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# INSECTA 2019

## International Conference



### Book of Abstracts

05<sup>th</sup> – 06<sup>th</sup> September 2019  
Potsdam, Germany



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## Preface

On September 5 and 6, 2019 the Leibniz-Institut für Agrartechnik und Bioökonomie e.V. (ATB) and Pilot Pflanzenöltechnologie Magdeburg e.V. (PPM) organize the 5<sup>th</sup> INSECTA for the first time in Potsdam, Germany. In 2015, PPM initiated the first national INSECTA in Magdeburg and since 2016 the INSECTA was jointly organized by PPM and ATB and addresses national and international experts from academia and industry. Our aim is to continue the success of recent congresses and to ensure that INSECTA will be the insect science and technology conference to attend and will foster interdisciplinary collaboration.

The INSECTA 2019 International Conference aims to give an overview of state-of-the-art technology of insects for food, feed and non-food application. Topics cover the whole value chain from research, rearing and processing to safety and waste valorization. Therefore, technological as well as safety and sustainability aspects are discussed by professionals.

In detail, the conference covers following topics i) insect rearing and production systems (substrates and cycled production, lab-scale optimization and up-scaling, industrialization and automation); ii) insect processing for food and feed (fractionation and processing techniques, nutrient composition and food products, pet-food, feed and aquaculture applications); iii) safety and environmental aspects (microbial detection and decontamination strategies, material flow analyses and environmental impact, legal aspects and quality management), and iv) non-food application of insects (insect biotechnology, biopolymers and lipids, waste valorization).

In total, the 5<sup>th</sup> INSECTA has attracted 104 presentations and participants from 35 countries. In the conference period of two days, 4 keynote lectures and 50 oral presentations will be held in 3 plenary and 12 parallel sessions as well as 54 posters will be presented in the poster session.

We hope the conference and this publication will provide the platform for bringing together those involved in insect production, processing and consumption worldwide to explore the frontiers of knowledge in the art, science and engineering of all types of processing methods, monitoring technologies, and quality management systems. Participants and the public will take advantage of the scientific and social INSECTA events to learn about the latest developments in the respective fields, to boost communication and to increase cooperation and coordination of research efforts among different research groups.

Finally, as the Chairmen of the Conference, we would like to take this opportunity to sincerely thank the contribution from the authors, the reviewers and the editorial team.

Oliver Schlüter & Thomas Piofczyk

Potsdam, August 2019

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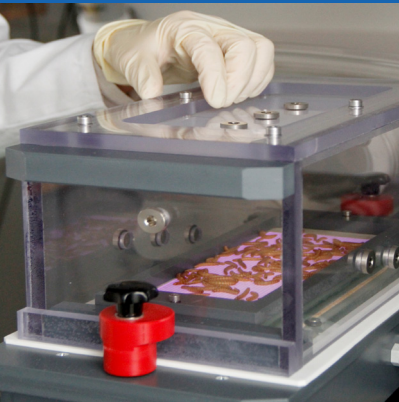
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## PROGRAM – September 05, 2019

07:55 a.m.	Bus shuttle from “Kongresshotel Potsdam” to conference venue “Neues Palais”	
08:10 a.m.	Bus shuttle from “Luisenplatz” to Conference Venue “Neues Palais”	
08:00 a.m.	Registration	Foyer
09:00 a.m.	Welcome – Opening remarks	Room 1.12
09:30 a.m.	<b>KEYNOTE:</b> <i>Prof. Dr. Ir. Arnold van Huis</i> Progress and prospects of insects as food and feed	Room 1.12
10:00 a.m.	<b>KEYNOTE:</b> <i>Heinrich Katz</i> EU Guidance document on best hygiene practices for the insect sector	Room 1.12
10:30 a.m.	Coffee break and poster session	

Parallel sessions from 10:50 a.m. – 6:20 p.m. in rooms 1.12 and 1.02

Session 1	Room 1.12	Session 2	Room 1.02
<b>Safety and environmental aspects</b>		<b>Insect rearing and production systems</b>	
10:50 a.m.		10:50 a.m.	
<b>Safety of insect based food and feed</b> <i>Winnie Nyakerario Akara, Germany</i>		<b>Development of efficient feeds for production of black soldier fly larvae derived from industrial by-products</b> <i>Anton Gligorescu et al., Denmark</i>	
11:10 a.m.		11:10 a.m.	
<b>Regulatory environment of edible insects in the EU: What have the past two years looked like, what does the future hold?</b> <i>Nicolas Carbonnelle, Belgium</i>		<b>Influence of moisture content of feeding substrate on growth and composition of <i>Hermetia Illucens</i> larvae</b> <i>Lotte Frooninckx et al., Belgium</i>	
11:30 a.m.		11:30 a.m.	
<b>Consumer acceptance of deep-fried, cornmeal-based fritters with 10 % meal of <i>Gryllus assimilis</i>, <i>Locusta migratoria</i>, and <i>Tenebrio molitor</i> in comparison to ordinary German food-stuffs</b> <i>Nils Th. Grabowski et al., Germany</i>		<b>Insects are what they eat: Impact of organic waste substrate on growth performance and nutritional status of <i>Hermetia illucens</i> larvae</b> <i>Chrysantus M. Tanga et al., Kenya</i>	
11:50 a.m.		11:50 a.m.	
<b>Readiness to adopt insects as food in the Czech Republic: Preliminary results</b> <i>Martin Kulma et al., Czech Republic</i>		<b>Self-selection of foods and by-products by the yellow mealworm (<i>Tenebrio molitor</i>) and impact of nutrient intake on biomass gain</b> <i>Juan Morales-Ramos et al., USA</i>	
12:10 p.m.		12:10 p.m.	
<b>Research in Myanmar's edible insect sector: Rapid knowledge growth as Myanmar emerges</b> <i>David Allan et al., Myanmar</i>		<b>Evaluating different organic by-products as rearing diet for <i>Acheta domesticus</i></b> <i>Costanza Jucker et al., Italy</i>	
12:30 p.m.	Lunch break and poster session		



Session 3	Room 1.12	Session 4	Room 1.02
<b>Safety and environmental aspects</b>		<b>Insect processing for food and feed</b>	
<b>01:40 p.m.</b>		<b>01:40 p.m.</b>	
<b>The microbial community of <i>H. illucens</i> larvae: What do we know so far?</b> <i>Jeroen De Smet, Belgium</i>		<b>Edible insects in a traditional value chain: Consumption patterns and the effect of domestic cooking on nutrition security</b> <i>Catriona Lakemond et al., The Netherlands</i>	
<b>02:00 p.m.</b>		<b>02:00 p.m.</b>	
<b>Will insect pathogens be a risk for black soldier fly (<i>Hermetia illucens</i>) production? Advancing research for reliable production</b> <i>Ward Tollenaar et al., The Netherlands</i>		<b>Alternative solvents for lipid recovery from BSF and evaluation of the protein quality</b> <i>Harish Karthikeyan Ravi et al., France</i>	
<b>02:20 p.m.</b>		<b>02:20 p.m.</b>	
<b>Pressed organic waste and sewage sludge for black soldier fly: Preliminary data on larvae rearing and safety evaluation</b> <i>Marco Meneguz et al., Italy</i>		<b>Proteins from black soldier fly (<i>Hermetia illucens</i>) as emulsifiers in oil-in-water emulsions produced by premix emulsification</b> <i>Junjing Wang et al., Spain</i>	
<b>02:40 p.m.</b>		<b>02:40 p.m.</b>	
<b>Rapid authentication of edible insect powders by means of infrared spectroscopy and multivariate analysis</b> <i>Jorge Mellado-Carretero et al., Spain</i>		<b>Enrichment in C3 of <i>Hermetia illucens</i> prepupae from oilseed co-products</b> <i>Bertrand Hoc et al., Belgium</i>	
<b>03:00 p.m.</b>		<b>03:00 p.m.</b>	
<b>Attenuated total reflected infrared spectroscopy combined with multivariate analysis. A novel tool for insect food product design</b> <i>Silvia de Lamo-Castellví et al., Spain</i>		<b>From the larva to the feed: Safety and nutritional aspects</b> <i>Verena Böschen, Germany</i>	
<b>03:20 p.m.</b>	<b>Coffee break and poster session</b>		

<p><b>Session 5</b></p> <p><b>Insect rearing and production systems</b></p> <p><b>03:40 p.m.</b></p> <p><b>Cost-effective insect rearing through automation and side-stream valorization</b> <i>Filip Wouters et al., Belgium</i></p> <p><b>04:00 p.m.</b></p> <p><b>Where do the larvae come from? Morpho-physiological and behavioral reproductive aspects of black soldier fly adults and their industrial applications</b> <i>Aline Malawey et al., USA</i></p> <p><b>04:20 p.m.</b></p> <p><b>Intraguild predation and cannibalism among adult <i>Ruspolia differens</i>: Towards overcoming challenges for mass-rearing</b> <i>Forkwa Fombong et al., Belgium</i></p> <p><b>04:40 p.m.</b></p> <p><b>Modularity of insect production and processing as a path to efficient and sustainable food waste treatment</b> <i>Sergiy Smetana et al., Germany</i></p> <p><b>05:00 p.m.</b></p> <p><b>G × E interactions: Larval performance of distinct black soldier fly genotypes grown on different feed substrates</b> <i>Christoph Sandrock et al., Switzerland</i></p> <p><b>05:20 p.m.</b></p>	<p><b>Room 1.12</b></p>	<p><b>Session 6</b></p> <p><b>Insect processing for food and feed</b></p> <p><b>03:40 p.m.</b></p> <p><b>Insects as food – A pilot study for industrial production</b> <i>Johan Berg et al., Sweden</i></p> <p><b>04:00 p.m.</b></p> <p><b>Physical functionality of <i>Tenebrio molitor</i> and using this knowledge to improve food applications</b> <i>Simon Hvid et al., Denmark</i></p> <p><b>04:20 p.m.</b></p> <p><b>Effect of temperature and insect:meat ratio on structure formation in hybrid batters</b> <i>Jana Scholliers et al., Belgium</i></p> <p><b>04:40 p.m.</b></p> <p><b>Volatile profile and odor attributes of mealworm, grasshopper, and silkworm dried by different methods and incorporated into cookies</b> <i>Maryia Mishyna et al., China</i></p> <p><b>05:00 p.m.</b></p> <p><b>Crickets from farm to fork: Farming, composition, safety and use</b> <i>Laura Gasco et al., Italy</i></p>	<p><b>Room 1.02</b></p>
<p><b>05:20 p.m. Coffee break and poster session</b></p>			
<p><b>Session 7</b></p> <p><b>Insect rearing and production systems</b></p> <p><b>05:40 p.m.</b></p> <p><b>Simulation of multi-stage air-separation of insects, faeces, and feed residues in a Zigzag-separator</b> <i>Andreas Baur et al., Germany</i></p> <p><b>06:00 p.m.</b></p> <p><b>Designing a cage for <i>Hermetia illucens</i></b> <i>David Deruytter et al., Belgium</i></p> <p><b>06:30 p.m.</b></p> <p><b>07:00 p.m. – 12:00 a.m.</b></p>	<p><b>Room 1.12</b></p>	<p><b>Session 8</b></p> <p><b>Insect processing for food and feed</b></p> <p><b>05:40 p.m.</b></p> <p><b>Effect of sex and developmental stage on nutritional quality of insects</b> <i>Lenka Kouřimská &amp; Martin Kulma, Czech Republic</i></p> <p><b>06:00 p.m.</b></p> <p><b>The different forms of vitamin B<sub>12</sub> in edible insects – Sources and causes</b> <i>Anatol Schmidt et al., Austria</i></p>	<p><b>Room 1.02</b></p>
<p><b>06:30 p.m. Bus shuttle from conference venue to evening event at Biosphere Potsdam</b></p>			
<p><b>07:00 p.m. – 12:00 a.m. Conference dinner at Biosphere Potsdam</b></p>			

## PROGRAM – September 06, 2019

08:00 a.m.	Bus shuttle from “Kongresshotel Potsdam” to conference venue “Neues Palais”	
08:00 a.m.	Bus shuttle from “Luisenplatz” to Conference Venue “Neues Palais”	
08:00 a.m.	Registration	Foyer
08:30 a.m.	<b>KEYNOTE:</b> <i>Dr.-Ing. Volker Heinz</i> Impact of insects on food and feed value chains	Room 1.12
09:00 a.m.	<b>KEYNOTE:</b> <i>PD Dr. Thomas Holzhauser</i> Edible insects – Assessment of allergenic potential and management of allergenicity	Room 1.12

Parallel sessions from 9:40 a.m. – 12:40 p.m. in rooms 1.12 and 1.02

Session 9	Room 1.12	Session 10	Room 1.02
<b>Safety and environmental aspects</b>		<b>Non-food applications of insects</b>	
09:40 a.m.		09:40 a.m.	
<b>Allergenicity assessment of edible insects and their protein hydrolysates</b> <i>Giulia Leni et al., Italy</i>		<b>The potential of the black soldier fly bio-converted rearing substrate as a plant growth enhancer</b> <i>Inbar Shouster-Dagan et al., Israel</i>	
10:00 a.m.		10:00 a.m.	
<b>Feeding study for the mycotoxin zearalenone in yellow mealworm (<i>Tenebrio molitor</i>) larvae – Investigation of biological impact and metabolic conversion</b> <i>Nina Kröncke et al., Germany</i>		<b>Insects as an alternative source for chitin or chitosan</b> <i>Lise Soetemans et al., Belgium</i>	
10:20 a.m.		10:20 a.m.	
<b>Effects of selected insecticides on black soldier fly (<i>Hermetia illucens</i>) larvae: Bioaccumulation, mortality, and growth</b> <i>Nathan Meijer et al., The Netherlands</i>		<b>INFACT: From Insect to surFACTant</b> <i>Sabine van Miert et al., Belgium</i>	
10:40 a.m.		10:40 a.m.	
<b>Insect digestion does not generate active oxygen compounds, demonstrating a nutritional advantage over meat consumption</b> <i>Adi Jonas-Levi, Israel</i>		<b>Novel polycistronic expression systems for specialized protein production</b> <i>Marc F. Schetelig et al., Germany</i>	
11:00 p.m.	<b>Coffee break and poster session</b>		

Session 11	Room 1.12	Session 12	Room 1.02
<b>Industrial perspectives</b>		<b>Insect processing for food and feed</b>	
<b>11:20 a.m.</b>		<b>11:20 a.m.</b>	
<b>Industrial bioconversion of meat waste by larvae of <i>Lucilia Caesar</i> L. (<i>Diptera Calliphoridae</i>) flies</b> <i>Mikhail Smahliuk &amp; Mikhail Zhuravlev, Russia</i>		<b>Biorefinery approach for conversion of organic side-streams into multiple marketable products using insects – InDIRECT project</b> <i>Leen Bastiaens et al., Belgium</i>	
<b>11:40 a.m.</b>		<b>11:40 a.m.</b>	
<b>Sustainable business model patterns – An integral part of insect-based business models</b> <i>Maria Real, Germany</i>		<b>Impact of different meat starter cultures on pH reduction, microbial community dynamics, and glutamic and aspartic acid production during meal-worm fermentation</b> <i>An Borremans et al., Belgium</i>	
<b>12:00 p.m.</b>		<b>12:00 p.m.</b>	
<b>Insect welfare in food and feed production</b> <i>Andreas Baumann, Switzerland</i>		<b>Effect of high hydrostatic pressure (HHP) processing on colour and textural properties of meal-worm species <i>T. molitor</i> and <i>Z. morio</i></b> <i>Philippa Victoria Grylls &amp; Richárd Pintér, Hungary</i>	
<b>12:20 p.m.</b>		<b>12:20 p.m.</b>	
<b>Edible insects in the German market – From online niche to retail mainstream</b> <i>Timo Bäcker &amp; Christopher Zeppenfeld, Germany</i>		<b>Evaluation of storage stability of dehydrated edible insects: Moisture adsorption isotherms, isosteric heat, estimated shelf life of flours of <i>Rhyncophorus phoenicis</i> and <i>Imbrasia truncata</i> larvae</b> <i>Aymar Rodrigue Fogang Mba et al., Cameroon</i>	
<b>12:50 p.m.</b>	<b>Closing Remarks</b>		<b>Room 1.12</b>
	<b>VDI BEST YOUNG SCIENTIST'S PRESENTATION AWARD</b> Sponsor: VDI – Magdeburger Bezirksverein		
<b>01:15 p.m.</b>	<b>Refreshments for farewell</b>		
<b>01:45 p.m.</b>	<b>Bus shuttle from conference venue to ATB</b>		
<b>02:00 p.m.</b>	<b>Visit ATB (optional)</b>		
<b>03:00 p.m.</b>	<b>End of conference</b> <b>Bus shuttle from ATB to “Potsdam Main Station”, “Luisenplatz” and “Kongresshotel Potsdam”</b>		

## Progress and prospects of insects as food and feed

Arnold van Huis

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The decline of the diversity and biomass of invertebrates around the world is worrying as insects support crucial ecological services. Edible insects are mainly involved in two ecological services: provisioning (nutrition and health) and supporting (decomposition and recycling). During the last five years the number of articles dealing with edible insects increased exponentially. The attention shifted from harvesting insects in the tropics to farming insects all over the world for food and feed applications. The black soldier fly as feed is often targeted as they can play a role in recycling many organic waste streams of low economic value and transforming them in high value proteins and fats. Microbial communities and their relationship with insects deserve full attention as it may help in the conversion of low-quality organic side streams. At the same time there are health benefits not only for farmed animals (the fat), but also for plants (using the left-over substrates). Other technological applications for fat are bio lubricants or biodiesel and for proteins bioplastics. The environmental impact of producing insects compares favourably to that of the common production animals. However, this as well as the profitability depend very much on the availability, quality and price of substrates used for rearing the insects. A new area is that of genetic improvement for which much progress can be expected due to the short life cycle of insects, e.g. can we select strains fit for specific waste streams? Another interesting area of research is health effects considering that insects have the largest anti-microbial peptide reservoir of all animals. A main challenge for insects incorporated in food is to go from the occasional snack to staple foods. Food safety needs full attention as it will determine the easing of legislation, which in turn will increase its potential to become a major industry. Consumers are becoming more and more aware that insects as food are a viable option, but strategies need to be finetuned to overcome the reluctance of eating insects. Insects need to be processed into ingredients, that can be applied for safe and appetizing products. The sector of insects as food and feed is developing fast. Organizations are being formed to embed the industry in a more conducive environment. The sector can only progress when the insect industry, the academic world, governmental organizations and public society closely cooperate.

## EU Guidance document on best hygiene practices for the insect sector

Christophe Derrien<sup>1</sup>, Heinrich Katz<sup>2</sup>

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With the insect as food and feed (for food producing animals) growing at an accelerating rate in regulations, science and technology several factors need to be simultaneously addressed. An important factor being hygiene in production of insects-based products. The overarching objective of IPIFF's Guide on Good Hygiene Practices is to help insect producers for food and/or animal feed purposes (hereafter referred to as 'insect producers') to achieve a high level of consumer protection and animal health through the production of safe products. To this end, the Guide provides guidance to insect producers to effectively apply EU food and feed safety legislation, while providing an incentive for them to develop a robust food and feed safety management system.

In addition, this Guide specifies requirements enabling to:

1. ensure that insect producers conform to their stated food and feed safety policy and demonstrate their commitments in this regard;
2. help the insect producers to effectively communicate food and feed safety issues to the regulatory authorities, and when needed, to their suppliers, customers and relevant interested parties (i.e., consumers) in the food and feed chains.

To achieve the above objectives, the Guide has drawn on the skills and expertise of companies directly involved in the production of insects, either for human consumption or for animal feed purposes. Furthermore, consultations also involve several European representative organizations of the food and feed business sectors and other interested parties.

## Impact of insects on food and feed value chains

Volker Heinz

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Although livestock farming has a long tradition as part of the human food supply it is surprising that not much more than 10 animal species form the basis of the global production of animal protein or fat. To feed the global livestock of chicken, pigs and ruminants about 50% of the worlds agricultural harvest is needed which results in ca. 4% of the worlds animal biomass (humans account for 2.5%). Since the total anthropod biomass is about 40% (which is a bit less than 1 bln t carbon) the question is not new whether insects species could be a proper and sustainable addition to the short list of livestock species.

Discussions on foodprint of agricultural productions systems and meat and other animal products in particular unleashed a wave of start-up companies active in all kind of alternative products circumventing animal welfare issues and reducing the CO<sub>2</sub> footprint.

This presentation will address the current livestock production systems related to the planetary boundaries of the ecosystem and where and in which way biomass conversion by insects can make up ground. The discussion will include feed and food value streams with regard to technological aspects and product safety and quality aspects with specific focus on using insect proteins and fat to mimic 'traditional' food products. LCA studies will be used to compare novel products against the standards.

## Edible insects – Assessment of allergenic potential and management of allergenicity

Thomas Holzhauser

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Insects (Insecta, e.g. beetles, locusts, cockroaches) belong to the phylum Arthropoda, so do arachnids (Arachnida, e.g. mites) and crustaceans (Crustacea, e.g. shrimps, crabs, lobsters). Several pan-allergenic proteins have been described in insects, mites, and crustaceans ([www.allergen.org](http://www.allergen.org)), including tropomyosin and arginine kinase.

Allergic episodes to edible insects, including severe anaphylactic reactions, have been reported mainly from areas of entomophagy. However, prevalence data about allergy to edible insects are lacking. Recent studies demonstrated an allergenic potential of insects both related to primary allergic sensitization and due to cross-reactivity after sensitization to other arthropods such as crustaceans or mites. Accordingly, crustacean and mite allergic individuals might be at risk for unexpected allergic reactions to insect proteins in foods, such as from mealworm, cricket or black soldier fly.

No causative immunotherapeutic treatment of insect food allergy is available to date. Strict avoidance of the allergenic source remains the most effective measure to avoid an allergic reaction. This also requires knowledge about allergenic cross-reactivity of insect proteins and reliable labeling of foods with regard to the presence of insect proteins.

Especially in the case of sensitization to thermally stable allergens, such as tropomyosins, thermal treatment (cooking, boiling, frying) may not fully abolish the allergenic activity. More harsh conditions, as e.g. extensive hydrolysis, may substantially diminish the allergenic activity, but this needs to be assessed with appropriate methods and on a case by case basis. For known pan-allergenic structures, suitable methods for allergenicity assessment are in principle available and applicable. Even though, prediction of allergenicity of insect-derived foods remains limited due to a lack of validated methods and reference proteins having clinically validated allergenic potency. For unknown potentially allergenic structures, novel methods for allergenicity assessment are proposed or partly developed, but further research will be necessary to improve the existing toolbox for allergenic risk assessment.



**Oral**

**Session 1–12**

## Safety of insect based food and feed

Winnie Nyakerario Akara

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With the increase of world population, animal protein is becoming more expensive and scarce as many people want meat on their plates but not everyone can afford it. Thus a need for alternative sources of animal protein exists. This is particularly noticed in the fish and poultry sector where there is growing scarcity of resources needed to produce healthy and nutritious feed, yet the solution is simple. In the global south, the potentials regarding insect rearing for feed and food are endless, the market remains highly untapped though as they are lacking in the research and technology required to fill the gap. Therefore, they are left heavily dependent on European and Western countries to demystify the process as insects could help address the global challenge of food security.

The longstanding ban on feeding processed insect proteins to aquaculture animals in the EU has recently been lifted. But before insects can be used as an option in these countries, possible food safety concerns should be investigated, so as to provide an overview of the possible chemical, microbiological, and allergenic hazards and prions that may pose as health risks upon the use of insects for feed or food. Chemical contaminants present in food and feed originally derived from insects, occur as a result of the presence of the particular contaminant in the environment and/or the substrate. These include, among others, heavy metals, veterinary drug and pesticide residues. The main chemical exposure route is through the substrate on which the insects are grown and, therefore, the level of concern will vary according to substrate used. This is of significant importance as bioaccumulation of such toxic substances and heavy metals can be high in invertebrates and they can easily be transferred up the food chain if not detected.

In the industry, through experience, it has become common and decent practice to clean the rearing trays with hot water after each cycle, and the input substrate treated with heat to kill any bacteria. This practice should however, be done on a case by case basis, depending on the substrate provided as heating can be bad for microorganisms that would otherwise be beneficial to insects and their processes. The source of the substrate should be known, and if there is reasonable suspicion of heavy metals or dioxin-polluted material, the substrate should be declined. Black soldier flies are used to living in rough environments – they are robust and able to deal with high and low pH values, and can deal with both microbes and mold. Probable accumulation of chemical hazardous compounds and microbial contamination most likely depends on the insect species, growth stage, substrate use, farming and processing conditions. Research should be done on a case-by-case basis, with efforts concentrated on a selected set of insect species that are promising for use as feed or food, farmed with an appropriate substrate, in a hygienic environment and under controlled conditions. Conclusions on obtained results should be limited to the species under investigation and to the specific farming and processing conditions.

## Regulatory environment of edible insects in the EU: What have the past two years looked like, what does the future hold?

Nicolas Carbonnelle

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The classification of insects as novel food has been clarified through the adoption of Regulation (EU) No 2015/2283 that has become applicable on 1 January 2018 in replacement of Regulation (EC) No 258/97.

The novel food status of edible insects implies that they are subject to safety assessment and pre-market approval before being placed on the market. Regulation (EU) No 2015/2283 provides two different procedures that can be used to place edible insects in the European market. In both procedures, the applicant is required to submit a set of information to the Commission that may involve the EFSA in the food safety assessment. The presentation analyzes the provisions of the regulation and its implementing rules, as well as the guidelines issued by EFSA, and explores the practical implementation of the authorization procedures a year and a half after the entry into application of the regulation. A state of play of the pending novel food applications relating to insects and insect-based ingredients is also provided.

Besides the novel food regulation, the commercialization of insects intended for human consumption raises other legal and regulatory questions pertaining to the methods of production of edible insects and to the labelling and presentation of the products. As regards methods of production, the presentation will address the organic production of insects intended for human consumption. In relation to the labelling and presentation, the current relevant legal frameworks will be addressed, to discuss the mandatory and non-mandatory elements of labelling as well as the challenges that the regulatory environment places ahead for simple products as well as for more complex consumer products.

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## Consumer acceptance of deep-fried, cornmeal-based fritters with 10% meal of *Gryllus assimilis*, *Locusta migratoria*, and *Tenebrio molitor* in comparison to ordinary German food-stuffs

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In contrast to nations with traditional entomophagy, eating insects in the Western countries may be challenging since many consumers label them as disgusting, particularly if the entire animals are offered. Homogenates are better-accepted, so consumers are more willing to taste insect meal-based products like bread or pasta. The goal of this study was to compare the acceptance of these homogenized products to that of ordinary foodstuffs.

Imagines of Jamaican field crickets (*Gryllus assimilis*) and of migratory locusts (*Locusta migratoria*) as well as mealworm (*Tenebrio molitor*) larvae were processed to flour. A fritter base containing 900 g nixtamalized corn flour, 100 g insect flour, 3 l water, 9 teaspoons salt and 1 teaspoon black pepper was prepared, based on Southern African mealie paps, specifically the Zambian *nshima*. After cooling, the dough was cut in pieces and deep-fried in peanut oil. Each participant received one fritter per insect species and a questionnaire in which he was asked to rate appearance, smell, taste, and mouthfeel of the fritters using the German school grade system (1 = very good to 6 = insufficient). Besides asking for age and gender, participants were also asked to rate ordinary German foodstuffs (beef, pork, schnitzel, lamb, rabbit, venison, chicken, turkey, fish, seafood, eggs, milk, cheese, yoghurt, tofu, spinach, potatoes, pasta, rice, and spelt).

At a health day event of the authors' faculty, n = 79 questionnaires were filled out. Overall (mean of appearance, smell, taste, and mouthfeel), *G. assimilis* and *L. migratoria* fritters were rated  $2.2 \pm 0.7$ , those of *T. molitor*  $2.4 \pm 0.7$ . Thus, they ranged behind eggs, beef, potatoes and pasta, dairy products, chicken, and on a similar level like fish or rice. All other foodstuffs ranged behind the fritters. Several foodstuffs were rated significantly in a different way in relation to gender and age, but not the fritters. Still, women tended to rate them better, and rating of cricket fritters decreased with age, while that of locusts and mealworms increased. Differences may be due to many factors incl. age-related changes in tasting ability.

## Readiness to adopt insects as food in the Czech Republic: Preliminary results

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In the Czech Republic, the people are generally neophobic and insects are considered to be mainly noxious pests. On the other hand, recent search for alternative protein sources caused an increased interest in entomophagy even in the Central Europe. As no current data of willingness of the Czech consumers to eat insects are available yet, this study aims to investigate the readiness to accept insects as food. An online survey of more than 1000 consumers was conducted to reveal their current experience with entomophagy, their willingness to adopt insects into their future diet and also determine which species in which form are the best suitable for this purpose. The preliminary results indicate, that majority of the public have no experience with entomophagy and do not even have an interest to try or taste the insects. On the other hand, the collected data also showed that almost a third of respondents are willing to include mostly processed insects (mainly Orthoptera and Coleoptera larvae) or products containing insect meal (regardless of the species) in their diet. On the contrary, no interest was generally found in eating whole raw insects. Finally, almost 80% of the respondents claimed, that they would accept the meat from the farm animals, which were partly fed by insect meal.

## Research in Myanmar's edible insect sector: Rapid knowledge growth as Myanmar emerges

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While much is known about edible insect sectors in Thailand, Laos PDR and Cambodia, little has been published on Myanmar. Long isolated, practices well known to Myanmar people have had little systematic study. Broad national reforms since 2011 enabled meteoric telecommunications sector growth (5% to 95% internet penetration in 5 years) allowing new channels like online edible insect trading. Ironically such routes have emerged even before the first PhD theses on cultural practices, local species and seasonal calendars for edible insects have been online or circulated externally, or before any value chain studies.

Spectrum – Sustainable Development Knowledge Network – is analysing and researching critical edible insect sector knowledge gaps, with three main focus areas:

1) Traditional entomophagy practices, 2) Existing insect related business models, value chains and farming practices, and 3) Potential for insects to support balanced feed systems for livestock and aquaculture. Work is proceeding with Yangon, Mandalay, Taungyi and Yezin Universities. The ASEAN Food and Feed Insect Association (AFFIA) supports Spectrum and the Universities with regional sharing.

Spectrum's work linked us with key Myanmar researchers. Their dissertations are summarised and greatly increase species and nutritional information.

Spectrum's findings are mapped from 270 interviews in 7 areas via quantitative and qualitative research on three wild harvest edible insect value chains. Methods involved market surveys; in-depth interviews (with collectors, farmers, distributors and sellers); observations and recordings through media; physical sample collection; seasonal calendar analyses and value chain mapping from source to end markets.

Research documents the state of the sector and will guide convening of a high-level roundtable panel for the development of a strategic plan and investment strategy. It will inform education materials and workshops for target communities and support pilot farms trialing smallholder rearing methods for vulnerable households in Myanmar.

## Development of efficient feeds for production of black soldier fly larvae derived from industrial by-products

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Black soldier fly larvae (BSFL) are known to inhabit a wide range of substrates, but their performance is highly affected by the quality of these substrates. Consequently, the production of BSFL for commercial purposes requires highly optimized diets able to maximize feed conversion and larval biomass production.

The presentation aims to show some of the work conducted by the Danish Technological Institute as a part of our feeding services. This will include:

Development of new diets derived from industrial by-products, based on their macronutrients profile. The protein, fat, carbohydrate and ash profiles of different by-products (i.e. beach casts, apple pomace, spent grains, wheat, rape seed cake, beetroot tops, malt and butter cookies residues).

Test of new diets at laboratory scale, with focus on production outputs (larval biomass, substrate reduction and feed conversion rate (FCR), on both fresh weight and dry matter (DM) basis) and larval survival rate. The production outputs differed between the tested diets with FCR ranging between 2.4 and 3.6, while the survival rate was similar (79%–82%). Based on these parameters the diet with the highest potential for being used in the production of BSFL was identified.

Optimization of new diets at pilot scale. The new diet was used to produce a batch of BSFL, during which two different feeding strategies were tested and the production outputs (larval biomass, substrate reduction and FCR) and larval survival rate were assessed. The results from this pilot production were further compared with the results from a BSFL production reared on chicken feed (control), maintained under similar conditions. The FCR was found to vary between 2.5 and 4.9 when larvae were reared on the new diet, and 2.8 when reared on chicken feed.

Characteristics of: 1) BSFL (protein, fat and ash content) reared on the new diet and chicken feed and of 2) insect frass (NPK) derived from the two production setups (new diet and chicken feed). The larvae reared on the new diet was found to have a higher protein (42%) and fat (25%) content compared to the protein (37%) and fat (17%) profiles of BSFL reared on chicken feed. Moreover, the NPK profile of the insect frass from the two production setups was found to differ with diets.

## Influence of moisture content of feeding substrate on growth and composition of *Hermetia illucens* larvae

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Black soldier fly larvae (*Hermetia illucens*) are able to convert organic side streams into high-value biomass, mainly consisting of proteins, fat and chitin. Food waste consisting of pre- and post-consumer products is already being collected separately by several waste management companies. This 'energy mixture' is usually valorized as feedstock for anaerobic digestion. However, it could be better valorized if used as feeding substrate for black soldier fly larvae. The present study aims to investigate the influence of the chemical composition (moisture, fat, protein, ash, fiber and carbohydrate content) of the 'energy mixture' on growth, feed conversion, waste reduction and composition (moisture, fat, protein, ash, chitin and carbohydrate content) of black soldier fly larvae and rearing residues.

Larvae were daily fed 100 mg of the 'energy mixture' or chicken feed with different moisture contents ranging from 80% to 55%. Larvae fed with substrates containing the highest moisture content grew faster and reached a higher maximal weight per larvae, than larvae fed with less moist substrates. Fat content was lowest in larvae fed with substrates containing the highest moisture content. Protein content did not differ between larvae fed with substrates of high or low moisture content.



## Insects are what they eat: Impact of organic waste substrate on growth performance and nutritional status of *Hermetia illucens* larvae

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Black soldier fly (BSF) *Hermetia illucens* is one of the most efficient recyclers of organic wastes into high-nutrient biomass that constitutes a sustainable fat (biodiesel) and high-quality protein ingredient in animal feeds. This research aimed to evaluate the influence of 8 substrates: chicken manure, rabbit manure, cow manure, spent grains, human feces, pig manure, mixed fruit and vegetable waste and restaurant waste on BSF larvae development, survivorship and nutritional composition. The 5<sup>th</sup> instar larvae and substrates were evaluated for proximate, amino acid, fatty acid, vitamins, flavonoids and minerals. Growth performance and survivorship of the larvae varied between 12–15 days and 75.8–92.8%, respectively. The nutrient composition of the larvae varied considerably depending the substrate. Crude protein and fat content varied significantly ranging between 38.5–62.7% and 14.0–39.2%, respectively. The amino acid, fatty acid, vitamins, flavonoids and mineral showed significant variation across the substrate tested. Low concentrations of heavy metals such cadmium and lead were detected but within acceptable limits. Our findings revealed that the black soldier fly larvae ingested low-quality nutrients from the substrates, amplify and metabolize them into novel derivatives with potential animal health benefits. Thus, the potential of BSF larvae as nutritious high-quality ingredient for poultry, fish and pig feeds is discussed.

## Self-selection of foods and by-products by the yellow mealworm (*Tenebrio molitor*) and impact of nutrient intake on biomass gain

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*Tenebrio molitor* L. is one of the most important species of industrialized insects in the United States. Although *T. molitor* can be mass produced using exclusively wheat bran as food, this diet lacks sufficient nutritional value, resulting in extended development time and reduced survival (MORALES-RAMOS *et al.* 2013). Adding nutritional value to *T. molitor* diet without increasing costs can be accomplished by supplementing it with agricultural by-products, which often contain valuable nutrients.

In this study 16 by-products were tested in combination with 4 foods as potential diet ingredients for *T. molitor*. Self-selection was used to determine optimal intake of each ingredient by *T. molitor* larvae. 7 self-selection experiments were established using different combinations of 8 ingredients. Each experiment consisted of 10 repetitions. Experimental units consisted of multiple-choice arenas with a radial distribution of 8 perforated plastic vials each containing a different ingredient. One hundred *T. molitor* larvae (12<sup>th</sup>–13<sup>th</sup> instar) were introduced to each unit and allowed to feed for a period of 21 days at 27°C, 75% RH, and dark conditions. The consumption of each ingredient and the larval live weight gained were measured at the end of the experiments. The percent consumption of each ingredient within each experiment was calculated. The intake of nutrients by larvae was determined using the nutrient matrix operation (MORALES-RAMOS *et al.* 2014). The impact of nutrient intake on biomass gained was analyzed using multiple regression.

Ingredients consumed at higher than 10% ratios were considered as potentially suitable for diet development. Larvae appeared to prefer corn dry distillers' grain with solubles (DGGs), rice bran (whole and defatted), brewery distilled grain, and canola meal among the by-products. Regression analysis of nutrient intake by *T. molitor* larvae yielded two different solutions using the stepwise and backwards elimination of model selection. The first identified carbohydrate, neutral detergent fiber and vitamin E and the second identified carbohydrate ratio, polyunsaturated fat ratio and phytosterol as relevant variables affecting ending live biomass gain.

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## Evaluating different organic by-products as rearing diet for *Acheta domesticus*

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Considering insects as a new alternative protein source, crickets are among the most cultivated species in many different regions of the world. Recently, they are receiving particular attention as food in the European countries where they have been reared since years as feed for pets and other insectivorous species. The mass rearing of the house cricket, *Acheta domesticus*, is commonly conducted on grain feed. This substrate has high costs and high environmental impact due to its production. Find an alternative low-cost and sustainable feed for the rearing of the house cricket is one of the main goal of the research project MAIC (Model for rearing edible insects). Rearing substrates were chosen within different by products from the agro-food industry (brewer grain, maize distiller, vegetables). Chicken feed was used as control. Each substrate was tested for *A. domesticus* rearing considering the growing performance of the insects, the feeding conversion and the final cricket meal yielded. Moreover, each diet was analyzed for its microbiological and nutritional characteristics. Also the presence of chemical substance (e.g. heavy metals) was considered. The meal produced with crickets reared on the different diets was analyzed in order to assess the hygienic-sanitary parameters and the final chemical composition. All the process is analyzed within a life cycle assessment approach to assess its environmental impact. Preliminary data acquired are illustrated.

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## The microbial community of *H. illucens* larvae: What do we know so far?

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The larvae of *Hermetia illucens* have become the mostly used insect in industry for the bioconversion of organic waste. This economic relevance is translated in a rapid increase in scientific research being published on this species. The last two years more papers were published on the topic than in the past three decades, dealing with many aspects of larval productivity. Of particular interest are the recent advances on the larval gut physiology<sup>1</sup> and its microbial community<sup>2,3</sup>. These indicate that the larval gut microbiota should be considered as an extra organ capable of degrading wastes and generate beneficial products for the larvae<sup>4</sup>.

Here, we present the results of our study exploring the microbiota of the substrate, the larvae and their residue, both on laboratory scale and in production scale facilities at several locations<sup>3</sup>. As the substrate composition differs greatly between rearing locations, large differences in the microbial load were observed. Little similarity between the microbiota of the larvae and the substrate at one facility or between the larvae at different facilities were observed. Nevertheless, 48 operational taxonomic units (OTUs) were omnipresent in all facilities, of which most belonged to the phyla *Firmicutes* and *Proteobacteria*. Our data are also compared to that of other recent studies to get a broad view on the presence of these 48 OTUs in these diverse datasets to explore the hypothesized claim of a core microbiota residing in the larval gut containing important symbiotic bacteria<sup>5</sup>.

We can conclude that the rapid increase in data on the microbial communities in the larvae warrants a thorough meta-analysis to advance from the current narratively describing towards a more in-depth understanding of the microbial community dynamics towards control and applications for industry.

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## Will insect pathogens be a risk for black soldier fly (*Hermetia illucens*) production? Advancing research for reliable production

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Black soldier fly larvae (*Hermetia illucens*) can contribute to creating a circular food system as they are very effective in turning low-grade food waste into valuable high-end proteins and fat. Being rich in oils and proteins also makes them particularly suitable for ingredient production for food and animal feed. That is why this sector is emerging and growing quickly. Compared with other production insect species for feed and food, black soldier flies (BSF) seem to be very resistant to diseases and up to now, not a single case of a disease outbreak in a black soldier fly plant is known. However, research on the health of both larvae and adults is needed to define early detection and identification of diseases as well as prevention and treatment procedures. The insights will support large-scale production and enable to continue creating reliable and high-quality output without unexpected production losses.

The project goal of the ongoing collaboration between University of Copenhagen, Protix and Bühler Insect Technology Solutions is to establish preventive measures that allow a safe and robust insect production by promoting the best practices to maintain insect health in production facilities. Therefore, we will develop diagnostics and guidelines for insect disease management in order to minimize risks and losses to the producers. To reach this goal we first need to investigate how BSF responds to various insect pathogens and how its immune system works.

We will present the methodology of testing several generalist and facultative pathogens on BSF larvae that could be a potential risk to production in European closed facilities. We are investigating whether the larval stage is susceptible to inoculation of high virulent strains of several different entomopathogens such as *Beauveria bassiana* and *Metarhizium brunneum*. We invite all colleagues in the industry to ask questions, review the project methodology and preliminary results and to share their own experiences by reaching out to us in order to enrich the industry's knowledge on this topic. More European collaborations on diagnosis and management of insect diseases are needed. Our team welcomes insect producers, veterinarians, food microbiologists etc. to join forces.

## Pressed organic waste and sewage sludge for black soldier fly: Preliminary data on larvae rearing and safety evaluation

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The interest on the waste management has grown in the last year to reduce cost and environmental impact. Black soldier fly (BSF) larvae can grow on different diet and waste (MENEGUZ *et al.* 2018; LALANDER *et al.* 2019). The study used pressed waste (PW) and sewage sludge (SE) based-diet, plus probiotics (yogurt), to evaluate the effect on growth performance and microbiological safety of the diet and the larvae.

Two diets were formulated, A: PW and B: PW+10%SE+1%yogurt. 1 g of eggs (<24h old) were used per replicate (5 replicates per treatment) and 8 kg of diet were allocated as batch feeding system in each replicate. The trial was stopped after 10 days.

The results showed no differences between the two diets in the final biomass (A: 1403.5±88.00 and B: 1353.2±236.20 g) and final larval weight (A: 89.3±10.1 and B: 88.6±4.2 mg), while residue left on B (49.8±1.8%) was higher than A (43.3±2.7%). The probiotic did not influence the SE digestion.

Bacterial counts (log(CFU)/g) were performed on the two diets with the following results: aerobic spore formers bacteria counted 9.4±0.03 (A) and 9.6±0.07 (B), total aerobic bacteria (6.1±0.16 (A) and 5.9±0.02 (B), anaerobic spore formers bacteria 3.6±0.06 (A) and 3.7±0.21 (B), Enterobacteria (A: 2.3±0.32 and B: 1.9±0.08), Staphylococci and Micrococci, Yeast, molds and coliforms <2. *Bacillus cereus*, *Salmonella sp*, *Listeria sp* were absent.

The larvae showed a low number of aerobic spore forming bacteria (<4), while enterobacteria/coliforms were higher: (6.2±0.07 (A) and 6.3±0.07 (B) for the former), and 4.8±0.22 (A) and 4.7±0.09 (B) for the latter. Also *E. coli* colonies were detected whereas pathogens (*Bacillus cereus*, *Salmonella sp*, *Listeria sp*) were absent.

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## Rapid authentication of edible insect powders by means of infrared spectroscopy and multivariate analysis

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Insects are being introduced as an alternative way to ensure world's food and feed security. Accordingly, analytical methods are required to determine food quality and safety of insect-derived products. Infrared spectroscopy provides a rapid chemical fingerprint of food products and could become a promising tool for authentication.

The present study describes a new approach to discriminate commercial edible insect powders of crickets, grasshoppers and worms using attenuated total reflectance infrared spectroscopy combined with multivariate analysis. An amount of insect powder (4 mg) was directly placed onto the sample stage of a portable spectrometer equipped with a single bounce ATR diamond crystal accessory. A pressure clamp was used to ensure optimal contact between samples and the diamond crystal. Spectra were collected (4000–800  $\text{cm}^{-1}$ ) to build up soft independent modelling of class analogy (SIMCA) models.

SIMCA models were developed to differentiate and predict insect species based upon data obtained from mid-infrared spectroscopy profiles. Insect species were successfully discriminated and significant biochemical differences were linked mainly to lipids and chitin. Moreover, no biochemical differences were detected among same specie clusters (buffalo worms and house crickets) regardless of their origin (i.e. farm). SIMCA models were also tested by internal validation and 100% correct predictions were obtained for most of the samples analyzed.

This research has proved the potential of infrared spectroscopy coupled with multivariate analysis as a powerful tool for the assurance of insect powder authenticity.

## Attenuated total reflected infrared spectroscopy combined with multivariate analysis. A novel tool for insect food product design

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Insects have become a new potential ingredient for food product design due to its high-quality content in protein and micronutrients. In Western countries, insect consumption is still a challenge for most of consumers. A strategy to introduce insects in Western diet is using edible insect powders as ingredients for innovative food design.

In preliminary studies performed by our research group, several insect powders (crickets, grasshoppers and worms) have been discriminated and classified using attenuated total reflected infrared spectroscopy combined with multivariate analysis. Nevertheless, it is necessary to check if this technology is capable of discriminating, predicting and quantify insect species used in food matrices. The objective of the present research was to study the potential of using attenuated total reflected infrared spectroscopy combined with multivariate analysis to discriminate raw salty dough and its 3D printed cooked version enriched with different insect powders. Several doughs were made using chickpea flour (16 to 46%), water (39%), olive oil (11%), curry, salt and different quantities of insects powder (*Alphitobius diaperinus* and *Locusta Migratoria*, up to 30%). The snacks were printed using a portable Focus 3D Food printer (Voronoi 3D model, around 4 g each piece) and cooked at 180°C for 12 min. An amount of dough and snack powder were directly placed onto the sample stage of a portable spectrometer equipped with a single bounce ATR diamond crystal accessory. Spectra were collected (4000–800 cm<sup>-1</sup>) and soft independent modelling of class analogy (SIMCA) was used for the chemometric analysis. The viscosity of the raw dough and the snack texture were also analyzed. SIMCA models built up from IR data obtained, clearly discriminated insect species used to prepare dough and 3D printed snack powders. Discrimination was mainly associated to lipids and chitin components. Quantitative predictions of insect powder used to prepare doughs and 3D printed snacks showed good correlation (from 10 to 30%).

This research has shown the potential of attenuated total reflected infrared spectroscopy combined with multivariate analysis to discriminate and quantify different insect powders used to produce novel foods.



## Edible insects in a traditional value chain: Consumption patterns and the effect of domestic cooking on nutrition security

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Consumption of indigenous edible insects contributes to food and nutrition security. This study aims at getting insight in consumption in rural and urban areas, to map nutrient variability over harvesting locations, and to study the effect of traditional processing on protein digestibility and mineral bio-accessibility.

A survey on insect consumption included 200 urban and 175 rural respondents. Samples of *Eulepida mashona* and *Henicus whellani* were collected in five rural districts in Zimbabwe (three locations per district) for nutrient content analysis. Samples of both species were subjected to boiling and roasting, followed by assessing in vitro protein digestibility and mineral bioavailability (Infogest protocol).

A frequency of insect consumption of >3 times a week was found for 64% of rural vs. 15% for urban respondents. Insect consumption was prevalent in both rural (90%) and urban (80%) areas. For rural respondents taste is the main motive followed by nutritional value (89% vs 51%), while for urban respondents taste and nutritional value were equally important (74%).

The protein content was high and relatively stable over sampling location for *E. mashona* (52–57%) and *H. whellani* (60–71%). Fat content was low (5–11%), Fe (25–55 mg/100 g) and Zn content (16–22 mg/100 g) were high and varied more with sampling location. Consumption of 50 g for both insect species will contribute on average to 30, 50 and 30% of the recommended daily intake for protein, Fe, and Zn.

In contrast to roasting, boiling of the insects reduced protein content up to 15% due to leaching. Protein digestibility for both non processed insect species was comparable, and roughly 10% lower than for whey proteins. Boiling and roasting reduced protein digestibility up to about 25%. Reduced digestibility with processing can be explained by protein modification and presence of phytochemicals. *E. mashona* had a higher Fe and Zn bioaccessibility than *H. whellani*. Fe and Zn bioaccessibility were reduced due to boiling/roasting up to about 60%.

Points of importance in the contribution of these indigenous edible insects to nutrition security were harvest location, preparation method, protein digestibility and species dependent mineral bioaccessibility.

## Alternative solvents for lipid recovery from BSF and evaluation of the protein quality

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In order to feed the growing population which is roughly estimated to touch 9.6 billion by 2050 and alleviate its negative impact on the food supply chain many research units, start-ups, think-tanks and other entities are coming up with innovative, greener alternative solutions to fix the anticipated demand-supply gap. Among the multifarious options being considered to handle the predicaments, utilisation of insects for food and feed applications are gaining significant traction. *Hermetia illucens* or the black soldier fly larvae (BSFL) for bio-conversion of discarded industrial by-products or side streams, municipal wastes from urban activities have been extensively studied and the economic value it imparts makes it a valuable contender among other solutions recommended.

In the case of oilseeds, the solvent extraction of oil with n-hexane is preferred as it promotes easier oil recovery and has a narrow boiling point (63–69°C). But, n-hexane reacts with free pollutants to form ozone, photo chemicals and is said to affect the neural system when inhaled by humans. Though the idea of complete replacement of petrochemical solvents for oil extraction is far-fetched, it is imperative that efforts to reduce the dependency on them and find suitable, economically feasible alternatives for the same must be given due consideration. Several research articles have articulated the effectiveness of green solvents for oil extraction from various biomass.

The objective of this presentation is to elucidate an amalgamation of theoretical and experimental work to deduce an ideal solvent for defatting the insect matrix with significantly higher lipid yields and functional properties. Contemplation of an optimal green solvent from a wide array of solvents including alcohols, ethers, esters and terpenes for defatting the BSFL matrix by combining theoretical and experimental methods. Theoretical data for determining the interactions between the solutes and solvents were realized with tools such as Hansen solubility parameters (HSP), consequently supported with relatively precise data obtained from conductor-like screening model for real solvent (COSMO-RS) and finally with the application of hurdle technology for decisive selection of suitable solvent based on the absolute theoretical data retrieved. It was within the purview of this study to establish an industrial scale simulation for oil extraction and to develop kinetic modelling for a better understanding of lipid diffusivity in the solvent medium. Furthermore, to elucidate the lipid composition data by identifying the fatty acid profile, lipid class constituents, the bioactivity of BSF oil and compare the effect of solvent on the protein quality parameters (protein dispersibility index, urease index, etc.) in the defatted BSF flour.

## Proteins from black soldier fly (*Hermetia illucens*) as emulsifiers in oil-in-water emulsions produced by premix emulsification

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With the increasing acceptance of the concept of entomophagy and the potential of insects as a novel source of proteins, several species (yellow mealworm, cricket, black soldier fly, etc.) have been suggested to supplement human diet attributed to their great nutritional value. However, little is known about the techno-functional properties of these new ingredients. The goal of this work is to assess the properties of protein fractions from black soldier fly (*Hermetia illucens*), BSF, when used as emulsifiers to stabilize oil-in-water (O/W) emulsions and compare their performance to a widely used food protein, such as whey protein (WP). The O/W emulsions are obtained by a high throughput, low energy premix emulsification system. In particular, dynamic membranes with tunable pore size (DMTS), consisting of micron size silica beads supported by a nickel metal sieve, were used to refine a coarse pre-emulsion obtained by rotor-stator emulsification. Two food-grade oils (sunflower oil and lemon oil) and three different oil fractions (20, 30 and 40 wt%) were selected, while protein concentration was kept at 1 or 2%. Flux during each emulsification cycle was recorded, and emulsions were analyzed by means of droplet size ( $d_{3,2}$ ) and distribution (span), Zeta potential and their stability was followed for 7 days.

Commercial BSF protein extracts (Hexafly) were further enriched following a sequence of solubilization and precipitation to have a final protein content of 65.40% of dry matter (Kp factor of  $5.60 \pm 0.39$  (JANSSEN *et al.* 2017)). Results show that BSF protein was able to stabilize sunflower oil at different oil fractions similarly to WP, but with slightly bigger  $d_{3,2}$  and span. Stability of these emulsions for 7 days shows a minor increase of  $d_{3,2}$  and span. As for lemon oil, BSF protein has shown better emulsifying performance than WP at high oil fraction based on the results of  $d_{3,2}$ , span and zeta potential. However, all the lemon oil emulsions stabilized with BSF protein were not stable for more than 3 days. The transmembrane fluxes in DMTS during the emulsification process varied between  $200 \text{ m}^3\text{h}^{-1}\text{m}^{-2}$  and  $670 \text{ m}^3\text{h}^{-1}\text{m}^{-2}$ . The results indicate the high potential of BSF protein extracts to stabilize O/W emulsions produced by membrane emulsification.

### Reference:

JANSSEN RH, VINCKEN JP, VAN DEN BROEK LA, FOGLIANO V, LAKEMOND CM (2017): Nitrogen-to-protein conversion factors for three edible insects: *Tenebrio molitor*, *Alphitobius diaperinus*, and *Hermetia illucens*. Journal of agricultural and food chemistry, 65(11), 2275–2278.

## Enrichment in $\omega$ 3 of *Hermetia illucens* prepupae from oilseed co-products

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Belgian fish farmers from the Walloon region are currently looking for alternative feed sources as they mainly produce rainbow trout, a carnivorous species with high protein and lipids requirement. Among the available new feed sources, *Hermetia illucens* (L. 1758) is a promising insect species with a polyphagous diet, a fast larvae growth rate and a short life cycle. Moreover, several studies have shown that the larvae composition in macronutrients is function of their diet and could be manipulated. The current composition of *H. illucens* larvae is characterized by a high-saturated fatty acids level ( $\pm 76\%$ ) with mainly lauric acid ( $\pm 55\%$ ) and do not meet the nutritional requirements of trout. The objective of this study was to adjust the profile in polyunsaturated fatty acids of *H. illucens* larvae by manipulating their diets. Nevertheless, the legislation governing insect rearing is constantly evolving but remains restrictive concerning the allowed feed ingredients for insect development. Consequently, this study focused on diet formulation from local plant co-products to reduce the ecological footprint of insect and fish productions. Considering the seasonality and low-fat content of the co-products, the diet formulations were supplemented with oilseed meal cakes. An experimental rearing has been developed and nutrition tests were conducted. After selecting populations (100 individuals/population) of young *H. illucens* larvae ( $\pm 0.01$  g), different diets enriched with flax and two rapeseed meal cake (chemical vs mechanical extraction) were tested in a controlled environment (60% relative humidity  $-27^{\circ}\text{C}$ ). Six incorporation rates per meal cake (10 – 20 – 40 – 60 – 80 – 100%) were tested in triplicate ( $n = 3$ ). The larvae were collected at prepupal stage and each population were lyophilized and cold ground into flour. Lipids from prepupae flours were extracted by Folch's method while fatty acids have been esterified and analyzed by gas chromatography. Protein and ash levels were also quantified respectively by Dumas' method and combustion. The proportions between saturated fatty acids (SFA) and unsaturated fatty acids (UFA) were specifically evaluated as well as the percentage of  $\alpha$ -linolenic acid (ALA – C18: 3 $\omega$ 3). The incorporation of meal cake has reduced the ratio SFA/UFA from 4.11 to 1.44 and increased ALA percentage from 0.72 to 15.83. The increase in the proportion of UFA in *H. illucens* prepupae flour should allow an increase of their incorporation in artificial diets for rainbow trout.

## From the larva to the feed: Safety and nutritional aspects

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As sustainable protein source insects are in the focus of feed and food production. With respect to the industrial-scale production [1, 2] the yellow mealworm (*Tenebrio molitor* L., *Coleoptera: Tenebrionidae*) is a convenient candidate. With respect to the insect composition, we can find in the industry different technologies for fractionating the insects or producing insect meal. At present, information about the fractionation process from industry are still limited. In general, it is possible to use the whole insect without a drying process in front of fractionation. The other possibility is the utilization of the drying process and a fractionation of the dried insect through a mechanical treatment. All these final products from these ways are different in raw fat content, raw protein content and in this context different in protein solubility or fat spoiling. Furthermore, the products have differences in the hygiene status.

The aim of this study was to compare different insect extraction methods. The results have shown that the processing or fractionation method has an important influence on the protein and lipid quality as well as the bacterial count. With respect to the fractionation the further processing of the protein flour in feed composition at first can be minimised the bacterial count and can make a better pellet quality with a reduced energy demand.

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## Cost-effective insect rearing through automation and side-stream valorization

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For the moment, the prices for insect products are quite high in comparison to other protein rich food/feed sources. This is mostly due to high labor costs (60.9% of all production costs). Last year an automated feeding line developed at VIVES for mealworms (*Tenebrio molitor*) was presented. The first prototype could be used to rear up to 50 tons of mealworms per year. Using this system, instead of feeding by hand, labor costs could be reduced by 75%. Consequently, the minimal selling price for a producer to get out of the costs dropped from 5.26 €/kg live weight to 3.71 €/kg. In addition, if this system would be upscaled to a production of 600 tons, the price per kg live weight could further be reduced until 2.48 €. Besides saving costs by automation and upscaling, the use of cheaper feed, using side streams, could also have a substantial impact given that feed costs represent 29.3% of all production costs. Our simulation showed that for an automated production facility with a production capacity of 600 tons of mealworms per year a price drop of the feed with 50% would mean that the minimal selling price reduces with 30%. Consequently, the price of 2.48 €/kg live weight mentioned above drops to 1.78 €/kg. Our simulations show that, due to the implementation of automation and the valorization of side streams, insect rearing systems can become cost-effective production systems.

## Where do the larvae come from? Morpho-physiological and behavioral reproductive aspects of black soldier fly adults and their industrial applications

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The current techniques applied to mass produce BSF do not take in account the negative impacts of such artificial environments on the fitness of males and females, individually. The researchers around this fly is mainly focused on their immature stage, with scarce data about how biotic and abiotic aspects could affect the reproductive performance of this specie. This study aimed to: 1) assess morphological features of the sperm and how females have adapted to it; 2) physiological aspects of the sperm under heatwave stress and senescence and how males would respond at molecular level; 3) thermoregulation of males and females and; 4) Do males and females control time of copula based on the physiological status of their mate? Light microscopy and SEM were performed to describe the general morphology of the reproductive tract of males, spermatogenesis process in testis from different age groups, the sperm storage of females, and measure the size of the sperm. TEM was used to assess the ultrastructure of the sperm in order to detail structures related with the reproductive adaptations of this brachycera group. The sperm viability was measured across ages and three temperatures using a cell counter (Nexcelom Bioscience, LLC) and the expression of heat shock proteins was assessed in young and old males. The thermal-preference of males and females was analyzed using a thermal gradient metal plate with a heat and a cooling source in each extreme. The copula duration of adults under the same and different age groups, were recorded and correlate with biometric measurements of males and females from each assay, respectively. Understand the rules of the system (i.e. sperm morphology, physiology) and how it operates under certain conditions (i.e. environment stress, nutrient restriction) are the pillars to successfully up scale the production of BSF for an industrial setting. The optimization of the techniques applied for mass produce insects will become an important asset in order to meet the suppliers' necessity – especially regarding to optimize production at a low cost, which will reflect on the price of the final product. If the insect industry wants to compete with the livestock and poultry industry, it needs to find a balance in offer a high-quality product with competitive prices.

## Intraguild predation and cannibalism among adult *Ruspolia differens*: Towards overcoming challenges for mass-rearing

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*Ruspolia differens* has received considerable interest in recent years primarily due to its widespread consumption as an edible insect across Sub Sahara Africa. Currently, these grasshoppers, which are considered as delicacies, are only available in large quantities twice a year during the rainy seasons. Therefore, it comes as no surprise that there is great research interest in studying their farming potential for possible exploitation in the future by mass-rearing. However, due to cannibalism, such attempts have proven challenging. This study evaluated trials that may curb predation and cannibalism by providing alternative preys to reared *Ruspolia*. Groups of ten young adult females or males were each kept in well-aerated cages (50 x 50 x 50 cm<sup>3</sup>). In the no-choice set-up, twenty L2 larvae each of black soldier fly (*Hermetia illucens*), stemborer (*Chilo partellus*), fruit fly (*Bactrocera invadens*) and desert locust (*Schistocerca gregaria*) were administered separately to both male and female cages for six successive days (first three days living prey and next three days dead prey), with a 24 h washout period between each diet change. In the choice set-up, a mixture of twenty living preys was fed for 12 consecutive days (first six days without and next six days with artificial diet added). The control diets, artificial diet only and artificial diet + grass, were fed for 6 days consecutively. For each condition, the proportion of cannibalism was recorded. Cannibalism was significantly reduced in cages where prey had been administered compared to the controls. There were indications of a hunting behaviour as the predators tended to feed more on living than on dead insect preys. Thus, the addition of live insect prey could reduce the prevalence of cannibalism. This observation can serve a dual practical purpose: (i) preventing colony collapse due to cannibalism and (ii) provide a safe means to eliminate insect pests, which could serve as prey.



## Modularity of insect production and processing as a path to efficient and sustainable food waste treatment

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Currently up to half of the food produced is wasted. The wasted food is treated with commonly used methods which in their turn negatively affect the environment due to emissions associated with biomass decay and need for extensive transportation, storage and processing. Modular systems of food waste treatment, applicable for a range of cases along the food supply chain foreseen as a potential solution. This study is aimed at the design and assessment of insect-based system in economic and environmental aspects for the conditions of Germany. Selection of insects as bio transforming agents is associated with their ability to convert low value food waste into biomass (rich in proteins and fats) and frass which can be sold for other purposes. *Tenebrio molitor* and *Hermetia illucens* are exemplary considered to feed on side streams composed of brewery grains, potato peels and overdate food from retail. For *T. molitor*, no life cycle assessment (LCA) is performed, as this species does not fulfill the design requirements of modular system. Modular system was designed as a semi-continuous container-based system with cleaning in place system and microwave drying, capable of utilizing up to 3.89 t of food waste with a payback period of 2–3 years depending on the selling price of larvae. For *H. illucens* the results of the economic analysis and LCA show the dependent performance of the system on the type of waste. If *H. illucens* is feed on brewery grains or overdate food, they are more environmentally and economically efficient than incineration and anaerobic digestion. Compared to composting, *H. illucens* is environmentally more efficient and economically comparable to the operating cost of composting. If potato peels are considered as feed for insects the commonly used waste treatment methods show overall better results.

## **G × E interactions: Larval performance of distinct black soldier fly genotypes grown on different feed substrates**

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Black soldier fly (BSF) larvae are considered as promising dietary protein source for aquaculture and poultry feeding, as well as for efficient nutrient recycling through organic waste treatment. Life history performance and body composition of BSF larvae has been shown to be extremely variable, thus hampering predictions of nutritional quality of insect-based feeds and economic performance in general. Feed substrate for BSF larvae is one major driver for the observed variation, as documented in a number of reports. Yet, pronounced differences are still indicated for comparable feed substrates across studies. These have mostly been attributed to different experimental settings whereas potential influences of BSF genetics have largely been neglected, primarily because appropriate genetic monitoring tools have been lacking so far. Building on a global population genetics survey of BSF based on newly developed polymorphic nuclear genetic markers, a fully crossed factorial design was designed to compare performances of four genetically distinct BSF strains grown on three feed substrates with six replicates each. Various growth and compositional traits were determined. Both factors, feed substrate and BSF genetics, as well as the interaction between the two, were found to have a highly significant impact on virtually all investigated life history traits (mortality, growth dynamics, average larval weight) and body compositional characteristics (dry matter, organic matter, crude protein, ether extract) of harvested larvae. The same applied for feed conversion ratio, nitrogen efficiency, degradation of fibre as well as nitrogen and carbon emissions. Frequently detected stronger differences between genotypes within feed substrates than within genotypes across feed substrates show that BSF genetics is key for interpreting and triggering outcomes. However, it can also be inferred that larvae of different genetic origin have different nutritional requirements, further suggesting that optimal feeds may vary across BSF strains, or vice versa, that based on the choice of the latter, different available feeding substrates may be exploited with different success. Our results imply that, depending on targeted objectives for implementing BSF products, efficiency and sustainability of BSF productions may be substantially increased by thoroughly considering BSF genetics and interactive effects with feed substrate composition. Ample indication for BSF genotype-specific effects also provides valuable insights for pinpointed BSF breeding strategies.

## Insects as food – A pilot study for industrial production

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Despite the many papers reporting on disgust factors of eating insects in Western cultures, the interest of insects as food is increasing, not least because they are nutritious, sustainable and tasty! The time has come to take the next step by making insects available not only as delicious restaurant food, but also for industrial production of foods and meals based on insects. The sensory attributes are of greatest importance to increase understanding of insects as a main ingredient in production and shelf life.

By the use of factorial designs with mealworms as main ingredient, the aim of this study was to evaluate the sensory impact of additions such as salt, oil/water and antioxidant agent. Also the impact of particle size of the mealworms was evaluated.

Cooked fresh mealworms, cut or ground into different particle sizes, oil, water, salt and rosemary were blended according to a factorial design. The resulting products were evaluated by descriptive sensory analysis in addition to instrumental measurements of viscosity and colour. Nutritional contents were calculated.

Results showed that particle size of the mealworms had a great impact, i.e., an increased particle size increased the yellowness and the perceived coarseness. Further, both viscosity and crispiness increased. An increased particle size also led to decreased odour, probably due to decreased exposure of particle surface. Increased salt content did, as expected, increase saltiness. It also increased the nutty flavour, probably due to the polarity of sodium chloride. Different ratios of oil/water did not seem to impact the sensory properties. Due to the anti-oxidative effects of carnosic acid and carnosol, addition of rosemary had a significant impact on shelf life in terms of decreased rancidity and colour changes. All samples were high in protein content.

All factors, but especially particle size of the mealworm fraction, influenced the sensory attributes.

## Physical functionality of *Tenebrio molitor* and using this knowledge to improve food applications

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*Tenebrio molitor* (common mealworm) is one of the most promising insects, when it comes to food in the future. The common mealworm rearing is well studied and is already put to use in several production facilities today. However, the characteristics and functionality of the mealworms as an ingredient in food is not as well studied.

We have tested the physical properties of three kinds of grinded *Tenebrio* meal; i) high fat mealworm ii) normal fat mealworm iii) fat reduced mealworm

The physical properties tested include water binding, lipid binding, foam stability, emulsion stability, solubility with more. The focus of the study is to compare the three different *Tenebrio* meals, to estimate how significant the fat content in the meal is, when it comes to physical properties.

Using this knowledge, have helped improving recipes for different applications, mainly in different bread applications. A consumer test and visual evaluation of these products will be presented.

Finally, the substitution of wheat flour with *Tenebrio molitor* meal in bread baking have been tested with focus on overcoming the decreased raising abilities, due to the decreased gluten content. An enzymatic approach is used, and key results will be presented.

## Effect of temperature and insect:meat ratio on structure formation in hybrid batters

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Processing insects into hybrid meat products may be a good way to reduce meat consumption, while simultaneously introducing insects to our food. The effect of heating temperature (T: 70 and 80°C) and insect:meat ratio (0:100, 25:75, 50:50, 75:25 and 100:0) on structure formation in hybrid batters was therefore studied.

During batter preparation, insects (*Zophobas morio* larvae) and/or meat were ground in the different ratios in presence of sodium phosphate buffer (0.3 M, pH 6). The final batters contained 0.6 M NaCl and 1.6% nitrogen. Viscoelastic properties of the batters were studied during a temperature sweep (1 h at 70 or 80°C, cooling to 20°C and 1 h at 20°C), which was immediately followed by a frequency and stress sweep. Furthermore, the raw batters were heated isothermally at 70 or 80°C and stored (3°C, 7 days) after which cooking loss, total expressible fluid (TEF) and hardness were studied. Results were statistically analyzed through two-way ANOVA and a Tukey's post hoc test ( $p < 0.05$ ).

Insect batters (100:0) and meat batters (0:100) possessed lowest and highest gel strength and structure stability, respectively, with intermediate values for the hybrid batters (25:75, 50:50, 75:25). However, increasing the meat content in the latter (25 to 75%) had little effect on the viscoelastic properties. Furthermore, increasing T resulted in higher gel strength and structure stability in all batters, with similar values for hybrid batters heated at 80°C compared to meat batters heated at 70°C. However, for both heating temperatures, all hybrid batters had a similar hardness compared to the insect batters, which was considerably lower than that of the meat batters. As for the water holding capacities, meat batters heated at 80°C showed the highest cooking losses. Meanwhile, the insect and hybrid batters (70 and 80°C) and the meat batters (70°C) all had similar properties, with little or no cooking loss and only little TEF.

These results suggest that the insect:meat ratio has little effect on the properties of hybrid meat products and that by heating these products at higher T (80°C), similar viscoelastic and water holding properties may be obtained compared to regular meat products (heated at 70°C), while the texture remains inferior.

## Volatile profile and odor attributes of mealworm, grasshopper, and silkworm dried by different methods and incorporated into cookies

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Odor evokes positive emotions or causes negative associations that may be crucial for the consumer acceptance of novel foods. In this regard, our study aimed to analyze the effect of different drying methods (lyophilization, oven- and microwave drying) on volatile profile and odor attributes of mealworm larvae (*Tenebrio molitor*), adult grasshopper (*Locusta migratoria manilensis*), and pupae of silkworm (*Bombyx mori*) as well as of 15% insect-based cookies.

The results showed that the volatile profile differs between raw insects, but 2-methyl- and 3-methyl butanal, dimethyl disulfide, limonene, and acetic acid were common major volatile constituents. All drying methods induced the release of alkanes (heptane, undecane), alcohol (2-methyl 3-buten-2-ol, 1-penten-3-ol), aldehydes (propanal, butanal, 2-methyl 2-butenal), esters (ethyl acetate, acetic acid butyl ester, propanoic acid butyl ester, 2-propanoic acid butyl ester), and ketones (2-butanone, 3-penten-2-one) with insect- and drying method-dependent variabilities in intensity. Volatiles, such as 2,5-dimethyl- and 2,6-dimethyl pyrazine, 2-ethyl-6-methyl pyrazine, which are typical for roasted foods, were detected in microwave-dried powder, suggesting the presence of Maillard reaction products that have been confirmed by FAST index. 2-methyl propanal, 2-methyl butanal, pentanal, 1-penten-3-ol, 2-heptanone were only determined in insect-based cookies, while their intensity varied as a function of insect and a method of drying. Also, drying led to powder darkening at different levels that was consequently reflected in the darker color of cookies with mealworm, grasshopper, and silkworm, comparing to wheat cookies.

Odor intensity of microwave- and oven-dried mealworm and grasshopper, and microwave-dried silkworms was ranked by untrained panelists as the strongest that is in accordance with the results of GC-MS analysis. Microwave-dried silkworm, and oven-dried mealworm and grasshopper powder received the lowest overall odor liking scores, however, incorporation of insect powder into cookies increased odor attractiveness.

Thus, the choice of a drying method allows manipulating odor characteristics and intensity that can be used in novel food formulation.

## Crickets from farm to fork: Farming, composition, safety and use

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Over the last years, the interest for insects as food has increased. In Italy, insects are mainly used for pet food purposes and the concept of entomophagy still face issue. In a “from farm to fork” view, this study investigated the whole production of house cricket (*Acheta domesticus* L.) tackling growth, influence of age of slaughter on cricket proximate composition, and microbiological safety aspects.

Twenty thousand 24 days-old crickets were allocated in 10 containers (climatic chamber: 27°C, 70% RH) and fed a commercial cricket diet. Crickets were killed at two different ages (nymph (N) and adult (A), 52 and 61 days-old respectively) and frozen (−18°C). Crickets were successively dried (40°C for 72 hours) grinded and proximate composition assessed. On dried and cricket meal (CM), microbiological analyses were performed. Finally, biscuits were prepared using different inclusion levels of CM in substitution of the 25 (CM25), 50 (CM50), and 75% (CM75) of fat.

Crickets reached a final weight of 0.33 g (N) and 0.43 g (A). Proximate composition was not influenced by the slaughter age (crude protein: 51.8 (N) and 49.5 (A)% on dry matter (DM); fat: 28.0% (N) and 30.5% (A) DM). The fat content resulted higher than values usually reported in literature (about 15%). Since no differences emerged between samples, the subsequent investigation were carried out without distinguishing them. Since dried crickets microbiological analyses showed high values (CFU/g – total aerobic bacteria count:  $5.2 \times 10^6$ ; yeasts and moulds  $2.1 \times 10^4$ ; Enterobacteria:  $1.1 \times 10^5$ ; coliforms:  $2.4 \times 10^4$ ; aerobic spore formers bacteria:  $1.1 \times 10^6$ ), cricket were sterilized and grinded. As repeated microbiological analyses on CM still showed too high values, meal was rehydrated and sterilization process repeated. Final results for all the microbiological parameters investigated showed values from  $10$  to  $10^2$ . The cricket biscuits were characterised by a higher humidity and a darker colour. Also the texture was affected with an increased of hardness and chewiness. The biscuits that gave the best results were produced with CM25 as they were those in which the physical characteristics and hardness were more similar to the control (without cricket).

## Simulation of multi-stage air-separation of insects, faeces and feed residues in a Zigzag-separator

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For the industrial rearing of mealworms, it is necessary to find a practicable solution to separate faeces and other residues from mealworms. To serve hygienic aspects and to avoid seized up mealworms in sieves, a smooth air classifier with multiple stages (11 inclined paths) was constructed, to achieve an increased accuracy of the separation. For the scaling, the settling velocities of the components faeces, mealworms and exuvia were calculated.

Based on these result, simulations were performed to increase the efficiency of the separation by changing operation modes. The used software was Fluent (Ansys 2019 R1). The simulations was done with a k-epsilon RANS (Reynolds Averaged Navier Stokes) turbulence model. For the particle simulation the Discrete Phase Modell (DPM) has been used under consideration of gravity, pressure and collision with de DEM (Discrete Element Method) collision.

Mainly two modes were observed: the continuous separation and the batch separation. The efficiency is highly dependent on the operation mode, due to different combinations of inlets and outlets for air, pressure and feedloads.

With open inlets and outlets, which is the case during a continuous separation, the pressure and the flow behavior is highly dependent on the feedload/ mass flow. Fluctuations of the mass flow or a too big feedload leads to a collapsing airflow in the separation area and an increasing performance. With a circulating air closure, a more stable operation can be achieved. For the lab scale plant, a mass flow of ca. 1 kg/min can be reached.

A closed inlet and/or outlet in the batch mode results in a more reliable and robust separation. Also the mass flow can be higher at ca. 5 kg/min for the lab scale plant. But adding the feed with living insects requires additional methods and suitable airlocks, which have to be designed. The optimum performance level must be determined in further simulations.

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## Designing a cage for *Hermetia illucens*

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Rearing black soldier flies (BSF) on an industrial scale requires a constant and reliable source of eggs or newborn larvae. These can either be bought from a hatchery or, what is currently most common, bred on site. In any case, the space, labor and energy needed to breed them should be minimized. It is known that black soldier fly do need fairly specific climatic conditions to reproduce and that without these conditions no eggs or only infertile eggs are deposited. Although the basic conditions are roughly known, many aspects of black soldier fly breeding are still unknown or not optimized.

At the experimental insect pilot plant of Inagro and within the Entomospeed project, BSF has been bred for several years. The acquired knowledge was used to design a new BSF cage for our pilot facility. The initial requirements were:

- 1) Maximize the number of eggs per cubic meter,
- 2) Both batch and continuous breeding should be possible,
- 3) A single person has to be able to handle the cage,
- 4) Easy cleaning and hygienization,
- 5) Durable design that can last for years,
- 6) Versatile enough to be usable in all conditions.

After two years, the result is a durable aluminium cage of 120 by 80 by 140 cm with a plastic removable bottom plate and mesh on the sides. Two drawers are installed in the bottom plate for pupae and attractant. The drawer design ensures that the flies cannot reach the attractant and that the pupae can be added without entering the cage (minimizing possibility of escape). Wheels ensure that they can be easily transported to and from the climate room (e.g. to the wash station). Further experiments were performed to assess optimal climate conditions, light source (intensity and wave length), fly density, cage colour, interior design, etc. A proposal is made to scale up the cage to meet the industrial demand.

## Effect of sex and developmental stage on nutritional quality of insects

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Nutritional value of insects depends on many factors. We investigated the effect of sex on the nutritional value of the house cricket (*Acheta domestica*) and nutrient contents of subadults and adults of *Blaptica dubia*, *Blaberus discoidalis* and *Blatta lateralis* roaches.

Both sexes of house cricket were rich in protein and lipids. The proximate composition was partly influenced by sex; females contained a significantly higher amount of lipids (18.3–21.7 vs 12.9–16.1 g/100 g dry matter,  $p=0.0001$ ) and fewer proteins than males (61.2–64.9 vs 66.3–69.6 g/100 g dry matter,  $p=0.0001$ ). On the contrary, males contained more chitin ( $p=0.0015$ ) than females. Nutrient quality expressed as essential amino acid (72.3–77.1), thrombogenicity (1.22–1.45), and atherogenicity indices (0.53–0.58) did not differ between sexes ( $p > 0.05$ ).

In case of roaches, levels of crude protein, fat, ash, chitin, calcium and phosphorus were analysed. All adults contained more crude protein (60.2–67.8 g/100 g dry matter > 48.9–54.7 g/100 g dry matter) and ash (4.2–5.1 g/100 g dry matter > 3.6–4.0 g/100 g dry matter) but less fat (14.5–21.4 g/100 g dry matter < 23.6–36.3 g/100 g dry matter) than subadults. Chitin content ranged from 5.3 to 8.6 g/100 g dry matter and the calcium to phosphorus ratio was 1:1.9–23.5. Moreover, the amino and fatty acid profiles were also determined. The lowest amino acid levels were found in case of sulphuric amino acids cysteine and methionine. Essential amino acid index was found at the level of 0.4–0.9. The main fatty acids in analysed cockroaches were oleic (38.0–44.2%), linoleic (8.5–15.3%) and palmitic (21.6–26.8%) acids. The obtained results thus indicated that quantity of protein and lipids varied between subadults and adults of cockroaches within the species, while quality of these nutrients remained more or less stable.

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## The different forms of vitamin B<sub>12</sub> in edible insects – Sources and causes

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Insects are considered as highly nutritious, not only regarding major nutrients (e.g., fat & protein) but also in minor nutrients, such as vitamins. Among them, vitamin B<sub>12</sub>, also called Cobalamin is of the utmost importance for a balanced diet. It mostly occurs in food of animal origin, and edible insects are believed to be a good source of vitamin B<sub>12</sub>.

The proposed work will start from a recently published report on the content of vitamin B<sub>12</sub> in four edible insect species and the possible presence of biological non-active forms of vitamins B<sub>12</sub>, so-called Pseudocobalamins (SCHMIDT *et al.* 2019). By introducing a comprehensive ultra-high performance liquid chromatography method, comprising mass spectrometry, the entity of Cobalamins and related compounds in selected insect species was investigated. Mass spectrometry data not only confirmed Pseudocyanocobalamin as a major component, but also indicate the presence of other, lesser-known non-active forms of vitamin B<sub>12</sub>, such as nor-Pseudovitamin B<sub>12</sub> and Cobalt free corrinoids.

Considering that vitamin B<sub>12</sub> and related compounds can only be synthesized by a limited number of microorganisms, the gastrointestinal microbiota of a selected species was investigated using next-generation DNA sequencing. If the content of biologically active vitamin B<sub>12</sub> forms in edible species would be intentionally modified, conclusion from both analytical approaches must be drawn jointly.

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## Allergenicity assessment of edible insects and their protein hydrolysates

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In the last years, edible insects have been explored as a promising alternative source of proteins in order to overcome the future food demands connected to growing world population. From 2018, insects have been definitely declared to belong to the category of Novel Foods, making necessary deep investigations to assess their safety. The presence of allergens represents one of the main hazards connected to their consumption, also due to the potential cross-reactivity with Arthropoda pan-allergens.

In the present work we assessed the presence of potential allergens in *Hermetia illucens* and *Alphitobius diaperinus* larvae and explored the enzymatic hydrolysis as a way to produce hypoallergenic protein extracts. We performed a detailed protein characterisation of both insects by high resolution mass spectrometry on a LTQ-Orbitrap instrument, which represented the base for an in silico allergenicity assessment. The relevant potential allergens identified by similarity to known allergens belonged to two distinct classes, associated to distinct animal groups: crustaceans, which are very relevant for food allergies, and other Arthropoda, already known as being responsible for inhalatory or stinging allergies (midge, silverfish, cockroach and mites). Among them, the proteins belonging to tropomyosin family emerged as the most representative. Western blotting analysis with Tropomyosin I antibodies (isolated from rabbit) outlined a positivity in both insects, demonstrating that there is cross-reactivity between insect tropomyosin and the other tropomyosins to trigger an immunological reaction. The immunoassay was carried out also on protein hydrolysate produced from both insects with Protease from *Bacillus licheniformis*. After the enzymatic hydrolysis the protein-antibody binding disappeared completely, suggesting that this biotechnological process is a valid method not only to extract protein from insects but also to reduce the allergenic risk connected to their consumption.

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## Feeding study for the mycotoxin zearalenone in yellow mealworm (*Tenebrio molitor*) larvae – Investigation of biological impact and metabolic conversion

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Along with the increase in the global human population, there is a rising demand for protein rich refined food of animal origin. Conventional livestock will not be able to cover this growing demand. Thus, there is an increasing need for protein from alternative sources in the future. Additionally, efficient sources for feeding carnivorous livestock are required. Insects could be used as an additional source of animal protein incorporated in food and/or feed. The processed proteins of yellow mealworm larvae (*Tenebrio molitor*) are, together with six other insect species, legally allowed in animal feed. Before considering *T. molitor* larvae as suitable for consumption, the possible presence of contaminants must be identified. One of the contaminants of most concern are mycotoxins. This report focusses on the effect of the presence zearalenone (ZEN) and its metabolites on *T. molitor* larvae. Seven different diets were prepared, namely the; control, spiked (S1: 558.5 µg/kg ZEN; S2: 2254 µg/kg ZEN), artificially contaminated (A1: 427.0 µg/kg ZEN; A2: 2283 µg/kg ZEN) and naturally contaminated (N1: 602.3 µg/kg ZEN; N2: 919.3 µg/kg ZEN). The amount of ZEN and its metabolites in the feed, larvae and the residue (combination of left over feed and faeces) were measured by HPLC-MS/MS. A significant difference in individual larval weight gain was found between the diets containing naturally contaminated wheat (N1: 7.80 mg; N2: 9.25 mg) and the other diets after eight weeks of exposure. No ZEN or ZEN metabolites were detected in the *T. molitor* larvae after harvest. However, ZEN, α- and β-ZEL were found in the residue samples. Lastly, ZEN- and ZEL-sulphates were only detected in the control and the naturally contaminated samples. Thus, suggesting that mainly transformation to of either ZEN to ZEL or ZENS to ZELS takes place during larval metabolism. For all diets, survival was high and an average survival of 99.8% was found. For ZEN the study indicates a low risk for toxin accumulation in the edible parts of the insect. However, the intense formation of the more potent metabolite α-ZEL should be addressed in further studies.

## Effects of selected insecticides on black soldier fly (*Hermetia illucens*) larvae: Bioaccumulation, mortality, and growth

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Certain persistent insecticides, which may be present in the substrates (even at levels below the legal limit) on which reared insects feed, may affect these insects' growth or survival rate; or may bio-accumulate in these insects. The objective of this study was to assess potential effects on black soldier fly (*Hermetia illucens*) larvae. The pesticides to be analyzed in this study were selected using a step-wise approach; based on national and commercial monitoring data, and such that different modes of action would be represented. The experiment was performed in two phases. In phase 1 batches of commercial maize-based feed were spiked to the European Union maximum residue level (MRL) for that pesticide. Depending on the effects on the insects at these levels in phase 1, higher or lower concentrations were used in phase 2. The results of both phases showed that six of the nine tested insecticides (chlorpyrifos, propoxur, tebufenozide, endosulfan, imidacloprid, piperonyl butoxide (PBO)) did not negatively affect the survival rate or increase in biomass, compared to the control treatments. In addition, the concentration of most analyzed insecticides was relatively low in the larvae (< 5%) compared to the concentration in the feed. Imidacloprid and endosulfan appeared to have a beneficial effect on the growth rate of BSF, but the reason for this is unclear. Fipronil accumulated in BSF larvae to extraordinary levels, with an average recovery of its metabolite fipronil-sulfon in the larvae 12.5 times higher than in the feed. The insecticides spinosad and cypermethrin affected growth and survival negatively, with the effects of the latter being augmented by addition of the synergist PBO. Total recovery of these two pesticides in larvae and excreta was significantly elevated compared to recovery of the other pesticides, suggesting that accumulation played a key role in their toxicity. We recommend for policy-makers to reconsider the MRLs of spinosad, cypermethrin and fipronil, due to the risk of these substances at the current legal limits for the growing insect industry. In the meantime, BSF farmers are recommended to target analyses of their incoming feed materials for these insecticides, before using it as substrate for the larvae.

## **Insect digestion does not generate active oxygen compounds, demonstrating a nutritional advantage over meat consumption**

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Meat consumption is highly correlated with cardiovascular diseases and several cancers. The most common explanation for this is the strong positive correlation between the amount of consumed meat and animal tissues and the concentrations of active oxygen compounds found in the consumers' digestive tract and blood.

In this study we examined the formation of active oxygen compounds during digestion of three types of edible insects, *Hermetia illucens*, *Apis mellifera* and *Locusta migratoria*, and during digestion of turkey meat, which was used as a control. We used an in vitro system that simulates protein digestion in the human stomach, and measured the active oxygen compounds formed by two methods, thiobarbituric acid reactive substances and ferrous oxidation in xylene orange.

Our results showed for the first time that during digestion of the tested insects the active compounds remained below threshold in contrast to their increase in the control group. In addition, it was found that the methods for preparing turkey meat caused an increase in the amount of active compounds, with frying causing the largest increase followed by baking and cooking, while the amount of active compounds in the examined insects that were fried, baked or cooked remained below threshold. The length of time stored by refrigeration or freezing, or as dry flour, also caused an increase in the amount of oxidizing compounds in turkey meat, but no changes were observed in the examined insects.

The findings of this study suggest a nutritional advantage for the consumption of whole insects and insect-based flour. We are now working on verifying these results in an in vivo model system of laboratory mice.

## The potential of the black soldier fly bio-converted rearing substrate as a plant growth enhancer

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Since 2015 BioBee Sde Eliyahu is developing production of high value animal feed products from larvae of the black soldier fly (BSF, *Hermetia illucens*). In their mass production, the BSF larvae are treating and processing organic material from food industry waste, bio-converting the waste into body mass. A significant by-product of this bioconversion process is the used rearing feedstock that also contains larval frass and exuvia (empty insect's skins after molting). This substance is treated thermally and ends up as a dry nutritious powder, with the potential traits of plant growth enhancer.

In a series of semi-field trials, the effect of the treated substrate was evaluated on cucumber plants. The trials were conducted under greenhouse conditions on young potted plants and included several application dosages compared to commercial Vermicompost and an untreated control. In addition, a phytotoxic effect of the BSF substrate at the different dosages was tested. The plants that were treated with the BSF substrate had higher growth index compared to the plants treated with Vermicompost and the control plants. That was demonstrated in plants height, number of leaves and their length. With respect to yield, in the BSF substrate treatments the yield was obtained earlier and accumulated to higher weight compared to Vermicompost and control treatments. There was no visual evidence of phytotoxicity in the plants that were treated with the BSF substrate. The findings above indicate that BSF used and treated feedstock substrate can be regarded as a soil conditioner and plant growth enhancer. A new series of trials and observations are currently being performed in order to validate and confirm the initial findings.



## Insects as an alternative source for chitin or chitosan

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Chitin is an insoluble polysaccharide, present in crustacean, insects and fungal, with a wide range of possible applications. Recently, there has been a lot of interest in the use of insects for food and feed applications. Large scale breeding will generate side streams, one being a chitin-rich waste streams. Larvae of the black soldier fly for example, shed their exoskeletons five times to grow into their final larval stage. These new side streams can be utilized to extract chitin or chitosan.

The demand for chitin/chitosan is growing fast and is expected to expand with an exponential value CARG of 13% throughout 2017–2027. Currently, crustacea waste streams are the main source for extracting chitin. Production of crustacea-based chitin involves multiple processing steps and is not favourable in terms of environmental aspects. Insects and side streams from insect breeding however, create a new, alternative, seasonable independent chitin source.

Chitin is often converted into a more water-soluble derivative, like chitosan. Deacetylation of crustacean chitin is traditionally performed with high NaOH concentration (40–60 w%), high temperatures (60–120°C) and long reaction times (1–8 h). Limited information on purification and deacetylation of chitin originating from insects was found in literature. Due to a different origin, an optimal deacetylation procedure can alter from the one of crustacean chitin and perhaps be more economically favourable.

This study focused on the extraction of chitin and its further conversion to high molecular chitin from the black soldier fly larvae using different approaches. The aim was (1) to tailor the traditional methods (based on crustacea) towards insect chitin, (2) decrease the amount of process waste and (3) to produce chitosan with different characteristics (degree of deacetylation, ...). Chitin originating from insects and shrimp (crustacea) were compared. Chitin extracted from the black soldier fly larvae proved of similar quality compared to shrimp chitin. The result proved that a process with less chemical and/or milder process parameters was feasible on lab as well as pilot scale.

The study is part of the InDIRECT project ([www.bbi-indirect.eu](http://www.bbi-indirect.eu)) that receives co-funding from BBI-JU under EU-Horizon2020 research and innovation program under GA-No720715.

## INFACT: From Insect to surFACTant

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Black Soldier Flies (BSF) can be cultivated on organic waste streams, thereby providing a useful system for converting waste into biomass. After harvesting the larvae, bio-based chemicals like chitin, proteins and fats, can be extracted and implemented in technical applications e.g. cosmetics (VERHEYEN *et al.* 2018).

BSF-fat contains about 50% of lauric acid and the fatty acid (FA) profile resembles the profile of coconut oil (CO) and palm kernel oil (PKO). Because BSF can be reared massively on small surfaces using organic side streams, they may provide a durable alternative to CO and PKO, which have a large impact on land-use and loss of biodiversity.

In the INFACT-project, FA derived from the BSF and CO are coupled to amino acids (gly and lys) to generate amino acid-based surfactants. The surfactants are made by chemical procedures, and a more green thermal amidation process is also investigated (LIU *et al.* 2018). This type of surfactant (produced commercially using CO) is non-toxic, mild and biodegradable and can be used in cosmetics and plant protection applications.

After hydrolysis of the fat and conversion of the FA into acid chlorides, gly-FA surfactants are synthesised with the Schotten-Baumann reaction (SBR) at a yield of approximately 65% and > 95% purity. Yields for the thermal amidation protocol are more variable (average 55%) and the purity is variable but always > 70% and after additional purification steps a final purity > 90%.

Lys-FA surfactants have been synthesised with the SBR (WU *et al.* 2014), but purity is < 50%. The mixture is complex, containing double and single bound surfactants and a lot of free FA. Using a TBTU coupling reagent (GOMES *et al.* 2008), better yields of (> 90%) double bound surfactants are obtained, but the product also contains a large proportion of currently unknown constituents.

The gly-FA surfactant (chemically and thermally synthesised) has been evaluated for solubility in water, surface tension reduction, CMC, foaming and emulsifying capacity and performs similar to the same type of surfactant when synthesised starting from CO. An initial evaluation of the gly-surfactant in cosmetic-formulations (shower gels, hand creams) is ongoing. Future work will evaluate the lys-surfactants in cosmetic applications as well.

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## Novel polycistronic expression systems for specialized protein production

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Modern techniques in insect biotechnology offer immense opportunities to create economically and ecologically sustainable solutions for protein production. While large insect rearing factories are built to produce protein for food and feed, the challenge of further upscaling those productions to significant quantities is taken up by several companies. Another exciting field is the production of specialized proteins that could be expressed and isolated from insects. Besides the selection of the proteins and the optimal production host, an efficient and scalable expression system is needed. Here, we evaluated the use of novel gene editing approaches and viral 2A peptides for polycistronic and stoichiometric protein production to make the systems both faster in their generation time and more versatile. In the Mediterranean fruit fly, *Ceratitis capitata*, we were able to increase the CRISPR/Cas9 targeting efficiency and integration of point mutations to specific genes to up to 90% to improve the ease of use of this technology for laboratory experiments. To increase the versatility of expression systems needed for protein production systems in insects, we also experimented in a proof-of-principle approach with multiple 2A peptides and differentially localized fluorescent proteins. Experiments were conducted in cell lines as well as in transgenic strains of the spotted wing drosophila, *Drosophila suzukii*. We found efficient ribosomal skipping for all tested 2A constructs, resulting in localized and predictable overproduction of proteins in insects. This highlights the potential of such technologies to be used in controlled protein production systems in the future.

## Industrial bioconversion of meat waste by larvae of *Lucilia Caesar* L. (Diptera Calliphoridae) flies

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Today, there are known technologies for manure bioconversion using the culture of the *Musca domestica* L. (Diptera, Muscidae) house flies, and technologies used for the break-down of food waste by the larvae of *Hermetia illucens* (L.) (Diptera, Stratiomyidae) flies. A common disadvantage of breeding these species is the relatively low protein content in feed meal obtained from the larvae of these species. Introducing industrial cultivation of the *Lucilia caesar* L. flies' culture, we get high protein content in larvae, and also have a shorter cycle of development of the larvae (unlike *H. illucens*). Substrate after bioconversion becomes a high-value amino acid fertilizer. This discovery promises to make the use of these species more valuable.

This paper innovatively proposes to mass cultivate the imago of *L. Caesar* flies and their larvae in an industrial setting. It is suggested to cultivate the species during the industrial waste utilization process, with further use of larvae for feed meal production. Research results were obtained from more than 5 years of industrial cultivation of *L. Caesar* flies and can be used as grounds for creating sites for bioconversion of meat and food waste.

As part of this work, an experiment was conducted to include a protein-lipid concentrate (PLC) from *L. caesar* larvae, instead of fishmeal, in the diet of weaning piglets. As a result of the experiment, animals of the experimental and the control group did not have significant live weight differences. Calcium and phosphorus levels in the diet of animals with PLC have improved compared to the control group. Additionally, animals of the experimental group have showed an improvement in the qualitative and quantitative composition of micro flora, which helps to reduce the number of dysbacteriosis cases and diarrhea. Data obtained from this experiment confirms the possibility of including protein-lipid concentrate in piglet diets to replace fishmeal in an amount of up to 2% of the total weight of the compound feed.

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## Sustainable business model patterns – An integral part of insect-based business models

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This presentation will reflect part of a doctoral thesis (published 12/2018) which dealt with the Insect Economy. This is a concept that is defined as a subset of innovative and sustainable products, processes, and services resulting from the Bio-Economy which bases on the use of insects as biological resources.

The thesis itself builds on a sound understanding of the problem context of the societal challenges, the Sustainable Development Goals (SDGs) and on the underlying academic frameworks, namely sustainable entrepreneurship, sustainable innovation and sustainable business models, and the underlying policy framework, namely the Bio-Economy. It followed two mayor research objectives: (1) identifying, describing and characterizing business opportunities around insects; and (2) assessing and classifying sustainable business opportunities.

The focus of the presentation at Insecta will lie on the second research objective. More specifically, the business opportunities concentrated on insects as feed, food, pet food, and waste converters as those are currently very dynamically developing opportunities (TAPONEN 2017). The data were collected through 20 qualitative interviews with CEOs.

The interview questions focused on eight dimensions to assess sustainable business opportunities. Three out of them are Business Model Canvas, Business Model Innovation Grid (BMIG) (BOCKEN *et al.* 2014), and Sustainable Business Drivers. For the data analysis, MAXQDA software was used.

Out of the eight dimensions, the BMIG resulted to be the most insightful dimension. The BMIG is a practice-oriented tool which aims to inspire businesses to reconceive the way they operate and become more future proof. Due to its structured format, the tool is suitable for data classification and was used to develop a typology.

The Business Model Innovation Grid constitutes among others of 52 mechanisms for sustainable business model innovation. During the data assessment 17 out of 52 mechanisms for sustainable business model innovation were recognized as part of the 20 assessed business models. For the data classification, these mechanisms were structured in the form of a typology which resulted in four types of sustainable business opportunities based on insects.

The results can be seen as a source of inspiration to identify business opportunities that provide opportunities for sustainable development. Thus, it helps entrepreneurs to see that contributing to sustainability does not need to be a philanthropic ambition, but societal goals and business goals can be tied together. In research, it may provide an approach to assess and classify other insect-based solutions from a sustainability perspective.

## Insect welfare in food and feed production

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Large-scale rearing and processing of insects raise novel questions including those about animal welfare and best ethical practices. Up to now, no animal welfare policies exist that apply specifically to insects. However, concerns arise from the possibility that insects could experience pain, suffering or other form of negative emotional states during rearing, harvesting and inactivation. Currently it is not confirmed that insects can have such responses. However, both from a productivity standpoint as well as considering evolving human ethical standards it seems prudent to develop rearing and processing techniques that minimize stress.

Brambell's five freedoms offer a comprehensive framework to establish good animal welfare practices. The concept is well adaptable to insects, because it defines ideal physiological states rather than standards for acceptable welfare. The five freedoms state that all animals under human care should have (i) freedom from hunger and thirst, (ii) freedom from discomfort, (iii) freedom from pain, injury or disease, (iv) freedom to express (most) normal behavior, and (v) freedom from fear and distress. Unfortunately, negative emotional states are often not directly observable. But it can be assumed that the presence of such states, if they do exist, will coincide with the presence of other physiological states that are observable, namely those associated with stressful conditions.

In our work, stress was assessed by quantifying levels of a stress-related insect hormone. Larvae of the black soldier fly (*Hermetia illucens*) were exposed to a variety of stimuli associated with existing and potential insect processing procedures. Both lethal and non-lethal treatments were applied. We have demonstrated that black soldier fly larvae exhibit a distinctive range of stress response that non-linearly scales with stimulus intensity. Even though there is still no conclusive evidence whether or not insects are conscious, the results still support recent findings from other work that they likely exhibit evolutionary precursors of emotions. Therefore we suggest that the development of insect-based food and feed production techniques should aim to minimize insect stress in order to ensure continued productivity and ethical treatment of the animals.

## **Edible insects in the German market – From online niche to retail mainstream**

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Insects are a promising alternative to conventional mammalian livestock offering high-quality nutrients at a lower environmental impact. However, the adoption of insects as food requires to overcome traditional taboos and acquired aversion from all stakeholders: from producers over retailers to consumers. Further, widespread adoption needs widespread availability. SWARM produces convenience snacks with ground crickets. While initially focusing on a narrow target group online, SWARM products are now widely spread in the major food retail chains in Germany. The presentation covers the marketing of edible insects, direct consumer reactions, and the process of getting insects on the shelves of brick-and-mortar retail.

## **Biorefinery approach for conversion of organic side-streams into multiple marketable products using insects – InDIRECT project**

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'Management of waste as a resource' was described in a European roadmap (COM (2011/571)) as a milestone to be reached by 2020. On the one hand, there are diverse under-spent side-streams situated at the farmers and post-harvesting level as well as in the associated retail sector. On the other hand, there is a need for new resources, for instance proteins as alternative for soy proteins.

InDIRECT, a three-year EU research project started November 2016, envisioned to develop biorefinery processes as part of new value chains to convert under-spent side-streams from the agrifood-sector and processing sector into useful marketable products. Cascading processes (recovery of multiple compounds from the same feedstock) were envisioned to increase the conversion efficiency and maximise the values of the feedstock. One of the biorefinery approaches considered is a two-step process that can cope with the heterogeneity of side-streams. In a first step the heterogenic feedstock is converted to homogenous biomass using insects. Insects are able to convert a variety of feedstocks into a more homogenous biomass, being their own biomass. In a second step, the insect biomass is further fractionated into a lipid, protein and chitin fraction, that all three have potential towards marketable end products. Fractions from lesser mealworm and black soldier fly were produced for application tests in feed and chemical applications. A general overview of the findings will be presented.

The multidisciplinary InDIRECT consortium consists of 9 partners from four countries (Belgium, The Netherlands, France & Italy), comprising 2 research organisations (VITO & University of Parma), 5 industrial partners (Nutrition sciences NV specialized in feed additives, two insect farms Millibeter & Protifarm R&D, IMPROVE specialized in plant proteins, and CHEMSTREAM focusing on chemical application), a non-profit organisation that works in close contact with farmers and processing industry (Innovatiesteunpunt) and a project supporting company (Temperio). The project ([www.bbi-indirect.eu](http://www.bbi-indirect.eu)) is coordinated by VITO.

This project received funding from the Bio Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation program under grant agreement No 720715.



## Impact of different meat starter cultures on pH reduction, microbial community dynamics, and glutamic and aspartic acid production during mealworm fermentation

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Previous research indicated that it is possible to apply a fermentation process on a paste made from mealworms. The addition of a starter culture, however, was indispensable to obtain a rapid acidification and to inhibit undesirable microorganisms (e.g. sulphite reducing clostridia) during fermentation. Although numerous starter cultures exist for the meat sector, no starter cultures are designed for the fermentation of insects. However, meat starters are expected to grow and metabolize well on the insect substrate too. In this study, six commercially available meat starter cultures were tested for their ability to ferment a mealworm paste. During fermentation, the evolution of pH and microbial counts were monitored and high-throughput 16S rRNA gene sequencing was used to unravel the changes in the microbial community composition. In addition, Ultra Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS) was applied to monitor the glucose consumption and the free amino acids Asp and Glu.

The application of the six starter cultures resulted in a fast prevalence of the species involved, coupled to a rapid acidification to a pH below 4.95. All starters were able to outcompete *Bacillus* and *Clostridium* species, who were dominating the control samples. Of the six starter cultures tested, *Lactobacillus curvatus*, was most promising, as its application resulted in the largest increase in the content of the free amino acids Asp and Glu. These amino acids are responsible for the well-appreciated umami flavour in fermented food products and might stimulate the acceptance of insects and their consumption.

## Effect of high hydrostatic pressure (HHP) processing on colour and textural properties of mealworm species *T. molitor* and *Z. morio*

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High hydrostatic pressure (HHP) is a non-thermal minimal processing technology which uses high pressures to inactivate and kill microorganisms and enzymes, creating a shelf-stable product which has little changes to its organoleptic properties. Insects for use in food applications is a promising alternative to livestock based products which make use of far more land, water and resources, and has a detrimental effect on the environment. One of the challenges of insects for use in food applications is the undesirable colour changes which result during processing. In order to make processed insect products appealing to consumers, it is essential to find ways to inhibit the undesirable colour changes – HHP is a promising technology.

This study aims to determine the effect of high hydrostatic pressure (HHP) processing (100, 200, 300, 400, 500, and 600 MPa for a holding time of 300 s) on colour and spreadability of two mealworm species: *T. molitor* and *Z. morio* (after 120 s grinding). The colour ( $L^*$ ,  $a^*$ ,  $b^*$  values) of untreated and treated samples was measured over time (0, 5, 10, 15 and 20 min), and the total difference in colour ( $\Delta E$ ) and browning index (BI) was determined. The HHP treated samples, specifically the higher pressure treatments (400, 500 and 600 MPa) displayed less negative effects on colour change ( $\Delta E$ ) than the untreated and lower pressure treated samples. The browning index (BI) decreased as the pressure treatments increased, with the highest pressure treatment displaying the lowest BI value. HHP processing proved to have a significant effect on the inhibition of detrimental colour changes in *T. molitor* and *Z. morio*. There did not appear to be any significant effects on the spreadability of the treated samples, however if the chitin content is decreased there might be an effect.

## Evaluation of storage stability of dehydrated edible insects: Moisture adsorption isotherms, isosteric heat, estimated shelf life of flours of *Rhynchophorus phoenicis* and *Imbrasia truncata* larvae

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Edible insects are used as ingredients in food products such as cookies. Prior to that, they are often transformed into flour, which need to be stable during processing, packaging and storage. In this study, moisture adsorption isotherm of *Rhynchophorus phoenicis* and *Imbrasia truncata* larvae flours have been studied at 20, 30 and 40°C in order to evaluate, their storage stability and predict their shelf life under typical packaging and storage conditions. Insect flours were prepared by freeze drying and grinding of the whole insects. Moisture adsorption isotherms, i.e. curves representing the relationship between the equilibrium moisture content and the water activity, were obtained with the static gravimetric method and were fitted to six sorption models (BET, GAB, Hasley, Oswin, Peleg and Smith). GAB model enabled to predict behavior of the two insect flours and was chosen to determine the monolayer moisture content ( $M_0$ , i.e. moisture content theoretically ensuring microbial, enzymatic and chemical stability). The Clausius-Clapeyron equation was then used to estimate the net isosteric heat of sorption. Results showed that maximum water content corresponding to microbiological growth limitations ( $a_w < 6$ ) was 5.8 g/100 g dry matter (DM) flour for *R. Phoenicis*; 5.6 g/100 g DM for *I. truncata*. At 30°C, the  $M_0$  of *R. Phoenicis* larvae flour was 4.5 g/100 g DM; those of *I. truncata* larvae flours was 3.7 g/100 g DM. Highest net isosteric heat ( $q_{st}$ ) values obtained at low moisture contents, indicated high water binding energy at low moisture contents. The Lowest value of  $q_{st}$  was obtained for *R. Phoenicis* larva flour (1.9 kJ.mol<sup>-1</sup>.K<sup>-1</sup>) and the highest for value for *I. truncata* larva flour (21.0 kJ.mol<sup>-1</sup>.K<sup>-1</sup>). This result showed that low energy is required to dry *R. Phoenicis* than *I. truncata*. Using Heiss and Eichner model, shelf lives of insect flours were estimated at 3 months, if dried to a water content of about 3 g/100 g DW and stored in polyethylene bag (thickness: 80 µm; surface area: 0.266 m<sup>2</sup>; water vapor permeability constant:  $2.28 \times 10^{-6}$  kg µm.m<sup>-2</sup>.day<sup>-1</sup>.Pa<sup>-1</sup>). In conclusion, the studied insect flours must have a water content between 2 and 5.8 g/100 g DM to be stored at 20, 30 and 40°C without risk of chemical, microbiological or enzymatic alterations.

# Poster Session

## Biofortification of zinc and manipulation of its overall quantity in mealworm (*Tenebrio molitor*) larvae by using zinc-enriched substrate

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Current estimates predict that the human population might surpass 9 billion individuals by 2050 which presents the challenge of providing nutrition and battling malnutrition despite growing demands. According to the FAO, malnutrition in the form of zinc-deficiency affects about 30 percent of the world's population. In recent years, insects are being (re-)introduced worldwide as an alternative protein source to conventional livestock production. The yellow mealworm (*Tenebrio molitor* L.) is one of the most widespread species that are commercially produced; mainly on mixed-grain diets. Insects have been shown to accumulate heavy metals and micronutrients, making the yellow mealworm a promising candidate for zinc supplementation in human diets. Rearing the larvae in zinc-enriched substrate raised their overall zinc content to up to three times the control level. However, in-vitro gastrointestinal digestion of our test larvae shows that the bioaccessibility of zinc after drying of the larvae can be as low as 20–40%, depending on the drying process. One reason for the low amount of resorbable zinc might be phytic acid which has been shown to diminish zinc bioaccessibility in certain animals and is a major component in mixed-grain diets. Our research investigates the impact of a modified substrate composition on the quantity of bioaccessible zinc in mealworm larvae. The possibility to increase the larvae's zinc content and improve overall bioaccessibility of micronutrients by altering the substrate composition has the potential to combat zinc deficiency in humans and thus is a promising field of study where more research is needed.

## Growing conditions and morphotypes of African palm weevil (*Rhynchophorus phoenicis*) larvae influence their lipophilic nutrient but not their amino acid compositions

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Today, great attention is being paid to insects as a source of food and energy. The larvae of *Rhynchophorus phoenicis* (Rp), a coleoptera of Curculionidae family, also known as African palm weevil, are eaten in many African countries where they are considered either as a delicacy or used as alternative food by rural populations. These larvae were generally collected from the wild, but today, they now can be bred. African palm weevil larvae are a potential source of lipids and proteins of good quality for human consumption. To evaluate the influence of growing conditions on nutrient composition of larvae grown on different raffia palms, larvae (3 larvae batches per condition) were collected from the wild (yellow wild or white wild larvae) or from a breeding system (white larvae). Growing conditions influenced the morphometric parameters of the larvae and their lipid content and composition. As highlighted by principal component analysis, wild larvae exhibited greater morphometric parameters (weight, length) and higher energy, lipid, carotenoid and total fatty acid contents than larvae from breeding system. As compared to white larvae, yellow larvae contained more energy and more lipids ( $27.7 \pm 2.3$  g/100 g fresh weight vs around 19.8 g/100 g), less polyunsaturated fatty acids ( $0.5 \pm 0.1$  g/100 g vs 0.8 g/100 g) and tocopherols ( $2.25 \pm 0.23$  mg/100g vs around 4.5 mg/100g), and, explaining their color, more carotenoids (800  $\mu$ g/100 g vs 280–390  $\mu$ g/100 g). Rp larvae contained from  $7.8 \pm 0.3$  (white wild) to  $8.8 \pm 0.9$  (yellow wild) g/100 g proteins and high levels of indispensable amino acids, with no effect of growing conditions. Their nitrogen to protein conversion factors varied from  $6.08 \pm 0.3$  (white wild) to  $7.42 \pm 0.4$  (yellow wild), This work gives a new and reliable insight on nutrient content variations in palm weevil larvae. The lipophilic nutrient composition of *Rhynchophorus phoenicis* larvae depends on its growing conditions and morphotypes, but nor its amino acid compositions.

## Attributional and consequential life cycle assessment of industrial production of *Hermetia illucens* insect biomass for feed and food

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The lack of protein sources in several parts of the world is triggering the search for locally produced and sustainable alternatives. Insect production is recognized as a potential solution. This study is a life cycle assessment (LCA) of food industry side streams transformation via *Hermetia illucens* into intermediate products applicable for feed and food purposes. It relied on a dataset covering nineteen-month period (2015–2017) of *H. illucens* production and processing with measured variables of water use, feed inputs, electricity and heat consumption, production yields from an industrial producer (Protix, Dongen, The Netherlands). LCA applied attributional modelling for the estimation of the most impacting stages of insect production and on consequential modelling for the estimation of potential benefits or risks for the agrifood system. The consequential LCA included effects on the market, associated with upstream increase in feed (increase in commercial feed production) or downstream availability of insect product (substitution of fertilizer, protein concentrate for feed or chicken meat). Attributional and consequential LCAs are followed by sensitivity analyses, which identify the most promising directions towards sustainable insect production and estimate the magnitude of impact reductions if those directions are pursued by the industry. Analyses of the existing pilot process largely correspond with other findings in the literature, indicating fresh insect biomass is almost twice more sustainable than fresh chicken meat. Produced at pilot scale, protein concentrate (insect meal) while being competitive against animal-derived (whey, egg protein, fishmeal) and microalgae, has higher environmental impacts than plant-based meals. Further scenarios illustrate strategies for more sustainable use of environmental resources providing guidance for producers and funding agencies to direct the industry to an impact profile that is lower, than many existing protein sources.

## Greenhouse gas emissions of black soldier fly larvae grown on different feed substrates throughout larval development

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Protein derived from black soldier fly larvae (BSFL) is considered a valuable alternative diet component in aquaculture and poultry feeding. Through more efficient nutrient recycling from agri-food waste and mitigating over-exploitation of limited marine and terrestrial resources a twofold sustainability increase may be provided. Yet, imperative for overall life cycle assessments, more detailed data on environmental impact criteria of BSFL production, e.g. greenhouse gas emissions, are needed. In open circuit respiration chambers running at 27.5°C and 50% relative humidity 7 days old BSFL were incubated for 14 days during which four feeding events took place. Six identical pre-consumer food waste components were mixed in mirrored relative proportions of 1:3 for individual components in order to obtain two feed substrates representing a high fibre (A) versus a well digestible nutrient-rich feedstuff (B), respectively. Both feed substrates contained equal dry matter contents and were provided in equal amounts in each feed treatment. Three chamber replicates each comprising of triplicated boxes of 10'000 BSFL were monitored per feed treatment, including continuous measurements of CO<sub>2</sub> and CH<sub>4</sub> every minute, as well as N<sub>2</sub>O 4–8 times per day. Prior to each feeding event and at harvest larvae were separated from residue material (frass and non-digested feed remains) to assess phase-specific biomass gain. Separations that took place before harvest were temporary only, and larvae were placed back to their respective residue material again. While feed B yielded higher BSFL biomass production and lower feed conversion ratios, larvae of both feeding treatments survived equally and entered the prepupal stage by the majority within 14 days. All gas emission profiles were strikingly affected by feeding events for both feed substrates, resulting in temporarily extreme peaks. Also similar for both feed substrates individual developmental phases of BSFL exhibited quite characteristic responses, with strongly diverging patterns of decreasing biomass while maintaining substantial emissions towards the 6th larval instar. Overall, CO<sub>2</sub> emission profiles between feeding treatments were similar. Feed A resulted in higher CH<sub>4</sub> emissions, while higher N<sub>2</sub>O emissions were detected for feed B. CH<sub>4</sub> and N<sub>2</sub>O emissions during BSFL rearing may primarily coincide with particular conditions of type of feed, substrate depth and moisture, excess nutrients and associated microbiota. Total CO<sub>2</sub> equivalents were high and comparable to conventional livestock biomass production. Therefore, context-specific considerations of feed substrates and rearing regimes, and particularly larval growth period until harvest may positively influence outcomes of comprehensive sustainability assessments of a given BSFL feed protein production.



## Challenges and opportunities for insect rearing in Myanmar

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Entomophagy is common in Myanmar and insects represent an important part of the diet, especially for communities in mountainous areas. It is also an important livelihood source and insects are sold to big cities and traded with the neighboring countries, China and Thailand, which have vibrant markets. However, negative environmental effects of over-collection are reported, prices are high and insects from and in Myanmar remain a seasonal product. There are many good reasons for insect rearing. However, insect rearing in Myanmar still is in its infancy. The research investigated challenges and potential for establishing insect rearing in the country. Fieldwork was conducted in Yangon, Central Myanmar (Mandalay, Pyin Oo Lwin) and the mountainous Shan State (Taunggyi and Kengtung) to understand the farming systems, consumption and collection patterns and processing techniques. In addition experts and farmers in Thailand were interviewed, where the transformation from collection to rearing took place only a decade ago. Expert interviews, interviews with traders, vendors and consumers as well as focus group discussions with farmers served as valuable information source. The research revealed that over 50 insect species from different taxonomic groups are consumed in Myanmar and all insects (except for silkworm, bees and crickets from a few small farms) are collected from the wild or imported from Thailand and China. Insect rearing and processing is still underdeveloped in Myanmar. Despite the important role that insects play for livelihoods only a handful of individuals have started mini-farming in the country. The presentation puts some thought into the question of how collectors, farmers or small entrepreneurs can be turned into mini farmers, how rearing can be promoted and which obstacles exist and can be overcome to establish insect rearing in the country. It also wants to show how the insect sector in Myanmar can benefit from the well established and developing sector in Thailand.

## Modeling temperature, airflow and CO<sub>2</sub> distribution in insect production setups using Computational Fluid Dynamics

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The insect production sector is at a young stage and the output is expected to grow from approx. 2,000 tons in 2018 to about 1,200,000 tons in 2025. However, for the insect sector to fully develop and consolidate, a series of challenges such as upscaling and ensuring optimal production conditions (i.e. temperature, gas emissions and humidity) must be addressed (IPIFF 2019).

This poster aims to introduce the work conducted by the Danish Technological Institute (DTI) in Computational Fluid Dynamics (CFD) modeling and to present how CFD simulation can be used to develop and visualize solutions for ensuring optimal conditions in production setups.

Initially, heat development from mealworms was measured using closed respirometry under controlled conditions, at lab scale. Furthermore, CO<sub>2</sub> production was recorded in full size mealworm production trays (40 x 60 cm) across different sized mealworms, using flow respirometry. Based on both datasets, and direct temperature measurements from boxes in the pilot production laboratory, a CFD model was developed. The model shows the heat development and air flow in a modular production rack from the production at DTI. Based on this initial single-rack-simulation, a second model, showing the heat development and air flow circulation of a full-scale mealworm production (96 racks), was developed. Similarly, as in the case of heat development and air flow, the CFD can be used to model GHG emissions at different scales (i.e. modular unit and full-scale production).

CFD models could be applied to assess heat development, air circulation and GHG emissions, and thus to select highly efficient ventilation set-ups, avoid overheating or revealing problematic areas when designing insect production facilities or when scaling-up.

### Reference:

**IPIFF (INTERNATIONAL PLATFORM OF INSECTS FOR FOOD AND FEED) (2019):** The European Insect Sector today: challenges, opportunities and regulatory landscape.

## Optimization of black soldier fly reproduction using new odour attractant and egg collectors

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The production and commercialization of black soldier fly (BSF) larvae for feed purposes are of high interest due to their nutritive value, low environmental impact and high conversion efficiency. However, the reproduction of BSF in captivity is far from being optimal and requires a high degree of biological knowledge.

The current poster will show some of the work conducted by the Danish Technological Institute (DTI) for optimizing the reproduction of BSF in captivity, with focus on improving fly density as well as increasing oviposition at specific sites (outside the cage) and hatchability by using newly developed odour attractant and egg collectors.

The flies were maintained at three different densities (i.e. A:1,000 flies/cage, B:1,350 flies/cage and C: 1,650 flies/cage) in cages of L 60 x W 60 x H 60 cm (duplicates), with access to water and sugar and similar sex ratio (F: 54% – M: 46%). All cages were maintained under controlled conditions (temperature: 27°C, relative humidity: 60%, LED light intensity: 14/10) inside a controlled climate room. The egg collectors were weighed before and after being placed on the top of the cages and replaced every second day for 6 days. A small recipient with the odour attractant was placed on individual egg collectors in order to ensure a high odour persistence and checked every time the egg collectors were replaced.

The total egg production per cage was found to increase with fly density from 8.29 g/cage (A), to 11.74 g/cage (B) and 14.22 g/cage (C). The oviposition of eggs at specific sites using an odour attractant was very high, at rates between 93% (density C), 94% (density B) and 99% (density A). The rest of the eggs (1%–7%) were oviposited inside the cages. The new egg collectors were found to increase overall hatchability up 80%. This is believed to be associated with a decrease in egg handling and a higher air flow across the egg clusters. In addition, the new egg collectors are easy to wash.

## Effect of light regime on fitness parameters of farmed *Ruspolia differens* fed on a novel artificial diet

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*Ruspolia differens* is the most consumed grasshopper species across Sub Sahara Africa (SSA), where periodic large swarms mainly occur in the rainy seasons. The harvesting of these wild insects, which are often considered as delicacies, is not sustainable and limits their yearlong availability. One method to circumvent this is the development of mass-rearing technologies within SSA. However, to mass-rear this species, the optimal rearing conditions need to be determined. In our study, we assessed multiple performance traits for *R. differens* reared at two light schemes: dark (light/dark: 1 h/23 h), and light (light/dark: 12 h/12 h) from newly hatched nymphs to death of the adults. Initially weighed larvae were placed in Petri dishes with perforated lids, water was provided for using moist cotton wool balls and fed a weighed amount of a newly formulated artificial diet. This low moisture ( $a_w = 0.49$ ) diet was composed principally of wheat bran, fishmeal, cornmeal, sugar, bone meal and soy flour. Proximate composition of this new artificial diet was: 53.5% carbohydrates, 21% crude proteins, 7% ash, 5% fat, 4.5% crude fiber. The highest survival rate from nymph to adult was recorded with the dark-reared insects. Final mean weight was significantly higher in the dark-reared insects and obtained within a shorter developmental time. It took on average 8–9 moults to reach adults in the light-reared insects, as opposed to 6–7 moults for those reared in the dark. Adult longevity was also significantly longer in the dark ( $37 \pm 3.7$  days) versus light ( $10 \pm 3$  days) reared conditions. These findings are indicative for the nocturnal behaviour of *Ruspolia* and suggest that farming them in a predominantly dark environment seems a profitable venture for mass rearing as shorter developmental times can be achieved, as well as an added advantage of fewer energy demands in terms of lighting.

## Combining entomophagous tradition with modern farming and innovative, yet traditional foodstuffs in Thailand and Cambodia (IFNext project presentation)

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Traditional entomophagy includes insect collecting, processing, consumption, and preservation. The latter is typically practiced in dry and warm areas. In humid, tropical regions, this is not the case, possibly because many species may be obtained always fresh. Still, there is a long history of preserving foodstuffs in regions where, drying is not an option.

Cambodia and Thailand are affected by malnutrition, particularly of mothers and children in rural areas. IFNext ("Bringing insect farming to the next level") is a project that started in 2019 and seeks to improve the nutrition of mothers and children in those countries by designing insect farming starter kits so that families may rear their own insects, consume them, and sell surpluses on local markets. During the project, kits will be adapted to the specific needs of the farmers. The consortium agreed on the Mediterranean field cricket (*Gryllus bimaculatus*) to be reared by everybody, and another species with local importance, i.e. the silk worm (*Bombyx mori*) for Thailand, the Cambodian field cricket (*Teleogryllus mitratus*) for Cambodia, and the mealworm (*Tenebrio molitor*) for Germany. Farmers will be involved directly when choosing a novel preserved insect product, using traditional, low-energy techniques, e.g. fermenting, smoking, krupuk-style crackers, and home canning. Products will be developed and their compositional, microbiological, and sensorial quality monitored. Results will be published in scientific and popular magazines and on social media in English, Khmer, Thai, and German.

The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE).

## Optimal rearing of *Alphitobius diaperinus* on organic side-streams in the InDIRECT project

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Insects are regarded as promising alternative animal based proteins for feed and food. Protifarm focusses on rearing the *Alphitobius diaperinus* or lesser mealworm (LMW). In Ermelo, the Netherlands, a large scale rearing facility houses many billions of larvae, which are reared and processed for food applications. Feed for the LMW larvae is i) a large part of the production cost and ii) has an enormous impact on the sustainability of the rearing system. It is therefore crucial to use as much side-streams as possible in the ration of the LMW.

In the InDIRECT project, an EU research project, insects convert under-spent side-streams via bio-refinery into useful products. Insects are able to convert heterogenic side-streams into homogenous biomass. The insect biomass is further fractionated into marketable products like protein, lipid and chitin fractions. Efficiency of rearing the insects on side-streams is one aspect of an economical viable system and is taken into account.

Four side-streams (DDGS, corn gluten feed, rapeseed meal and rice bran) were provided by project partners. Each side-stream was combined with standard products available at Protifarm R&D (also side-streams). Combinations were investigated in dose-response studies which were performed according to standard Protifarm protocol. Per treatment, six or eight replicates were used. Yield per tray (experimental unit) and feed conversion ratios are investigated parameters. Chosen side-streams result in marked differences on performance of the LMW larvae, ranging from positive (rapeseed meal) to neutral (rice bran) to negative effects (DDGS) on performance of the larvae.

This study received funding from BBI Joint Undertaking in the framework of the European Union's Horizon 2020 research and innovation program, under grant agreement No 720715.

## Mini incubators for grasshopper eggs

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The development rate of insects' eggs and the time required till hatching depends on biotic and abiotic factors in the vicinity of the eggs as well as on the physiological factors of the laying female and the genetic factors of the parents. In order to examine the effect of biotic and abiotic factors on the development of eggs from wild grasshopper populations, it is necessary to neutralize the parental effects and to examine the development of eggs from a single pod. As a result, the number of eggs is limited and is about several dozens, resulting in a limited number of factors and replications that can be performed.

The purpose of this study was to set up an optimal incubation device for the development of single eggs of *Schistocerca gregaria*. We have successfully developed mini-incubators, 1.5 ml in volumes that are suitable for single egg incubation, with 100% survival and hatching rate of the eggs.

This development provides a cheap, easy-to-use and small-scale tool for further research of grasshopper embryology and hatching experiments.

## Producing black soldier fly larvae (*Hermetia illucens*) with common Western European horticultural residues

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The production of black soldier fly larvae is often suggested as a sustainable source of protein. It could play a key role in the conversion of low value residual biomass into high value insect protein and thus contribute to an improved usage of all the available biomass. To achieve this, the insect sector must be able to co-exist with other forms of animal production with minimal competition for feed sources (e.g. spent grains), especially if the insects themselves are then used as a feed in animal production.

The horticultural sector is an example of a sector with large quantities of residual biomass. Some examples of crops with potential are:

- (i) Brussels sprouts: 420,000 metric tons of stems and leaves a year (F, N and UK)
- (ii) Chicory: 150,000 tonnes of residual roots (B and N)
- (iii) Tomato : 100,000 tons of fiber rich residual material (B, N and UK)
- (iv) Cauliflower: 154,000 tons of residual foliage each year (B)

With B: Belgium, F: Flanders, N: the Netherlands and UK: the United Kingdom.

Because most of these side streams are only available seasonally, various preprocessing steps were evaluated to stabilize and/or enhance the biomass. Furthermore, due to the varying quality of the residues, a calculated approach is required in order to compose a balanced diet with maximum inclusion of the residual biomass. The minimal nutritional needs of black soldier fly larvae are roughly known. The macro nutrients of different horticultural residues were evaluated based on a proximate analysis and mixtures were made that met the proposed requirements (30% dry matter, 10–20% protein, 2% fat and 30–40 % total carbohydrates).

The mixtures were evaluated in pilot scale conditions based on the following parameters: (i) harvestability, how easily the larvae and the remaining substrate can be separated from each other, (ii) larval growth rate and survival (iii) feed conversion, the amount of feed required for the production of 1 kg of larvae (wet and dry basis) and (iv) processing capacity of the larvae, based on weight reduction of the original mixture. Initial results indicate that the pre-fermentation of chicory roots has no negative effect on growth (feed conversion ratio: 1.5–2) but does increase the harvestability of the larvae.



## Influence of artificial light on the reproduction of black soldier fly (*Hermetia illucens*)

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Black soldier fly (BSF) or *Hermetia illucens*, a fly species originating from Central- and South-Amerika, has strict requirements for its environment. This fly species naturally lives in tropical and subtropical conditions. When BSF is reared artificially, certain natural habitat factors need to be imitated to obtain that BSF will reproduce.

Previous research showed that the reproduction of BSF depends on light intensity and wavelengths which are present in natural light conditions. In regions with temperate climate, BSF is often reared in greenhouses. However, cloudy and shorter days in winter make it impossible to guarantee an optimal yield, which is desirable in a continuous BSF-culture. Moreover, it is quite a challenge to keep a greenhouse at the desired temperature on cold days. To ensure a suitable light intensity and wavelengths mating under artificial light, which mimics part of the spectrum of daylight, can be the solution, resulting in an optimum egg yield throughout the year.

In this study, reproduction of BSF was investigated under four different artificial light sources, namely a fluorescent lamp, a halogen lamp, a light-emitting diode lamp and a ultraviolet lamp. The experiments were always sight-by-sight comparison. The flies were kept in a 60 x 60 x 90 cm cage, which was located in a growth chamber to prevent contamination of other, unwanted light sources. The temperature in the growth chamber was kept between 25 and 27°C, relative humidity was kept at 60%. A density of 0,00324 flies/cm<sup>3</sup> was applied in the cage. The light-dark cycle was set to 16:8 hours.

Egg laying quantity as well as egg quality were investigated. The egg quality was determined by following the egg hatching and survival of the larvae. The results are processed using T-test.

## Enzyme activities in the digestive tract of locusts (*Schistocerca gregaria*) express cellulolytic potential

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**Background:** Knowledge about the digestive capacity of insects is still limited, which restricts the proper allocation of feed material to large-scale production of insects. It is of particular interest whether insects may digest non-starch-polysaccharides (NSP), since degradation of these types of carbohydrates is limited to microbial digestion in the forestomachs of ruminants and – to a smaller degree – in the caecum and colon of all livestock and of humans. As locusts in nature consume fibrous material (e.g. grass), one could assume their general potential to extract dietary energy from NSPs. Indeed, recent studies suggested a cellulolytic digestive potential of locusts, but a more detailed characterization is still lacking. Therefore, the present study aimed into assessing the digestive potential of grasshoppers for saccharose, lactose and cellobiose as the most simple representative of cellulose in comparison to maltose as a representative of starch.

**Material and methods:** Nine adult desert locusts (*Schistocerca gregaria*) were immobilized in a freezer (–22°C) and killed by decapitation. The entire digestive tract was removed, homogenized, diluted with distilled water, and centrifuged. The supernatant containing the water-soluble constituents including digestive enzymes was mixed with either one of four aqueous sugar solutions (maltose, saccharose, cellobiose, lactose) or with pure water (control, baseline). Release of glucose was monitored photometrically every 4 minutes using a glucose test kit over a time span of 24 minutes.

**Results:** Addition of maltose resulted in a clear release of glucose over the entire time of observation ( $p < 0.0001$ ). Linear release of glucose was observed also with saccharose ( $p < 0.0001$ ), cellobiose ( $p < 0.0001$ ), and lactose ( $p < 0.001$ ). Corresponding speed of glucose release accounted for 51%, 15%, and 7% as compared to maltose. Speed of glucose release was statistically different among all sugars tested ( $p < 0.001$ ).

**Conclusion:** Although digestive enzymes of locusts are obviously adapted mainly to amyolytic feed carbohydrates (e.g. starch) and saccharose, there is also some cellulolytic enzyme activity. This gives rise to the speculation that locusts may digest even other soluble non-starch-polysaccharides such as e.g. inulin, pectins etc.

## Influence of different nutritional sources on *Hermetia illucens* adult longevity

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*Hermetia illucens* is a saprophagous species able to bioconvert wide amount of organic substrates representing one of the most interesting insect species as protein source for livestock feeds. Literature is rich of information related to larval growth, including biology, development and nutritional needs, the chemical composition and the efficiency of bioconversion. On the contrary, few information is available on adult biology and performances during its lifetime. In the present study we evaluated the influence of different nutritional sources on the survival and longevity of *H. illucens* adults. Survival of starved adults was compared with adults fed with different feeding solutions. Effects on isolated newly-emerged specimens and on a cohort of males and females (50+50) were evaluated. Survival was significantly influenced by the nourishment, the sex and the interaction of the two factors. Longevity was significantly longer in both sexes when a sucrose solution was provided, compared to starved adults. Longevity in cages was always shorter than in isolated specimen for both males and females, with males living little more than females when a sucrose was provided. Ovary area was measured in females under different nutritional sources at 1, 3, 5, 7, 10, 14 days after the emergence. Statistical differences in the ovary dimension were observed between days, with a maximum development 3 days after the emergence, but not between females fed with the different nourishment or starved.

The research was supported by the Project "Insect Bioconversion: from vegetable waste to protein production for fish feed (INBIOPROFEED)" funded by Fondazione Cariplo (grant n° 2014-0550) and by the grant of the University of Milan Project "PROENTO – Insects: an alternative source of proteins".

## Chemical food safety related to using supermarket returns for rearing *Hermetica illucens* for feed and food uses

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Insects are considered an alternative source of proteins for feed and food production in Europe. One of the insect species with great potential for large scale production is *Hermetia illucens* (black soldier fly, BSF). To become economically feasible and to contribute to circular economy, BSF rearing should be performed on substrates with little or no alternative use for feed and food production. In Europe, plant-derived materials and supermarket returns without meat and fish are currently legally allowed to be used. Supermarket returns are a group of foods that were produced for human consumption, but are no longer intended for human consumption. The aim of this study was to investigate the chemical safety related to the use of supermarket returns as substrate for rearing BSF. A small scale experiment (in triplicate) and a large scale experiment (in duplicate) were done with rearing BSF larvae on four different types of supermarket returns and a control (5 treatment groups) at, respectively, Wageningen University and at Protix. BSF larvae were reared from day 4 since hatching till day 10. After harvest they were frozen and sent to RIKILT (Wageningen University and Research) for chemical analyses. A range of possible contaminants were analysed in both the larvae samples and the substrates, including four heavy metals, dioxins and PCBs, PAHs and mineral oils. Survival rates and dry matter increase were not significantly different between the five treatment groups. Concentrations of the chemical compounds in the larvae were generally low. However, the bio-accumulation factor (the ratio of contaminant concentration in larvae to its concentration in substrate) was high for some of the contaminants considered. More detailed results are available at the start of the conference and will be presented.

## Impact of side-stream based feed on the composition of the lesser mealworm larvae

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InDIRECT, a three-year EU research project started in November 2016, envisioned to develop biorefinery processes as part of new value chains to convert under-spent side-streams from the agro and processing sector into useful marketable products. One of the biorefinery approaches considered is a two-step process that can cope with the heterogeneity of side-streams by using insects to convert the side streams to a homogeneous biomass i.e the insect itself. In the next step, the insect biomass can be further fractionated into higher value products like protein-enriched fractions.

This study reports the impact of different feeds on the composition of the lesser mealworms grown on them. More specifically, changes in the composition of the lesser mealworm, that were grown on 18 different diets (composed out of side streams), were determined in terms of protein and lipid content, amino acids and fatty acid composition and chitin content. The nutrient composition of the larvae ranged between 35% and 49% in protein content, 14% and 28% for lipid and had a chitin content between 4% and 6%. The dietary composition ranged in protein content from 16% to 34% and from 2% to 19% in lipid content. The study showed that the lesser mealworm can concentrate these important dietary nutrients in its biomass which is reflected in a high protein and lipid content.

Although some variations were measured, the variations in larval composition could not be correlated with the variation in the diet composition. In terms of essential amino acids, the larval amino acids profile was comparable to that of beef. Results also showed that the larvae contained a high amount of linoleic acid (C18:2), which is an essential fatty acid. No correlation between the amino acid or fatty acid profile of the larvae and the diet was detected. In conclusion, the composed different feed diets did not significantly influence the insect composition thus ensuring a stable product independent of the diet.

The study is part of the InDIRECT project ([www.bbi-indirect.eu](http://www.bbi-indirect.eu)) that receives co-funding from BBI-JU under EU- Horizon2020 research and innovation program under GA-No720715.

## Lipid content in house crickets (*Acheta domesticus*) fed different diets formulated with by-products

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The house cricket, *Acheta domesticus* L., has been produced in the United States for over 60 years as pet food and fish bait, but recently a new industry has emerged producing house cricket powder as an ingredient for human foods. However, the market price of cricket powder is higher than other protein products due to high rearing costs. Efforts to develop cheaper diets using agricultural by-products are under way. In a previous study (MORALES-RAMOS *et al.*, unpublished) four diets formulated with by-products showed promising results when compared with a reference diet (PATTON 1967). In this study the lipid content of adult crickets reared in 4 diets formulated with by products is compared to that of crickets reared in Patton's (1967) diet 13.

House crickets were reared in 4 diet formulations and reference diet Patton's (1967) diet 13 for a period of 7 weeks at 27°C, 75% RH, and 12:12 h (L:D). In addition to food grade ingredients the content of by products was: diet 1 34% corn dry distiller's grain with soluble (DDGS); diet 2 38% DDGS, 5% rice bran, and 5% canola meal; diet 3 28% DDGS and 17% rice bran; and diet 4 30% DDGS, 20% rice bran (whole), 20% rice bran (defatted), and 10% canola meal. Adult crickets from each diet treatment and reference diet were frozen at -28°C and dried in a vacuum oven at 100 mbar and 50°C for 72 hours. Dry crickets were pulverized and 500-mg samples (3 per diet) were extracted with a solution of methanol: chloroform 2:1 and dried on a hot plate under a nitrogen stream. The weight of the dissolved lipids in each sample was weighed to determine the crude lipid content in mg/g.

The results of lipid content determinations in decreasing order were (mean ± standard deviation): Patton's (1967) diet 13 = 278.3 ± 28.1; diet 3 = 268.8 ± 44.5; diet 1 = 263.1 ± 29.0; diet 2 = 256.9 ± 43.9; and diet 4 = 243.8 ± 20.2 mg/g. Lipid content was not significantly different among crickets fed the experimental and reference diets indicating no detrimental effect of by-product ingredient content in the formulated diets. This study will continue with crude protein content determinations.

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## Heat treatment inhibits anti-protease activity of black soldier fly meal and could improve protein digestibility in fish

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The presence of anti-nutritional factors in black soldier fly meals (BSF; *Hermetia illucens*) depends on protein content and larval stage at harvesting. A protein and/or a protein complex could be one of the causal factors explaining in vitro anti-protease activity of digestive homogenates in fish. We hypothesized that heat treatment such as decontamination and/or drying processes could inhibit anti-protease activity and improve protein digestibility of BSF meals. Total digestive enzymes were extracted from the proximal intestine of 18 juvenile rainbow trout (3 pools of 6 fish;  $121.7 \pm 11.2$  g) and incubated in triplicate with increasing levels of homogenized fly meal following the method described by MOYANO *et al.* 1999. Fly meals were obtained from black soldier fly larvae fed on a Gainesville diet for 5, 10 and 15 days post hatch ( $n=3$  tank/larval stage;  $28 \pm 2^\circ\text{C}$ ; 50% RH). The larvae were either kept raw (R) or boiled in water for 4 min (B) prior being dried to constant weight at low (LT; freeze-dried; 12 h), conventional (CT; oven-dried at  $60^\circ\text{C}$  for 6 h) or high (HT;  $130^\circ\text{C}$  for 20 min) temperature. Anti-protease activity of BSF meals (expressed as the % of inhibition at 150  $\mu\text{g}$  of inhibitor per UA enzyme activity) was not greatly affected by low temperature processes (R-LT =  $11.2 \pm 6.0$ ; R-CT =  $17.5 \pm 5.3\%$ ). However, high heat treatments (B-LT and R-HT) were found to completely inhibit the anti-protease activity of the BSF meals. These trends were not affected by larval stages at harvesting. Nutritional trials with juvenile rainbow trout are currently underway *in vivo* to estimate the apparent digestibility of proteins and amino acids of the studied BSF meals. Our study should help to formulate insect-based diets to meet the digestive requirements of fish species and improve growth performance.

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## Insect oil as an alternative of palm oil and poultry fat in broiler chicken nutrition

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The study was conducted to evaluate the effect of total replacement of palm oil and poultry fat with *Tenebrio molitor* oil (TM oil) in broiler chicken diet on the growth performance and lipid fatty acid composition of liver and breast muscle tissues. A total of 72 seven-day-old female Ross 308 were used. The birds were randomly distributed to 3 different groups, 12 replicates per group and 2 birds per replicate. The experiment was 30 days in metabolic cages. The basal diet was formulated on maize and soybean meal basis. 5% of palm oil, poultry fat or TM oil was added to the diet. The growth performance parameters (BWG, FI, and FCR) were measured during day 7, 14, 21, and 30. Immediately after slaughter, samples from breast muscle and liver tissue were into dry ice for analyses. Data were tested using the General Linear Models procedure of SAS software. Means were separated using Duncans' tests following one-way ANOVA. In all periods of the experiments, BWG, FI or FCR were not affected by dietary treatments. The liver tissue of the chickens fed a diet supplemented with TM oil showed the lowest value of SFA ( $P = 0.004$ ) and the highest of UFA ( $P = 0.004$ ). In addition, TM oil significantly decreased MUFA ( $P < 0.001$ ) and increased PUFA ( $P < 0.001$ ) in comparison to PF and PO. Furthermore, SFA and UFA profile of the breast tissue was not affected by any of the dietary fat sources. However, TM oil reduced MUFA content ( $P < 0.001$ ) and increased PUFA ( $P < 0.001$ ). Moreover, n-3 and n-6 fatty acids were significantly increased in case of TM oil supplementation ( $P = 0.006$ ;  $P < 0.001$  respectively). In conclusion, the use of TM oil in broiler chicken diet did not show any effect on the growth performance. Moreover, TM oil supplementation improved the fatty acids profile of the liver tissue and the breast muscle.



## Insects full-fat meals as functional feed additives affect broiler chickens' growth performance and immune system traits

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Two independent experiments were conducted to investigate the effect of insect full-fat meals (*Tenebrio molitor* and *Zophobas morio* larvae), added “on top” of a complete diet (Exp. 1) or calculated into diets (Exp. 2), on the growth performance, and immune system traits of broiler chickens. 1000 one-day old female Ross 308 broiler chicks were used in both experiments. In the first trial, the birds were randomly assigned to 6 treatments i.e., NC (negative control) – no additives; PC (positive control) – NC + salinomycin (60 mg/kg diet); TM02 – NC + 0.2% TM full-fat meals; ZM02 – NC + 0.2% ZM full-fat meals; TM03 – NC + 0.3% TM full-fat meals; and ZM03 – NC + 0.3% ZM full-fat meals. In the second trial, 4 treatments were set: NC – no additives; PC – NC + salinomycin (60 mg/kg diet); TM03 – NC with 0.3% TM full fat meals; and a ZM03 – NC with 0.3% ZM full-fat meals. Data were tested using the GLM procedure of SAS software. Means were separated using Duncans' tests following one-way ANOVA. Insect full-fat meals increased the BWG and FI (Exp. 1;  $P = 0.024$ ,  $P = 0.022$ , respectively), and no effect on the FCR was recorded. In addition, in groups fed insect full-fat meals and PC comparing to NC decreased the IgY ( $P = 0.045$ ) and IgM, ( $P < 0.001$ ) levels significantly. In second experiment, IgM levels were also decreased ( $P < 0.001$ ) in groups fed insect meals. Moreover, the IgM levels were negatively correlated to the BWG ( $r = -0.4845$ ) and the FI ( $r = -0.4986$ ), with statistically significant values ( $P < 0.001$ ). In conclusion, the current results confirmed that both *T. molitor* and *Z. morio* may be applied as functional feed additives to achieve improved growth performance and changes in the immune system.

## Replacement of soybean oil by *Hermetia illucens* fat in young turkey nutrition

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*Hermetia illucens* (black soldier fly, BSF) is one of the most examined insect species which is currently considered as a novel poultry feed ingredient, BSF contains a high concentration of crude protein (up to 58%) and fat ~38% (DM). The available literature is mostly focused on the substitution of soybean meal in broilers nutrition, however, there is a lack of data in terms of replacement of commonly used energy sources by insect origin fat. The usage of super-critical CO<sub>2</sub> extracted oil from BSF as a soybean oil total replacement resulted in similar growth performance (BWG, FI, FCR; unpublished data) of broiler chickens. Whereas, there are no information about the application of insect origin fat in young turkey nutrition. Therefore, the aim of the study was to investigate the effect of partial or total soybean oil replacement by BSF fat obtained using super-critical extraction with CO<sub>2</sub> on the growth performance of young turkeys. 216 seven day-old female B.U.T. 6 young turkeys were randomly allocated to 3 dietary treatments contained 6 replication (12 birds each). The birds were kept in floor pens from day 7 till 35 d of age. The following treatments were applied: SO – 100% of soybean oil, HI<sub>50</sub> – 50% of BSF fat and 50% of SO, and HI<sub>100</sub> – 100% of BSF fat. The obtained results of soybean oil replacement by BSF fat confirmed previous experiments carried out on broiler chickens. The partial or total replacement by BSF oil have shown similar growth performance results to the SO treatment in the 7–35 d period ( $P > 0.05$ ). Moreover, the addition of BSF fat to the turkey's diet did not affect ( $P > 0.05$ ) selected internal organ weights, as well as the gastrointestinal tract length. In conclusion, the BSF fat may be an alternative source of energy used in young turkey nutrition.

## The effect of soybean oil replacement by *Hermetia illucens* oil on broilers growth performance and nutrients digestibility

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The aim of the study was to investigate the effect of partial or full replacement of soybean oil by *Hermetia illucens* oil (HI) obtained via super-critical CO<sub>2</sub> extraction on the growth performance and nutrients digestibility of broilers. In an experiment, lasted 35 d 1 day-old male broilers (Ross 308) were used. The birds were assigned to 5 groups per 10 replicates (16 birds per rep). Feed and water were supplied ad libitum. The following design of the experiment was used, SO – control treatment with 100% soybean oil; HI25, 50, 75, 100 – experimental treatments with partial, i.e., 25, 50, 75 or full 100 replacement of soybean oil by HI oil. TiO<sub>2</sub> (0.3%) as an internal marker was used. The growth performance parameters, i.e., BWG, FI, and FCR were measured at 14, 21, 28, and 35 d, as well as coefficients of apparent ileal digestibility of nutrients, were calculated at 28 and 35 d of age. In general, there were no statistically significant differences between control SO and experimental groups in terms of BWG, FI, as well as FCR. Only in the first two weeks (1–14 d) HI oil positively decreased FI ( $P < 0.001$ ), and FCR ( $P = 0.031$ ) values in comparison to SO control group. However, in the case of FCR the most efficient were groups with 75% or 100% of *H. illucens* oil in the broiler diets. The usage of *H. illucens* oil as a replacement for soybean oil negatively affect the crude protein ( $P < 0.001$ ) and AMEN ( $P < 0.001$ ) ileal digestibility at 28 d of birds age. However, the results of digestibility calculation at 35 d were not affected by *H. illucens* oil, in terms of crude protein ( $P = 0.854$ ), ether extract ( $P = 0.203$ ), as well as AMEN ( $P = 0.810$ ). Present data suggest that soybean oil may be replaced by *H. illucens* without negative effects on the growth performance during 35 d rearing.

## Influence of processing methods on the nutritional and physicochemical quality of *Acheta domesticus* and *Gryllus assimilis*

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Despite significant economic progress and poverty reduction, a quarter of Bangladesh's population remains food insecure<sup>1</sup> with high rate of malnutrition<sup>2</sup>. Bangladesh faces pressure to feed increased numbers of people while land availability is decreasing. Edible insects can contribute to solve this problem in Bangladesh, as it is widely accepted that insects provide high-quality proteins, fats and other micronutrients such as vitamin B<sub>12</sub>, iron, zinc, etc. comparable with meat and fish<sup>3</sup>. Despite the benefits of entomophagy, consumer disgust remains one of the largest barriers to the adoption of insects as viable sources of proteins in many countries including Bangladesh. Development of insect based products by invisibly integrating insects into the common diets could be a possible way to increase the acceptability of edible insect as a source of nutrients. However, its effective inclusion in food preparations relies on several factors including technological functionalities, changes in nutrient composition during processing, and consumer's acceptance. To understand the behavior of insect proteins, fats and other nutrient during processing, two insect species *Acheta domesticus* and *Gryllus assimilis* were subjected to three different processing conditions, in particular: (i) freeze drying and (ii) oven drying to obtain a powder and (iii) blanching to obtain a paste. The proximate composition of powders and paste was analyzed, together with a number of micronutrients and quality characteristics. Although there were small differences between the batches for the main nutrients, the influence of the processing methods was rather limited. For other parameters, processing methods had a considerable impact on nutritional value and/or quality. It is therefore important to take this knowledge into account when introducing insects into food products.

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## Bioaccessibility and cellular uptake of iron from processed yellow mealworms

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The consumption of insects is expected to have many advantages which have contributed to the current trend to include edible insects in western diets. Edible insects contain high levels of trace elements such as zinc, selenium, and iron. Therefore, their inclusion in human diets might be a strategy to combat nutritional deficiencies such as that of iron. However, little is known about the actual bioavailability of iron from insects.

Here, an *in vitro* digestion/Caco-2 cell culture model is presented to determine bioaccessibility and cellular uptake of iron from freeze-dried larvae of *Tenebrio molitor* (mealworms). The processed mealworms were digested *in vitro*, and iron concentration of the digests was determined by Inductively Coupled Plasma – Mass Spectrometry to assess iron bioaccessibility. Cellular iron uptake was indirectly measured as ferritin synthesis in Caco-2 cells. Values obtained for mealworms were compared to bioaccessibility and cellular iron uptake from digests of minced pork. Furthermore, it was analyzed whether processed mealworms could increase bioaccessibility and cellular uptake of iron in co-digestion with iron sulfate.

The freeze-dried mealworm samples contained approximately 10 times more iron ( $17.7 \pm 4.1$  mg/100 g dm) than minced pork ( $1.6 \pm 0.4$  mg/100 g dm), however, the iron concentration of the bioaccessible iron fraction measured after *in vitro* digestion was only about 2 times higher in the mealworm digests ( $104.0 \pm 44.1$  µg/L) compared to the digests with pork ( $63.4 \pm 20.9$  µg/L). Both digests induced ferritin synthesis in the Caco-2 cells, with a significantly higher cellular uptake for digests from pork.

After digestion of 16753.5 µg/L (300 µM) iron sulfate, only 0.05% of the total iron from the initial digestion mixture was bioaccessible and there was no detectable cellular uptake of iron compared to the control. Co-digestion with mealworms resulted in a higher bioaccessibility of iron (3.1%) and in a significantly increased cellular iron uptake.

Overall, it can be beneficial to incorporate freeze-dried mealworms in human diets, because an enhancing effect on iron bioaccessibility and cellular uptake was observed.

## Reduction of off-flavor in *Allomyrina dichotoma* larva using a combined method of extraction and filtration process

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The aim of this study was to investigate the effects of a method of extraction and filtration process on reduction of off-flavor in an edible insect, *Allomyrina dichotoma* larva. The volatiles were initially isolated by solid-phase microextraction (SPME) and then afterwards they were analyzed by gas chromatography-mass spectrometry-olfactometry (GC-MS-O). Comparison of the GC-MS profiles of the processed edible insect revealed that an adequate combination of processing steps allows to reduce the level of off-flavor volatiles. The major components of earthy and moss off-flavor in *Allomyrina dichotoma* larva were identified as 3-octanone and 3-octanol and their concentration were decreased from 751.2 ppb to 67.8 ppb and from 656.2 ppb to 155.9 ppb respectively when *Allomyrina dichotoma* larva went through filtration process after extracted. These results may support that the process conditions could be used as an efficient method for reducing of off-flavor in *Allomyrina dichotoma* larva. Furthermore, edible insects could be considered a candidate as a food ingredient for processed food products.

## Ensiling *Hermetia illucens* larvae for storage purposes

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Rearing and use of insects for food and feed is still at an early stage, and therefore, there is a temporal discrepancy between production and demand for larvae. Presently, artificial drying is the most commonly used method to convert fresh larvae to a storable form. This procedure requires a lot of energy, increasing production costs and decreasing the sustainability of larvae production compared to other protein sources for animal nutrition. Thus, investigation of alternative conservation methods is needed. One possibility is the anaerobic storage using the effects of a bacterial lactic acid fermentation, whose principle lies in the utilization of the materials water-soluble carbohydrates as substrate for naturally occurring lactic acid bacteria. The resulting low pH value ensures to suppress harmful microorganisms and thereby preserves the nutritive value, as is common in forage conservation (corn or grass silage for example). Since the amount of water-soluble carbohydrates in insect larvae is very limited, we hypothesized that the addition of barley with or without different silage additives, enables sufficient lactic acid production and therefore successful conservation of the larvae. In the present study, apart from a control containing only fresh larvae (1), 7 treatments of larvae and admixtures were tested in total. Vacuum bags or glass jars, to most closely simulate practical conditions with higher oxygen exposure, were used as laboratory ensiling containers. They were filled with the whole or chopped larvae and 40% (2) or 60% (3) barley meal (DM basis). Furthermore, treatments include larvae combined with 20% or 40% barley meal and sodium nitrite (4 + 5). The remaining treatments contained larvae, 40 % barley meal in combination with formic acid (6) or lactic acid bacteria (7), respectively.

The first results showed that an admixture of barley meal as water-soluble carbohydrate source ensures a sufficient lactic acid fermentation as indicated by pH values around 4.5.

Building upon the presented findings, the fermentation of *Hermetia illucens* larvae promises a successful preservation.

## Fractionation of insect biomass – BBI-INDIRECT project

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Biomass of insect larvae such as the black soldier fly and the lesser mealworm are rich in lipids, proteins and chitine, and can as such be considered as a feedstock for these compounds. The larvae can be used as a whole, or after fractionation. Within the BBI-H2020 InDIRECT project ([www.bbi-indirect.eu](http://www.bbi-indirect.eu)), two fractionation approaches have been studied.

A *first biorefinery* approach is based on a mechanical separation of the larvae generating (1) a juice fraction with a low chitin content (0.5%) and rich in lipids and proteins, and (2) a pulp fraction containing nearly all chitin besides some lipids and proteins. Further chemical supported fractionation of the juice resulted in a protein enriched fraction (up to 60% protein) and a lipid enriched fraction (up to 85%). Depending on the envisioned application and importance of functional properties and purity, the fractionation process can be tailored. The pulp fraction was further fractionated aiming at purified chitin and co-valorisation of a protein rich fraction. Chitin was converted to the more water soluble chitosan and chitosan oligomers. The whole fractionation approach was applied on larvae from the black soldier fly (provided by Millibeter, B) as well as on the lesser mealworm (provided by Protifarm, NL), indicating that the biorefinery approach that was developed is rather generic and not insect species specific. The approach was upscaled from lab to pilot scale to generate fractions for application tests related to feed and chemistry.

The *second approach* is based on enzymatic-assisted hydrolysis of the whole larvae biomass. The proteolytic activity of enzymes allowed to extract and solubilize in a single fraction up to 80% of total proteins, in form of peptides. The peptide-fraction is more digestible, rich in bioactive peptides and free amino acids which make it suitable as feed for aquaculture. The remaining pellet, rich in chitin, represents an economical value for the chemistry sector.

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## Target marketing of the entomophagy with segmentation by food preference

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We have a neophobia which is an instinct to resist novel food. In order to promote an entomophagy as a food business, it is important to establish a marketing strategy to overcome our neophobia. When we buy food, we are not only considering the price, but also referring various information (credence goods) related to the products. Labels of nutrition and ingredient, advertisements through SNS and mass media are the typical examples of credence goods we refer. In addition, since our food preference has a large diversity, a target marketing based on the segmentation of consumers will be needed. If we could clarify the relationship between the credence goods and consumer's preferences, an effective strategy to accelerate the entomophagy might be established.

In this study, we applied a conjoint analysis and measured consumer's preference of the entomophagy products. The profiles for conjoint analysis consist of 10 attributes (price, protein enrichment, calorie off, eco-friendly, organic product, fair-trade product, online reviews, recommendation by experts, TV ads, and brand), and the values of respective attributes were determined as utility scores. Moreover, we prepared a questionnaire with 18 questions asking food preference, and a factor analysis was carried out.

Total of 972 valid responses were obtained from undergraduate students. Factor analysis resolved a six-factor solution. Each of these factors has a distinctive grouping of questions that can be associated with a specific food preference (Factor 1: interest in production area, Factor 2: health awareness, Factor 3: curiousness, Factor 4: conformity, Factor 5: neophobia, and Factor 6: cost-consciousness). A non-hierarchical cluster analysis was performed by using the factor scores, and the respondents were grouped into 4 segments (conservative, curious, budget-minded, and health-conscious). The respondents who grouped into the curious and the health-conscious segments showed lower weight for price, indicating that these segments have a higher potential for accepting the entomophagy. Moreover, health-conscious segment had higher weight for the label indicating organic product. In our presentation, we will discuss about the differences in utility scores by respective segmentations in more detail.

## Comparing different defatting methods to extract protein and fat from the lesser mealworm

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Over the last few years, insects have entered the western food and feed industry. In the EU, legislation is changing and today, certain insects are allowed as feed for aquaculture and pets. Regarding the food sector, novel food law apply. Insects have an excellent nutritional value (comparable to beef) and thus can be consumed whole. However, fractionated insect biomass (lipid-rich, protein rich or chitin rich) may be beneficial as ingredients as since it gives more freedom in tailoring towards a specific product composition. In addition, certain fractions can possess bioactive or techno functional properties that have an added value to the food/feed application. Furthermore, fractionated biomass can also apply in other applications. Fat isolates for example can be used in energy or cosmetic industries.

Within the InDirect project ([www.bbi-indirect.eu](http://www.bbi-indirect.eu)), the lesser mealworm was investigated to recycle nutrients of side streams followed by fractionating of the insect nutrients in order to generate high added products. The lesser mealworm is currently bred on a large scale envisioned for human consumption. The main components are proteins (49% on dry weight (DW)), lipids (28% on DW) and chitin (5% on DW). Different standardized defatting approaches were compared to generate protein and lipid enriched fractions, i.e. heat treatment, solvent-based extraction, supercritical CO<sub>2</sub> extraction and acid based isoelectric point precipitation. Firstly, all treatments underwent preliminary optimization experiments. A brief investigation of the optimal time, temperature, solvent choice and the effect on protein solubility (a first indication toward techno-functional properties) was performed. The best conditions for the four treatments were compared. Evaluation was based on a high-quality ratio of yield versus purity, meaning even though the first aim was to generate pure isolates, the yield is also considered as an important factor. Results show that no treatment succeeded in generating a fat-free protein isolate. However, high protein isolated were achieved (up to 70% protein).

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## Evaluation of insect derived functional feed ingredients in poultry diets

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An indirect biorefinery approach was developed converting side-streams into crude extracts via a two-step process consisting of (1) conversion of biomass by insects and subsequent (2) biorefinery of the insect biomass. The aim of the first step is to convert heterogenic feedstock into a homogenous biomass via insects. Insects are able to convert a variety of feedstock into a more homogenous biomass, being their own biomass enriched in functional feed ingredients.

Black soldier fly biomass as well as biorefinery products thereof were evaluated for their nutritional value in poultry trials. Diets were formulated in iso-nutritional and iso-energetic feed formulation, based on linear programming software (WinMix™). The birds were one-day-old-chickens, and were grown for 42 days. At the start of the trial, the birds were allocated to the different pens by weight. This allocation was made in order to have an equal average weight and an equal standard deviation around the average weight for each treatment and pen. Feed intake was registered. During the trial, a veterinarian and a Felasa D certified person supervised the performed poultry experiment according to the international guidelines described in law EC/86/609 and further amendments. During the whole trial period the birds were fed ad libitum. A dose-respons of the retained insect fractions was tested, and following parameters were analysed: Daily weight gain, daily feed intake, feed conversion ratio, microbial and histochemical analysis in the gastrointestinal tract.

These parameters enable to determine the efficacy of the insect fractions on zootechnical performance, and in particular the feed conversion ratio as well as on survival rate and health status of the animal, as a measure of antibiotic use.

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## Vitamin B<sub>12</sub> in buffalo worms by UHPLC-PDA-MS

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The buffalo worm (*Alphitobius diaperinus*) used to be known mainly as the cosmopolitan storage pest and pathogen reservoir. However, recent intensive research and subsequent legislative changes on alternative sources of food and feed in EU have changed the perspective on this insect from the annoying vermin to promising nutrient source. Regarding chemical composition, they are a good source of high quality protein, lipids as well as other macronutrients and micronutrients. Moreover, buffalo worms are unable to fly, cannot climb on smooth surfaces, and might be fed on various substrates of organic origin (including those low in nutrients and waste or by-products from food industry). Due to their very high reproduction rate and quick life cycle, the biomass gain is even higher than in other edible Coleoptera. Therefore, rearing of such insects is assumed to be a non-complicated, environmentally friendly, cheap and effective way of producing insects as food or feed.

Recently, minor nutrients such as vitamins came into the focus of breeders and consumers. Especially vitamin B<sub>12</sub> is of the utmost importance for a balanced diet and of great concern to vegetarians and vegans. Based on a recently published ultra-high performance liquid chromatography method (SCHMIDT *et al.* 2019), the content of total vitamin B<sub>12</sub> in buffalo worms was determined to be 0.5 µg/100 g using photodiode array detection. Surprisingly, the confirmation of this result based on mass-spectrometry, using a comprehensive UHPLC-PDA-MS method, yielded a content of 0 µg/100 g of vitamin B<sub>12</sub> active compounds. False-positive results in the studied samples are likely caused by nor-Pseudocobalamin.

### Reference:

**SCHMIDT A, CALL L-M, MACHEINER L & MAYER HK (2019):** Determination of vitamin B<sub>12</sub> in four edible insect species by immunoaffinity and ultra-high performance liquid chromatography. *Food Chemistry* 281, 124–129. <https://doi.org/10.1016/j.foodchem.2018.12.039>.

## Potential of *Yarrowia lipolytica* and *Debaryomyces hansenii* strains to produce high quality food ingredients based on cricket powder

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This research was aimed to produce, using *Yarrowia lipolytica* and *Debaryomyces hansenii* strains, cricket powder hydrolysates endowed with improved safety, functionality, sensory and technological properties to be further used as food ingredients. The hydrolysates were produced using cricket powder doughs inoculated with *Y. lipolytica* and *D. hansenii* strains at level of about 6 log cfu/g and incubating them at 25°C for 72 h. The hydrolysates were characterized, comparing to the control, for their chitin content, fatty acid, protein, amino acid and volatile molecule profiles. In addition, the yeast cell loads were monitored over incubation time.

The results obtained showed that the strains of *Y. lipolytica* were able to grow in the matrix reaching cell load values higher than 8 log cfu/g while the strains of *D. hansenii* did not show growth. However, the hydrolysates were all characterized, in strain dependent way, by reduced chitin content, higher presence of antimicrobial substances (acetic acid, short chain fatty acids, chitosan, GABA) and health promoting molecules (arachidonic and linolenic acids, GABA, AABA, BABA). Indeed, in the cricket powder hydrolysates a major release of free amino acids was detected highlighting the highest matrix digestibility. Instead, the hydrolysates obtained by the use of GRAS yeasts were characterized the presence of many volatile molecules and specific aminoacids, typical of ripened fermented foods and able to impart good sensory properties. In this study, it was demonstrated the ability of each tested strain, based on its physiological and metabolic features in the system taken into consideration, to give rise to a cricket-based hydrolysate characterised by a specific physico-chemical, sensory and functional fingerprinting.

## Improve of insect derived proteins solubility by enzymatic processes

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Insects have a wide number of properties that make them interesting candidates as an alternative and additional source of protein in developed countries in view of environmental problems and the increasing global population over 9 billion by 2050, according to the United Nations Food and Agricultural Organization (FAO). Entomophagy in the western world often evokes fear and disgust reactions from consumers, but the inclusion of them as a powder or extract in food products could pave the way to enhancing consumer acceptance. Insect meal are samples with high protein content (approximately 60% on dry weight) but the highly insoluble nature of the native insect protein is an important weakness for their use in the food matrices. Enzymatic technology is a sustainable process to alter the functional properties of proteins including solubilisation.

In this study, enzymatic hydrolysis of mealworm (*Tenebrio molitor*) flour (TMF) was investigated as a method to produce soluble protein rich hydrolysates. Experiments were conducted using different proteases (0–4% w/w concentration of endoproteases, alkaline protease and exopeptidases) and combination of therefore, under variation of pH (6–8), temperature (50–70°C) and hydrolysis time (1–7 h). Protein solubilisation was monitored in final hydrolysates as total NTK protein after filtration.

Higher solubilisation yields were obtained using an alkaline protease, obtaining values of 24%, that combined with an endoprotease allows obtaining successful solubilisation yields higher than 35%. Otherwise, only 7% of TMF proteins are solubilized using enzymatic processes with exopeptidase, 10% with an endoprotease and 14% using a combination of thereof. Produced hydrolysates showed a protein content up to 70% dw.

As conclusion, enzymatic hydrolysis combining the action of endoprotease and alkaline protease is a sustainable technology to produce 100% soluble protein hydrolysates from TMF.

## Correlation of process sequence and valuable compound extraction yield during processing of *Acheta domesticus*

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Optimized processing of edible insects is a prerequisite for the production of safe, high-quality insect-based foods. The respective processing steps vary depending on the insect species and growth stage as well as the desired product properties. In this context, the production of standardized insect flour fractions, the avoidance of undesirable reactions during processing and storage as well as the production of durable and marketable ingredients from insects may be considered as the main reasons for developing environmentally friendly and cost-efficient processing concepts for producing standardized valuable insect-based food with defined formulations.

Facing this challenge, aim of this study was to develop a process route for the efficient extraction and concentration of valuable components from crickets (*Acheta domesticus*) by altering the process sequence during cricket processing. Since the drying process affects quality and nutritional attributes and, to a certain extent, also the microbiological stability, it represents a key technology involved in producing flours and flour fractions from edible insects. Conventional drying methods as oven drying were found to strongly reduce the protein solubility and to consequently decrease the protein extractability during protein concentration or isolation from conventionally dried crickets.

In this study, three fractions could be produced utilizing a modified production process: a fat free protein solution (79.2%), a low-protein solid (5.6%) and a liquid high-fat fraction (10.5%) with dry matter contents of 0.35%, 23%, and 12%, respectively. The approach aimed at recovering proteins from fresh crickets directly following rearing in order to avoid the energy consuming removal of water at various stages of the processing chain. A protein concentrate with an increased crude protein content of 80% and a lowered fat content of 1 % was recovered by subsequent drying and milling. The low-protein fraction contained 77% of crude protein and 4% of fat. Compared to flour produced from dried crickets (76% crude protein and 9 % fat) the protein content was increased while decreasing the fat content.

## From pilot plant to production – Processing line for protein and fat recovery from insects

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Producers of insects larvae with the aim of protein and fat recovery have often the situation that they start with a small amount of harvested larvae per week which grows in a short/mid-time period to a few tons per day. For the recovery process they need a highly flexible pilot plant in the beginning and a continuously working process line in a later stage. It is challenging to combine both requirements in one system.

GEA developed a concept which accompanies the producer in both phases: from startup phase up to continuous production in small scale.

The processing starts in batch production, with production capacities from 200–500 kg fresh larvae per day. With a growing production output the system can be switched to continuous production to produce up to 500 kg/h equal to 10 t per day fresh material.

We distinguish between pilot, small and medium scale plants. Minimum equipment for a pilot plant are grinder, heat exchanger and decanter, our basic module. Based on this basic module you can increase capacity by just adding process stages, e.g. a drier, a separator to polish the oil or an evaporator to treat the stickwater. The benefit is that no equipment needs to be replaced, its just adding other equipment to the existing. With this concept you can easily grow from pilot to small to middle sized capacities.

Important for the scale up to a final production scale is to use the same technology from the very beginning to ensure the same product quality.

So the pilot plant will be not only a perfect starter with variable capacities but also the perfect system to develop different product qualities for different markets.



## Persistence of food-borne bacterial pathogen in house fly (Diptera: Muscidae)

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Nowadays few data are available on food safety related to the use of insects as protein source for food-producing animals. The aim of this study is to evaluate the persistence of pathogenic bacteria in *Musca domestica* reared on a diet contaminated with four microorganisms causing some important food-borne diseases: *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Clostridium perfringens*. The substrate on which the eggs (collected from a stable colony) hatched was contaminated, only at the beginning of the experiment, with  $5 \log_{10}$  CFU  $g^{-1}$  of each bacteria. *M. domestica* was reared under controlled condition: RH 70%, photoperiod 16:8 h (L:D) and temperature 25°C. Sampling of three developmental stages (larvae, pupae and adults) of house fly and their relative substrates were carried out and analyzed to quantify the pathogens using plates count on selective media. The pH and  $A_w$  of the substrate were also evaluated. Untainted substrate was added when needed. Three replicates and a control were set up. The values of the pH and  $A_w$  did not change significantly during the experiment (keeping in the range of bacteria growth). For all the bacteria tested the contamination of the substrate and of larvae increase of about 2 log until stage 3. In pupa the contamination slightly decrease (about 1 log). Adults keep contamination for all the bacteria tested but the average contamination results below 3 log.

These observations suggest that from a microbiological point of view, it's essential to monitor the substrate since the earliest stages of rearing due to the inability of the larvae to eliminate pathogens.

## Dynamic of five food pathogens in *Tenebrio molitor* and *Zophobas morio* larvae reared on contaminated substrate

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During rearing of insects for food and feed, their microbial safety is of utmost importance, but little is known on the transmission of food pathogens from the substrate to the insects. As for other food and feed products, it is important to monitor possible safety hazards during their production process. One of the potential risks associated with the consumption of mealworms is the possible presence of microbial food pathogens. Mealworms are already mass-produced as feed for pets such as reptiles and birds. In addition, they are promising candidates for human consumption. This study focuses on two mealworm species: the yellow mealworm (*Tenebrio molitor* L.) and the giant mealworm (*Zophobas morio* Fab.) The aim was to 1) explore potential food safety risks associated with edible mealworms and 2) to investigate the transmission of different pathogens to mealworms larvae can occur, in case mealworms are fed with contaminated wheat bran as substrate. Larvae were reared on the substrate which most used, consists of wheat bran supplemented with potato for 68 days under controlled conditions (RH 65%, dark condition and temperature 28°C) Three hundred larvae for specie were divided into 2 groups. The first group was infected by contaminated food (potatoes) supplied for 5 days, which was then removed and replaced to sterile one. The second group was maintained as negative control. The oral infection was carried out with bacterial quantity of  $10^8$ – $10^9$  CFU/g of *S. typhimurium*, *L. monocytogenes*, *C. botulinum*, *E. coli* and *Bacillus cereus*. For each trial after 0, 1, 4, 8, 14, 22, 57, 68 days diets and larvae was assayed for pathogen populations, pH, and water activity. Mealworms larvae started to be positive 4 hours after contamination and maintained high levels of contamination for 8 day. After this point the larvae and substrate contamination begins to decline but remained positive throughout the duration of the trial. The control group remained negative for each pathogen. The results from this study demonstrate that when the high pathogens contamination occur in mass rearing of mealworms this can remain until the end of the larval period. Pupae obtained from infected larvae were negative for all pathogens.

## Promoting insect production and consumption in the central highlands of Madagascar

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Insects are a traditional, nutritious and affordable food for the rural population in Madagascar. ProciNut project aims to improve the nutritional status of poor communities by promoting production, processing and consumption of edible insects. A first field study was conducted in five villages and in the city of Ambositra, located in the central highlands of Madagascar, to understand the local insect harvesting and farming, processing and consumption system.

Results from focus group discussions, key informant interviews and market interviews show that insects are mainly collected from the wild in the rainy season which is also the lean season. They comprise a range of different species, including beetles, locusts, cicada and the wild silkworm *Borocera cajani*. Most insects are eaten on a seasonal basis, but are highly valued by consumers. However, the amount collected remains low compared to demand. Harvested insects are mainly used for home consumption, and only surpluses are sold on local markets. Other edible insects exist locally but are not consumed or poorly viewed by the communities, e.g. crickets.

Rearing of insects is not common in the region, but is considered by some villagers as a possible solution for achieving a higher and more continuous supply of insects as food. Also, processing of insects could result in higher off-seasonal amounts, but will depend on the species and the available quantities. In short, insect farming and processing could provide an additional protein and micronutrient source and tackle the widespread food and nutrition insecurity which affects 69% of the population in the region.

## Occurrence of selected human foodborne viruses in industrially produced insects for food

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Since the introduction of edible insects on the European markets, the microbiological quality and food safety of edible insects was investigated by a few research groups. That research was generally focused on bacteria and, to a lesser extent, fungi as biological food safety hazards. However, also foodborne viruses, should they occur, may pose a food safety risk. While EFSA has indicated in its risk assessment<sup>1</sup> that most insect-associated viruses should not pose a risk for human health, it is also stated that insects may harbor or transmit vertebrate viruses, including human pathogens. The latter has not been investigated for edible insects so far.

The goal of this research was to obtain insight in the occurrence as well as the quantity of four selected human foodborne viruses in a collection of samples from industrially produced insects for food. Viruses selected were Hepatitis A virus, Hepatitis E virus, Norovirus GI and Norovirus GII, which are all RNA viruses. Edible insects considered were the yellow mealworm (*Tenebrio molitor*), the house cricket (*Acheta domesticus*) and the tropical house cricket (*Gryllodes sigillatus*). A comprehensive collection of samples was obtained from different rearers in Belgium and The Netherlands, covering several cycles per rearer.

Detection and quantification of the viruses was performed using a TaqMan-based qPCR targeting each virus separately. After total RNA extraction, extracts were subjected to a reverse transcription (RT) PCR using a selective primer for each target virus. The cDNA obtained was subsequently used for qPCR, with inclusion of an internal amplification control (IAC). Cq values were compared with standard curves obtained from virus cDNA reference fragments. The results (which are currently being generated) will point out whether these viruses were present or not in the samples and if so, to what extent. This information can contribute to the assessment of insect food safety.

### Reference:

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## Labeling of food insect products in the European Union

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In recent years insects as food have become very important for people in the European Union. The marketing of edible insects was not only limited by the consumer's distrust of something new, but above all by the lack of specific legislation. The only regulation considering edible insects is Regulation (EU) 2015/2283 (Novel-Food-Regulation), which has been in force since 1 January 2018 and contains rules on the placing on the market of food insect products in the European Union. There are currently no other specific regulations for food insect products for example with regard to labeling. Only the general requirements set out in Regulation (EU) 1169/2011 concerning the provision of food information to consumers are applicable on food insect products. In order to facilitate the handling of edible insects, some countries of the European Union have published guidelines on this topic, which contain additional recommended labeling elements based on risk assessments. These include an allergen warning regarding the possible cross-reaction with shellfish, crustaceans, dust mites and a heating warning for products that have not yet been heated in the manufacturing process. It is also recommended to indicate the common and scientific name of the species. We present the labeling of samples that have already been investigated by the Chemical and Veterinary Investigation Office in Freiburg. We also show whether the manufacturers of insect food products already apply the recommended labeling elements for their products.

## Legal status of edible insects in African countries and Haiti

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Wherever practiced, the tradition of entomophagy has developed into a concise set of local dos and don'ts to ensure minimum risks for the consumer. However, traditions are constantly confronting their rationale as time progresses and conditions change, as may the upcoming insect farming. Surveying food law in Africa is a complex matter, be it because of availability of texts, be it by the extent of the food law itself. The objective of the present contribution was to survey African food laws with regard to edible insects.

For that, public health and trade authorities were contacted directly or via corresponding embassies and private network scientists in Africa. They were asked whether rearing, placing on the market, and if consuming edible insects is legal. If so, they were requested to name the corresponding legal base. If not, they were asked if edible insects were condoned or actively forbidden (if so, on what base).

While several countries failed to respond, in most countries that did answer, insects were condoned as foodstuff. However, no concise legal framework existed. Yet, the Democratic Republic of the Congo, South Africa, and Namibia are planning to include corresponding sections. In contrast, Madagascar refers to the general food law of the Codex Alimentarius, and Gabon contemplates edible insects in the Code Forestier de la République Gabonaise which regulates the sustainable management of forest resources.

In this way, the current African food law considers, if at all, traditional entomophagy. As insect farming becomes popular, traditional handling of insects to ensure food safety may not be sufficient for this novel situation. Thus, food law should also include edible insects.

## A new model for know-how diffusion is needed for the growth of insect industry

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A rapid growth in insect industry is an asset for the radical change needed to our food system to gain sustainability. Insects can serve as a solution to protein self-sufficiency, recycling side streams of the food industry into more valuable components, nutrient recycling and sanitizing bio waste. However, the change requires building and speedup of the entire innovation ecosystem of the insect industry. Knowledge diffusion through the networks is the missing part of the current innovation system of insect industry.

Recent attention has been paid for the new technology, markets, and creation of legitimacy. Product development and marketing alone are not enough for creating new markets, but a wider food culture change is needed for the social acceptance of insect foods amongst the Western cultures. Information should be collected through various consumer researches on product acceptability, selection factors and usability.

Expanding the current edible insect production into feed business requires remarkable up-scaling in production volumes. Technology leap is necessary for the growth because a radical decrease in production costs is urgent. There is, however, very little expertise in profitable insect production that could be used for planning new investments and making funding decisions.

New know-how is needed for the buildup of the novel industry. At present, the knowledge is often intellectual capital of companies, and thus not available to be utilized in education or training. Therefore, the formation of expertise needed for the change, is slow. Mechanism for know-how diffusion, tempting all the actors, and anticipating the future needs should be invented.

Our plan is to pilot a model for know-how diffusion. The model consists of a nationwide network of organizations operating in the fields of research, education and business. Expertise from research organizations encourages the students in their insect industry-related projects and theses. We utilize the intellectual capital of the network to solve the above-mentioned challenges.

## The state of the art of the insect business in the Netherlands

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The insect production as well as the use of insects as ingredients and valuable protein sources for the European feed and food market, is still in its infancy. This study aimed to identify the state of the art of the Dutch insect industry through analysing the industry and the involved stakeholders. This is done by adopting an ecosystem perspective (ADNER 2017) and applying the Ecosystem Pie Model (TALMAR *et al.* 2018).

During a workshop involving multiple stakeholders, different parties identified similar needs, risks and opportunities and prioritized in a similar way. Through mapping the stakeholders, it was shown that many different, small scaled parties must be involved in the business ecosystem of insect production in the Netherlands. Preliminary results showed however, that many of these stakeholders are essential in materialising the value proposition (i.e., insects as sustainable feed protein) but show only little dependence. This combination may lead to the risk that the insect feed ecosystem lacks clear leadership. This is extra troublesome because stakeholders are highly interdependent within this ecosystem. The final results will show the most urgent ecosystem bottlenecks, and how addressing these bottlenecks would influence the future development of the full insect industry in the Netherlands. Still, this study also highlights that connecting only the insights from the industry is insufficient to allow for the insect industry to be developing itself as an innovative ecosystem for business. It is essential to align all different stakeholders, in which also consumers (of feed and food products) should be considered, to develop, envision and materialize the ecosystem value proposition.

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## Analysis of subconscious mind on an entomophagy of Japanese younger generation

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According to the World Population Prospects, global population will be beyond 9 billion by 2050. Edible insects receive an attention as an alternative protein source which may make up for deficiencies in the future food supply. Insects have a big potential as a novel food because of their high production efficiency, high nutritional value, and low environmental load. In Japan, we have a traditional habit of eating locusts and bee larva, however the chance of entomophagy is decreasing due to the westernization of our diet. In Recent, the entomophagy is negative enough to be recognized as a bizarre food rather than a traditional food.

In the near future, Japanese people is most likely to face a psychological issue that how to solve the negative impression of the entomophagy for accepting it more easily. In particular, to clarify the youth's psychological structure about the entomophagy will be a key to solve this issue. In this study, we applied a factor analysis to identify the subconscious mind on the entomophagy and discussed about the differences in cognition and evaluation on the edible insects by presence or absence of eating experiences.

We prepared the questionnaire with 27 questions asking cognition and impression on the entomophagy. Total of 135 vailed responses were obtained from undergraduate students of Takasaki City University of Economics. Factor analysis resolved a four-factor solution. Each of these factors has a distinctive grouping of questions that can be associated with a specific subconscious mind on the entomophagy (Factor 1: acceptance of the entomophagy, Factor 2: rate insect as a food, Factor 3: anxiety about safety and hygiene of the entomophagy, Factor 4: negative image of the entomophagy). The scores of Factor 1 and 2 from the respondents who have experience of the entomophagy were significantly higher ( $p < 0.05$  by t-test) than those from the respondents with no experience. This indicates that a neophobia will be a simple but a major cause to inhibit acceptance of the entomophagy. We need to study an innovative approach to make the first step for eating insects.

## Novel food-omics: Exploring a fingerprinting approach to insects' species identification

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The use of insects based powders in the food and feed sector is of high interest worldwide, due the increasing protein demand and to the need of environmentally friendly production chains. Powdered insects are hardly recognizable and production costs and output vary widely between insect's species and within the same species according to farming substrates (EFSA 2015). This raises the possibility of commercial fraud with potential consequences for human health. Rapid and reliable methods for species identification could represent an important solution to limit this possibility.

Currently, laboratory methods aiming at this rely on PCR, with very accurate results but a major limitation, the ability to confirm the presence of a species but not to identify adulterated products.

A number of different techniques can overcome this issue (BÖHME *et al.* 2019; MEDINA *et al.* 2019) and Direct Analysis Real Time-High Resolution Mass Spectrometry method as well as Near-Infrared spectroscopy represent interesting analytical solutions due to their high discriminating ability.

In this work we have explored the performances of two techniques (DART-HRMS and NIR) to differentiate insects' powder of known origin and composition. We focused in particular on orthopteran and coleopteran.

In particular, analyses were performed to evaluate the characteristic fingerprinting profile of the insects samples and to identify chemical markers able to differentiate insects. All the collected data were used to build a statistical model able to discriminate samples according to composition (species) and to classify insects samples (both single species and multispecies) of unknown origins to identify adulterated powders. Results suggest the possibility to use DART-HRMS and NIR as rapid techniques for authenticity evaluation at least as a first screening technique.

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## Microbiological analysis of food insect products

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Applications for approval as novel foods have been submitted so far for seven food insect species. These species are house crickets (*Acheta domestica*), lesser mealworms (*Alphitobius diaperinus*), honeybee drone brood (*Apis mellifera* male pupae), crickets (*Gryllodes sigillatus*), black soldier flies (*Hermetia illucens*), migratory locusts (*Locusta migratoria*) and mealworms (*Tenebrio molitor*). They may continue to be placed on the market until a decision is taken in accordance with Article 35 (2) of Regulation (EU) 2015/2283 on novel foods, but not later than 2 January 2020. This regulation only contains rules on the placing on the market of edible insect products in the European Union, microbiological criteria are not mentioned. So far only the criteria for *Listeria monocytogenes* of the Microbiological Criteria Regulation (EU) 2073/2005 can be applied to food insect products. Further microbiological criteria have not yet been established. Based on the previously examined samples of food insect products at the Chemical and Veterinary Investigation Office in Freiburg, we present the microbial findings in these samples. The results are compared with the expected microbial hazards in insect products published by the European Food Safety Authority (EFSA) in their risk profile related to production and consumption of insects as food and feed. Furthermore, some European countries have published national guidelines on food insects, some of which contain recommendations for microbiological criteria. We present the comparison of the test results made by the Chemical and Veterinary Investigation Office in Freiburg with the recommendations of the national guidelines from the European Member States.

## **Preliminary results of survey on food safety aspects of drone brood from *Apis mellifera* L.**

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Drone brood removal is a common practice for the control of the honeybee parasite *Varroa destructor* in beehives of *Apis mellifera* L. in Switzerland and other European countries. At present, the removed drone brood is not used. The demand for alternative protein sources is growing and drone brood has a big potential to become a new food product and a new income for beekeepers.

In contrast to insects currently produced for food, honeybees feed in an open system in the environment, which is difficult to control. Therefore, food borne pathogens from the environment on drone brood need to be assessed carefully. We analysed samples of drone brood from eight Swiss apiaries regarding important food borne pathogens.

This survey gives a first overview on microbiological food safety of drone brood from *A. mellifera*. Based on our results, further studies will be conducted to develop a process of drone brood production that is safe regarding food borne pathogens.

## Processing of cricket flour: Impact on microbial safety

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The use of insects as food poses potential microbiological risks, as insects can be vectors for human, animal or plant pathogenic microorganisms. In 2015, the European Food Safety Authority (EFSA) estimates the microbiological risk in its risk profile for the production and consumption of insects as food and feed to be comparable to other unprocessed animal protein sources when conventionally authorised feed is used as insect substrate. The microbial condition of the substrate and the hygienic conditions during the processing steps influence the microbial risk of edible insects. For example, thermal treatment and appropriate storage conditions are strongly recommended for edible insects before placing them on the market. Information on the microbial load of insects including the occurrence of possible human pathogens as well as the impact of processing on the microbial diversity is rare.

Therefore, aim of this study was the evaluation of microbial diversity changes during production of flour from crickets (*A. domesticus*). These were washed and subsequently thermally treated by steaming for 10 min at 100°C. Afterwards, the heat treated crickets were dried for 3 h at 110°C and milled to cricket flour. After each process step the samples were analyzed using traditional microbiological plate count methods with non-selective and selective media and subsequent identification of microorganisms using MALDI-ToF MS.

The total viable count was reduced from 9.24 log<sub>10</sub> CFU/g<sub>DM</sub> to 1.98 log<sub>10</sub> CFU/g<sub>DM</sub> along the entire processing chain. Washing led only to minor changes of the microbial load of the crickets, whereas steaming showed the highest inactivation. Drying only achieved an additional microbial inactivation of approximately 1 log unit. No growth above the detection limit was observed on selective media for Bacillaceae, Enterobacteriaceae, Enterococcaceae, and yeast and molds in cricket flour. Identification of microorganisms by MALDI-ToF MS after growth on non-selective media revealed that the predominant microorganisms in cricket flour belong to the member of Enterobacteriaceae and also members of the families Staphylococcaceae, Enterococcaceae, and Moraxellaceae could still be detected in cricket flour.

The results indicate that the steaming process is essential for effectively increasing microbial safety since this process showed the highest inactivation. It is recommended to not only evaluate the total viable count but also to monitor changes in microbial community during processing to ensure microbial safety of the final product.

## Assessment of the N- and P-fertilization effect of black soldier fly by-products on maize

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In 2016, more than 50% of the fish for human consumption originated from aquaculture (FAO 2018). In contrast, fishmeal and fish oil production, mainly used as feed in aquaculture, decreased by 50% between 1994 and 2016 (FAO 2018). To meet the growing demand for an alternative animal protein source such as insect meals, the black soldier fly (BSF) industry is on an expansion course. Thus, the valuation of its by-products, foremost of BSF residue (frass), is getting more economic and ecological weight.

In a pot trial with maize, the N- and P-fertilizing effects of three by-products of the BSF production (frass, larval skins, adult flies) were assessed. By-products were applied in three nutrient-based application rates to avoid distortion by different gross nutrient amounts, and plant nutrients, physiological and yield parameters were measured at harvest date.

The applied N amount was sufficient to obtain optimally nourished plants in the controls. Ground flies had the highest N-fertilizing effect of all by-products, similar to commercial fertilizers used as controls, whereas its proportion of the BSF production systems' output is low. Frass is quantitatively the most important by-product but showed comparably low N-fertilization effects. Its low N availability was attributed to volatilisation losses, mainly driven by high pH and ammonium contents.

Frass can be characterized as a composite fertilizer with a nearly even N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O ratio and high dry mass contents. Possible application purposes could be as a garden fertilizer or as basic fertilizer in agriculture and horticulture. For a more accurate assessment of the proposed valuation as a fertilizer, the variety of beneficial effects related to insect frass and/or chitin that are reported in literature should be investigated further, as they could define additional possible applications. Phytotoxic effects, as suggested by other authors (ALATTAR *et al.* 2016), were not observed at the present application rates. However, the crops, application modalities and post-processing methods influencing its occurrence are a field of further research.

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## Compilation of black soldier fly frass analyses

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In 2016, aquaculture produced more than 50% of the fish for human consumption, while fishmeal and fish oil production, which mainly is used as feed in aquaculture, halved in the past 22 years (FAO 2018). To meet the growing demand for an alternative animal protein source such as insect meals, the black soldier fly (BSF) industry is on an expansion course (IPIFF 2018). Thus, the valuation of BSF frass is getting more economic and ecological weight. However, little information exists on the properties and the uniformity of this by-product.

That is why BSF producers were asked for frass analyses. Of the returned analyses and frass analyses found in the literature, an anonymized compilation of 22 analyses was created for macro and micronutrients.

With a relatively even N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O ratio at around 3% DM, BSF frass can be characterized as a compound fertilizer. The nutrient contents are high, most likely comparable to poultry manure, and the C:N ratio of around 13 is similar to solid manures. Frass nutrients, especially micronutrients, showed great variation which was attributed to the unknown variation in BSF feed and its production systems. The neutral to alkaline pH bears the risk of NH<sub>4</sub><sup>+</sup> volatilisation of the NH<sub>4</sub>-N proportion (17% of total N) after application. High dry matter contents of 66% on average are expected to attenuate this process.

Possible application purposes based on the NPK pattern are as a garden fertilizer (high valuation, low amounts) or for basic fertilization in agriculture and horticulture (low valuation, high amounts). With the increasing importance of BSF frass, public and anonymized nutrient tables can be helpful as a mean of orientation for farmers and producers, preferably supplemented by a fertilizer analysis. With additional, basic information on the production system, the nutrient compilation could be refined, so the variation of the parameters would be reduced.

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## Cloning and expression of an antimicrobial peptide from *Hermetia illucens* (Diptera: Stratiomyidae)

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The ongoing problem of antibiotic resistance has led researchers to seek new solutions to control and treat infections by various pathogens as an alternative to modern antibiotics. One potential candidate is are antimicrobial peptides (AMPs) which are less prone to trigger resistances in pathogens and are specific toward bacterial cells<sup>1</sup>. AMPs are small molecules produced by all living organisms, but insects, and in particular *Hermetia illucens* represent one of the most promising organism source for AMPs. After a transcriptome analysis and a functional annotation (Blast2go software), it was possible to identify 82 putative antimicrobial peptides which can potentially be derived from the BSF. All encoded sequences for AMPs were bioinformatically characterized in order to predict their physico-chemical properties as well as their antimicrobial and anticancer activity. The *in silico* analysis allowed to hypothesize, that the AMP encoded by the gene corresponding to the contig C15867, identified in the *H. illucens* transcriptome, is among the most promising. Hence, it was selected and recombinantly expressed. For this purpose, the pCRII–TOPO vector was used to clone the gene of interest between the BamHI and EcoRI restriction sites. Subsequently, the gene was transferred into the expression vector pGEX-4T1, which allows the production of the AMP as a fusion protein with the GST protein. The recombinant product was expressed in *E. coli* BL21 cells with 0.8 mM IPTG. Samples were collected two and three hours after induction. The cells were recovered by centrifugation, chemically lysed (8M urea), and both the obtained supernatants and the pellets were analysed by SDS-PAGE. The purification and the enzymatic (thrombin) hydrolysis of the GST tag will be done in future, in order to verify the antimicrobial activity of the peptide and its structure.

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## Development of purification techniques for the isolation and identification of antimicrobial peptides derived from the *Hermetia illucens* (Diptera: Stratiomyidae) larvae hemolymph

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Insects are the largest class within the animal world in terms of their number of species. In fact, more than one million have been described and it is estimated, that an equivalent number of species remain to be identified<sup>1</sup>. The evolution of the biological diversity within this class is, among other points, supported by their widespread resistance to a broad range of pathogens. For insects, the first line of defence against pathogens is the cuticle. After this barrier has been overcome by pathogens, a complex interaction of innate humoral and cellular immune reactions is induced in tissues and haemocoel. This generally results in a rapid elimination of microorganisms<sup>2</sup>. One aspect of the insects' immune response studied in recent years, is the synthesis of antimicrobial peptides by the fat body, as they are rapidly released into the hemolymph. Accordingly, we focused on the identification of novel AMPs from the hemolymph of the larvae *Hermetia illucens* (Diptera: Stratiomyidae). For this purpose, we developed an HPLC-method to fractionate the extracted hemolymph. The method was based on a reversed phase chromatography using a stepwise elution-method with varying methanol-H<sub>2</sub>O elution conditions. The AMP activity of the collected fractions will be tested and the most promising samples will be further fractionated and analysed to identify novel antimicrobial peptides. After verifying their antimicrobial activity, the AMPs will be further characterized by mass spectrometry and tandem mass spectrometry analysis. Moreover, the same experiments will be performed on the hemolymph extracted from larvae of the *Hermetia illucens* insect immunized with different bacteria in order to isolate and identify peptides induced by immunization.

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## InsectChitin – Chitin/Chitosan production of insect biomass

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Chitin is the second most abundant biopolymer after cellulose and is mainly found in insects and marine crustaceans. Although strong polar interactions make chitin highly resistant to highly concentrated chemicals, it also makes extraction very intricate. (TÜRCK 2014)

In 2011, 1600 tonnes of purified chitin (derived from marine biomass) were extracted worldwide, with Japan as main producer. Due to the wide range of products (purity, qualities), the price range varies between 10–1000 \$ per kg. (TÜRCK 2014)

The existing method for chitin extraction from marine biomass (crustaceans, zooplankton) is not very efficient (low yields: chitin about 6%, chitosan 2.5%) and not environmentally friendly. Due to the increased use of marine resources and their potential ecological and social implications, the sustainability of the bioplastic chitosan, from marine biomass, must be considered critically. (LINDENTHAL, SCHLAAK & SIEFERT 2010)

The extraction of chitin from crab shells and its conversion to chitosan occurs within three main steps: demineralization, deproteination, deacetylation (RIGBY 1936). Between every single main step, at least one washing step is necessary, which makes the preparation even more expensive. The relatively low product yields and the process intricacy show the need for development work in this area.

The chitin content of marine crustaceans of about 20–31% is comparable to that of insects (10–36% in dry matter). However, the conditions for the acid treatment (demineralization) in the extraction from insects are significantly more moderate than those in the extraction from marine crustaceans. The reason for this is the lower content of inorganic substances in the insects (<10%) compared to the marine crustaceans (20–40%) (KAYA, ERDOGAN, MOL, & BARAN 2014), so the acid treatment could possibly be omitted.

The intention is to develop a process for the chemical and enzymatic production of chitin or chitosan from insect biomass. Exemplary, chitosan-based flocculants for precipitation processes in wastewater technology will be developed in order to recover biodegradable sludges. It is also intended to produce the chitosane substances in a very pure form for the use in medical and other applications.

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