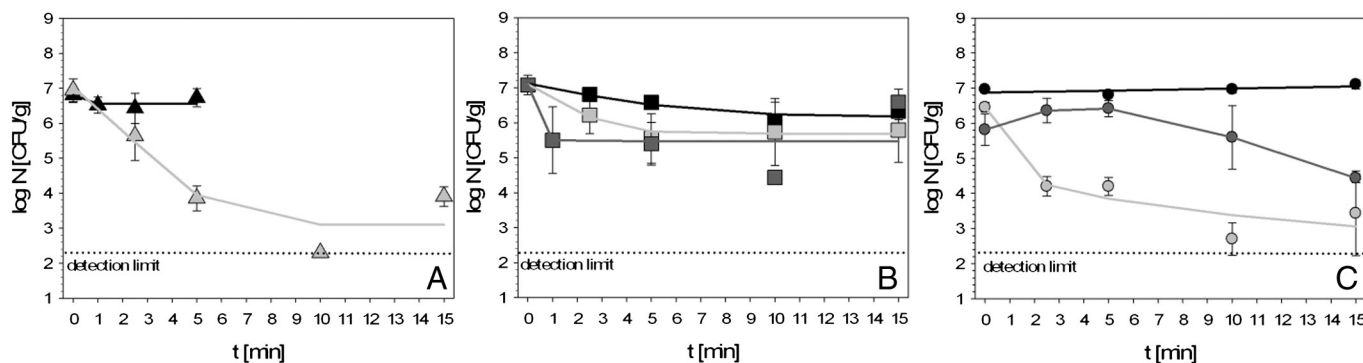


Fig. 2. Microbial surface activity of mealworm larvae after (A) direct (black triangle) and indirect (light gray triangle) cold plasma treatment, (B) high hydrostatic pressure treatment at 400 MPa (black squares), 500 MPa (light gray squares), and 600 MPa (dark gray squares), respectively, and (C) thermal treatments in a water bath at 45 °C (black circles) and 90 °C (light gray circles) and in a drying oven at 90 °C (dark gray circles) for up to 15 min. The lines represent the inactivation obtained from the applied models from the GlnaFit tool.

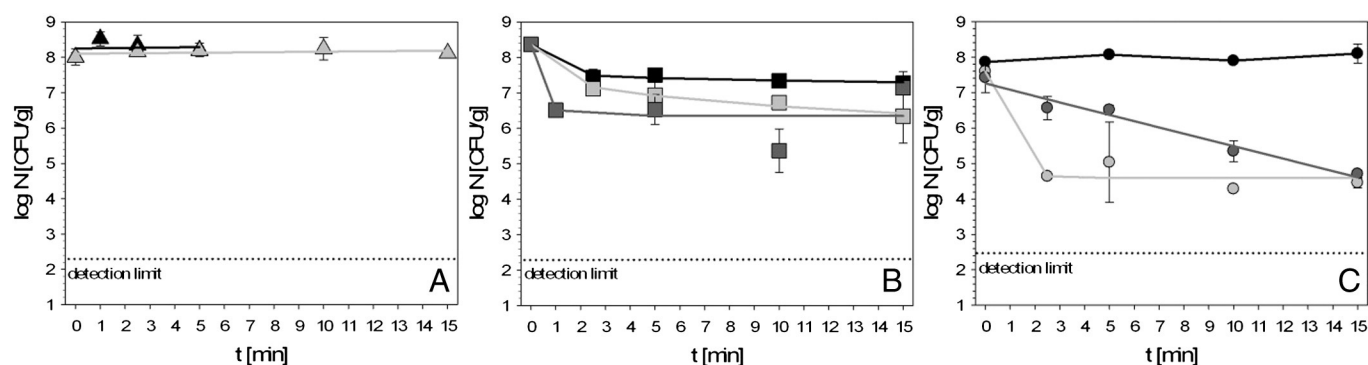


Fig. 3. Overall microbial activity of mealworm larvae after (A) direct (black triangle) and indirect (light gray triangle) cold plasma treatment, (B) high hydrostatic pressure treatment at 400 MPa (black squares), 500 MPa (light gray squares), and 600 MPa (dark gray squares), respectively, and (C) thermal treatments in a water bath at 45 °C (black circles) and 90 °C (light gray circles) and in a drying oven at 90 °C (dark gray circles) for up to 15 min. The lines represent the inactivation obtained from the applied models from the GlnaFit tool.

Since a treatment in the drying oven at 90 °C had little impact on the microbial surface activity of mealworm larvae but was effective for the decrease in total microbial count in contrast to indirect plasma treatment being very effective on the surface activity but not on the overall count, it is suggested to apply indirect plasma and oven drying at 90 °C for 5 min. Another approach for the improvement of the decontamination is a consecutive treatment of indirect plasma and treatment in a water bath or drying oven at 90 °C, respectively.

Comparing the inactivation kinetics modeled with GlnaFit (Geeraerd and Van Impe Inactivation Model Fitting Tool) version 1.6 (Tables 2 and 3) it can generally be said that all models show a good fit as indicated by the low root mean square errors (RMSE). All effective decontamination treatments fit the “log-linear model” (total decontamination in a drying oven at 90 °C), “log-linear and tailing-model” (surface decontamination with indirect plasma or high hydrostatic pressure at 600 MPa; total decontamination in a water bath at 90 °C) or “Weibull-model” (surface decontamination in a water bath at 90 °C).

The “log-linear model” is the traditional and most basic model for the description of microbial inactivation and assumes that the inactivation rate is proportional to the number of organisms (Richardson, 2004). It showed the best fit for the total decontamination of mealworm larvae in a drying oven at 90 °C.

Surface decontamination in a water bath at 90 °C was best described by the “Weibull model” with the scale parameter $\delta = 0.09$ min representing the time needed for the first log cycle reduction and the shape parameter $p < 1$ (concave curve) indicating the presence of (at least) two (sub)populations with different heat sensitivity to the treatment. The more sensitive population is inactivated at higher rates, the more resistant population remains unaffected or shows only minor inactivation (Pele, 2000). The difference in inactivation behavior between a heat treatment at 90 °C in a water bath and in a drying

oven can be explained by e.g. evaporative cooling effects and water reduction in the drying oven in comparison to a sealed environment in a bag in the water bath.

Surface decontamination with indirect plasma or high hydrostatic pressure at 600 MPa and total decontamination in a water bath at 90 °C was best described by the “log linear and tailing model”. The mechanistic theory of tailing comprises that a subpopulation is very resistant to the treatment, inaccessible, adapted, or genetically more resistant. The resistance possibly results from experimental artifacts such as heterogeneity of the treatment (Cerf & Metro, 1977). It is also possible that spore forming bacteria are responsible for the tailing effect.

It was also observed that the best fit of microbial survivor model type obtained for the same treatment differs for surface and total microbial decontamination except for a high pressure treatment at 600 MPa and a treatment at 45 °C in a water bath where the same model type had the best fit for both surface and total decontamination. Consequently there seem to be differing inactivation kinetics comparing surface and total decontamination. Although treatment time had been added in order to ensure that the thermal core of the larvae reached the treatment temperature, a thermal gradient within products during thermal treatments before the sample core temperature was reached cannot be denied. Additionally, in the drying oven a cooling effect of evaporative cooling could be responsible for differing microbial survival comparing surface and total decontamination.

4. Conclusions

Worldwide a variety of ways for the preparation and decontamination of edible insects as food is applied including for example traditional techniques also applied for meat products such as boiling, frying, smoking, but also drying and preparing as chutney. The effectiveness

Table 2

Statistical measures and parameter values obtained from GlnaFit Version 1.6 for experimental data of surface decontamination.

Treatment	Model type	RMSE	Log N_0 [CFU g ⁻¹]	K_{max} [min ⁻¹]	Log N_{res} [CFU g ⁻¹]	Shoulder length [min]	δ [min]	P [–]
<i>Cold plasma</i>								
Direct	Log-linear + tailing	0.22	6.81	25.54	6.56	–	–	–
Indirect	Log-linear + tailing	0.82	5.98	1.45	3.10	–	–	–
<i>High hydrostatic Pressure</i>								
400 MPa	Log-linear + tailing	0.20	7.12	0.38	6.17	–	–	–
500 MPa	Log-linear + tailing	0.17	7.1	1.04	5.69	–	–	–
600 MPa	Log-linear + tailing	1.10	7.08	6.50	5.47	–	–	–
<i>Thermal</i>								
45 °C	Log-linear regression	0.12	6.88	–0.03	–	–	–	–
(water bath)								
90 °C	Weibull	0.60	6.46	–	–	–	0.09	0.24
(water bath)								
90 °C	Log-linear + shoulder	0.40	6.20	0.64	–	8.66	–	–
(drying oven)								

Table 3

Statistical measures and parameter values obtained from GlnaFIT Version 1.6 for experimental data of total decontamination.

Treatment	Model type	RMSE	Log N ₀ [CFU g ⁻¹]	K _{max} [min ⁻¹]	Log N _{res} [CFU g ⁻¹]	Shoulder length [min]	δ [min]	P [–]
<i>Cold plasma</i>								
Direct	Log-linear regression	0.27	8.25	–0.02	–	–	–	–
Indirect	Log-linear regression	0.09	8.10	–0.01	–	–	–	–
<i>High hydrostatic pressure</i>								
400 MPa	Weibull	0.07	8.37	–	–	–	8.09	0.11
500 MPa	Weibull	0.10	8.37	–	–	–	1.25	0.27
600 MPa	Log-linear + tailing	0.90	8.37	5.44	6.35	–	–	–
<i>Thermal</i>								
45 °C (water bath)	Log-linear regression	0.12	7.90	–0.03	–	–	–	–
90 °C (water bath)	Log-linear + tailing	0.39	7.59	3.62	4.59	–	–	–
90 °C (drying oven)	Log-linear regression	0.22	7.26	0.41	–	–	–	–

of these preparation practices regarding their microbiological safety needs to be ascertained.

Furthermore it can be concluded that indirect cold plasma treatment is a very effective method for the surface decontamination of mealworm larvae and even might have a potential for live surface sterilization in the rearing process. However, for the inactivation of the overall microbial count including the gut microbiota, a complemental volumetric decontamination treatment has to be applied. The best results for the reduction of the overall microbial count have been achieved by thermal treatments at 90 °C. For an improved inactivation a combination of indirect plasma and thermal treatments at 90 °C is suggested. More research and data on the impact of cold plasma, high hydrostatic pressure and thermal treatments on the microbial surface and overall activity of mealworm larvae are needed in order to develop effective decontamination methods and ensure the microbial safety of mealworm larvae and other edible insects as food and feed.

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