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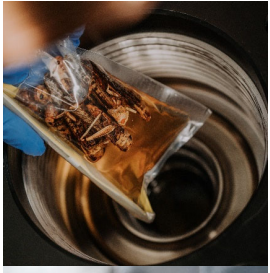

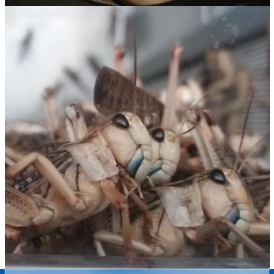

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Bornimer Agrartechnische Berichte

INSECTA 2022

International Conference

Book of Abstracts

14th – 16th September 2022
Giessen, Germany

ATB

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Heft 106
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September 2022

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Preface

The INSECTA 2022 conference aims to provide an overview of the state-of-the-art in insect utilization along with the prospects and constraints of the use of insects for food, feed and non-food applications in Europe and worldwide. This publication contains abstracts of all talks and posters presented at this conference.
The Leibniz-Institut für Agrartechnik und Bioökonomie e.V. (ATB) and the Pilot Pflanzenöltechnologie Magdeburg e.V. (PPM) alternate responsibility for conference leadership and co-conference leadership on an annual basis. PPM initiated the INSECTA 2015 in Magdeburg and took the leadership in 2016 (Magdeburg), 2018 (Giessen) and 2021 (Magdeburg). ATB was responsible for the organization in 2017 (Berlin), 2019 (Potsdam) and 2022 (Giessen). We are delighted to be guests at the Justus-Liebig-University (JLU) of Giessen and the Fraunhofer IME – Bioresources this year. The institute for insect biotechnology of the JLU was founded in 2015 as a structural goal of the LOEWE Center for Insect Biotechnology and Bioresources (LOEWE-ZIB). It is among the worldwide leading scientific entities in insect biotechnology (also known as yellow biotechnology) for development and application of biotechnological methods to translate insects or molecules, cells and associated microorganisms derived thereof into products and services for specific use in medicine, plant protection or industry. The Gala dinner will be celebrated in the new research building of Branch Bioresources of the Fraunhofer IME in Giessen, which exists since October 2020 and is becoming a pioneer in insect research.
INSECTA 2022 aims to sustain the success of recent congresses and to ensure its impact in insect science and technology for both industry and academia, as well as to bridge insect farming with insect biotechnology. Therefore, the annual meeting of the LOEWE-ZIB will take place in parallel. The theme of the conference is “INSECTA meets Yellow Biotechnology” with emphasis on insects as food, feed and non-food applications. Research and science-based innovation play a central role in enabling the related industry to address challenges faced by the sector at a global level. This conference aims to bring together companies and research innovation, helping to discover future perspectives of insect technology. Within the entire insect value chain, we have compiled a broad scientific program consisting of four central conference themes: i) Insect rearing and production systems, ii) Insect processing for food and feed, iii) Ethical and environmental aspects and iv) Non-food applications of insects.
Within the conference, we hope to offer a special platform for intensive and trustful professional exchange between newcomers and experts as well as academia and industry. In total, the conference has attracted 97 presentations from more than 20 countries. In the conference period of three days, seven keynote lectures, two company presentations, and 48 oral presentations are arranged in three plenary and 12 parallel sessions. Additionally, 40 posters and 11 company stands will be displayed for further discussion and exchange.
Finally, as the chairpersons of the conference, we would like to take this opportunity to sincerely thank the authors, reviewers, sponsors and supporters for their valuable contribution.

Oliver Schlüter, Julia Durek, Thomas Piofczyk, Andreas Vilcinskas

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BIS offers a total solution for insect farming based on in-house products and systems.

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ATB is a pioneer and a driver of bioeconomy research.

Our vision:
A circular, diverse, innovative and sustainable bioeconomy produces healthy food for all, operates on the basis of renewable raw materials and facilitates the realization of One Health for humans, animals and the environment.

Our research on insects aims at the sustainable exploitation of insects for food, feed and non-food applications.

It covers the entire supply chain and integrates smart and innovative technologies. Special focus is on the systemic evaluation of the material, energy and information flows while complying a balance of quality & safety.

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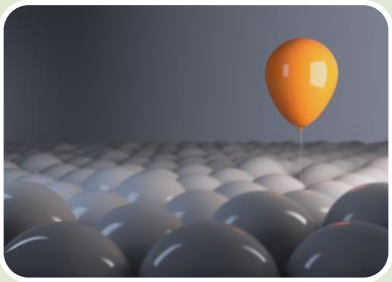

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Dr.-Ing. Sara Hadjiali | E-Mail: hadjiali@ppm-magdeburg.de |Tel.: +49-391-8189-132

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The VDI is an association for technically and scientifically active people (VDI: Association of German Engineers) and is one of the largest technically oriented clubs and associations in the world.

The VDI Magdeburger Bezirksverein supports the INSECTA conference since 2015 and grants the “VDI Best Young Scientists Presentation Award” every year. It is divided into 6 district groups and many thematically different working groups and has offers for all ages. One group related to INSECTA conference is the Arbeitskreis Nachwachsende Rohstoffe (circle of experts for renewable resources) that is managed by Sara Hadjiali. VDI Magdeburger Bezirksverein is organised by voluntary works.

The VDI Magdeburger Bezirksverein is supported in its work by numerous companies in the region and forms an important network and the VDI is also international networked.

Also VDI Magdeburger Bezirksverein has a sponsorship award for students at the universities in Magdeburg every year and the Gruson plaque of honor for members who deserved in the technical development and the work of VDI Magdeburger Bezirksverein Magdeburg.

More information can be found at homepage:

https://www.vdi.de/ueber-uns/vor-ort/bezirksvereine/magdeburger-bezirksverein

Program

September 14, 2022

02:30 p.m.	**Registration**	Foyer
03:30 p.m.	**Welcome – Opening remarks** *Oliver Schlüter* (Chair, ATB) *Andreas Vilcinskas* (Co-Chair, Justus Liebig University Giessen) *Priska Hinz* (Minister of state for environment in Hessen) *Alexander Goesmann* (Vize president of Justus Liebig University Giessen)	Auditorium
04:00 p.m.	**KEYNOTE:** *Jefferey K. Tomberlin* **Synergy between animal welfare & industrial production of insects: Opportunities created- not lost**	
04:40 p.m.	**KEYNOTE:** *Dorothee Tegtmeier* **The black soldier fly gut microbiome and its contribution to the bioeconomy**	
05:20 p.m.	**KEYNOTE:** *Martin Rühl* **Biotechnology in food and feed applications with insects**	
06:00 p.m.	*Eleonore Glitz* (Projektträger Jülich) **BMBF-Programm KMU-innovativ Bioökonomie**	
06:00 p.m.– 09:00 p.m.	**Get-Together**	Foyer

September 15, 2022

08:00 a.m.	**Registration**	Foyer
09:00 a.m.	**KEYNOTE:** *Karol Barragán-Fonseca* **Integrating social aspects in insect farming**	Room C112
09:30 a.m.	**KEYNOTE:** *Shikha Ojha* **Implementations of innovative technologies in the edible insect processing pathways**	
10:00 a.m.	**Company Presentation: Better Insect Solutions** *Lars-Henrik Lau Heckmann* **Better Insect Solutions – Setting the standard in insect farming**	

September 15, 2022

Session 1 ***Room C05a***

Insect rearing and production systems

10:15 a.m.

Are black soldier fly trial results influenced by larvae handling?
Sara Bellezza Oddon et al., Italy

10:35 a.m.

The influence of H. illucens larvae on digested municipal sewage sludge microbial activity and functional diversity
Piotr Bulak et al., Poland

10:55 a.m.

Bacterial babysitters: Decrypting the microbiota on the Black Soldier Fly's embryo surface and their origin
Carina D. Heussler et al., Austria

Session 2 ***Room C05b***

Ethical and environmental aspects

10:15 a.m.

The influence of the product name on the acceptance of insect based food
Anna Lürkens, Sonja Floto-Stammen et al., The Netherlands

10:35 a.m.

What motivates consumers to accept whole and processed mealworms in their diets? A cross-country study in Belgium, China, Italy, Mexico, and US
Daylan Amelia Tzompa-Sosa et al., Belgium

10:55 a.m.

Sensory analysis of edible insects in Czechia: does size really matter?
Martin Kulma & Lenka Kouřimská, Czech Republic

11:15 p.m. Coffee break and poster session

Session 3 ***Room C05a***

Insect rearing and production systems

11:45 a.m.

Increasing the ω-3 fatty acids content of Black Soldier Fly Larvae through substrate fermentation
Freek IJdema et al., Belgium

12:05 p.m.

The influence of dietary starch, fibre, and water on the nitrogen balance in black soldier fly larvae production
Tom van den Beuken et al., The Netherlands

12:25 p.m.

Can the use of brewing by-products modulate microbiota and mycobiota of black soldier fly larvae?
Ilaria Biasato et al., Italy

12:45 p.m.

Visualization and quantification of microplastics in the gut of black soldier fly larvae (*Hermetia illucens*)
Siebe Lievens et al., Belgium

Session 4 ***Room C05b***

Ethical and environmental aspects

11:45 p.m.

Sensorial properties of raw and heated, fresh and spoiled Mediterranean crickets (*Gryllus bimaculatus*)
Nils Th. Grabowski et al., Germany

12:05 p.m.

Feeding habits and acceptance of insect-based products by dog and cat owners – a survey
Fien Carron, Camila Baptista da Silva et al., Belgium

12:25 p.m.

Zero Waste Protein Factories: Leveraging Biology to empower the industry for waste-to-protein conversion on-site
Katharina Unger, Austria

12:45 p.m.

Economic viability of insect farms in the Netherlands
Hilde Niyonsaba et al., The Netherlands

01:05 p.m. Lunch break and poster session

Session 5 ***Room C05a***

Insect rearing and production systems

02:05 p.m.

Bioconversion potential of agro-industrial by-products by *Tenebrio molitor* – long term results
Anna Bordiean et al., Poland

02:25 p.m.

Nutritive value of different raw materials in yellow mealworms: optimizing *Tenebrio molitor* nutrition
Bruno Fasce, Juan José Pascual et al., Spain

02:45 p.m.

Statistical comparison of insect growth parameters at different test sites
Siebe Berrens et al., Belgium

03:05 p.m.

Too hot, too cold: Scaling effects and standardised research for industry relevance
Julie C. Wray, Meghan D. Cooling et al., Canada

03:25 p.m.

Development of a continuous reproduction system for the BSF
Seppe Salari & Lisanne de Goede, The Netherlands

03:45 p.m. Coffee break and poster session

Session 6 ***Room C05b***

Insect processing for food and feed

02:05 p.m.

Evaluation of black soldier fly larvae proteins as substitute of poultry by-product meal in extruded dry diets for dogs
Aman Paul et al., The Netherlands

02:25 p.m.

Development of an enzymatic treatment followed by tricanter centrifugation to produce insect meal with improved characteristics from *Hermetia illucens*
Sandra Balsells, Anna Margenat et al., Spain

02:45 p.m.

The oxidative stability of commercial *Hermetia illucens* meal
A. Mouithys-Mickalad, Ghina Kotob et al., The Netherlands

03:05 p.m.

Differences in process control starting with the killing of the larvae between *Hermetia illucens* and *Tenebrio molitor* in dry and wet processing – A detailed view of intermediates and end products
Verena Böschen et al., Germany

03:25 p.m.

Hygienic status of black soldier fly based feeds during processing – A detailed view of intermediates and end products
Patrick Sudwischer et al., Germany

Session 7 ***Room C05a***

Insect rearing and production systems

04:15 p.m.

The influence of dietary fat on the yield, bioconversion efficiency, and body composition of black soldier fly larvae
Ruilong Zheng et al., The Netherlands

04:35 p.m.

Multifaceted approach to high fiber waste management using black soldier fly larvae
Pratibha Yadav, Austria

04:55 p.m.

Making insect farming simple and accessible to everyone, anywhere, by complete decoupling of BSF breeding from rearing & processing
Yuval Gilad et al., Israel

05:15 p.m.

Studies of *H. illucens* mating behavior under high density conditions
Rachel Toaff-Rosenstein et al., France

Session 8 ***Room C05b***

Insect processing for food and feed

04:15 p.m.

Nutritional analysis of seaweed flies *Fucellia maritima* (Haliday, 1838) reared on five different substrates
Felipe Lourenço et al., Portugal

04:35 p.m.

Microbial characterization of Bombyx mori and possible reutilization of chrysalides as food ingredient: circular economy approach in sericulture
Filippo Marzoli et al., Italy

04:55 p.m.

Taurine content in insects
Lenka Kouřimská et al., Czech Republic

05:15 p.m.

Zinc twice: A trace element to benefit insects and consumers
Claudia Keil et al, Germany

07:00 p.m.– 12:00 a.m. **Conference barbecue at LOEWE Center for Insect Biotechnology & Bioresources (Fraunhofer IME)**

September 16, 2022

08:30 a.m.	**Registration**	Foyer
09:00 a.m.	**KEYNOTE:** *Heinrich Katz* **Insect production and processing in Europe – Recent developments and perspectives**	Room C112
09:30 a.m.	**KEYNOTE:** *Thomas Wilke* **Insects as sustainable aquaculture feed**	
10:00 a.m.	**Company Presentation: Royal Dutch Kusters Engineering** *Jeroen Kusters* **Insect rearing technologies**	

Session 9 ***Room C05a***

Insect processing for food and feed

10:15 a.m.

Effect of conservation, blanching and drying conditions on the safety and quality of *Tenebrio molitor* larvae
José C. Ribeiro, et al, Portugal

10:35 a.m.

The influence of different side streams on the nutritional composition of the yellow mealworm (*Tenebrio molitor*)
Isabelle Noyens, Geert Verheyen et al., Belgium

10:55 a.m.

Detection of changes in physicochemical properties during storage of high pressure treated mealworm paste
Giacomo Rossi et al., Germany

11:15 a.m.

DTI – Insect industry prospects
Mads H. Juul & Neda N. Moghadam, Denmark

Session 10 ***Room C05b***

Non-food applications of insects

10:15 a.m.

The fertilization potential of *Hermetia illucens* larvae post-breading residue
Monika Kaczor et al., Poland

10:35 a.m.

Can further composting improve the fertilizer value of frass?
Arthy Surendran & Rob Lillywhite, United Kingdom

10:55 a.m.

Compliance of frass with legal requirements
Lotte Frooninckx et al., Belgium

11:15 a.m.

Insects in the fight against antibiotic resistance: in vitro characterization of black soldier fly antimicrobial peptides
Laurence Van Moll et al., Belgium

11:35 a.m. **Coffee break and poster session**

Session 11 ***Room C05a***

Insect rearing and production systems

11:55 p.m.

New species of culturable bacteria from insects
Juan Guzman & Andreas Vilcinskas, Germany

12:15 p.m.

Ozone treatment to control mite infestation in reared crickets (*Acheta domesticus*) oviposition substrate
Michela Bertola et al., Italy

12:35 p.m.

Dose-response effects of starch in chicken excreta on *Musca domestica* larval performance and bioconversion efficiency
Lennard Pisa et al., The Netherlands

12:55 p.m.

Higher larval density leads to lower survival and fat content, smaller size, longer development time and increased investment into immunity in the black soldier fly
Leonard O. Opare et al., Estonia

Session 12 ***Room C05b***

Non-food applications of insects

11:55 p.m.

The chemical diversity in arachnid venom as a source of biomolecular innovation in medicine and agriculture
Tim Lüddecke et al., Germany

12:15 p.m.

Venomics of the native myrmicine ants *Myrmica rubra* and *Myrmica ruginodis*
Sabine Hurka et al., Germany

12:35 p.m.

Educational leadership chair in the production and primary processing of edible insects
Marie-Hélène Deschamps & Grant W. Vandenberg, Canada

12:55 p.m.

Insect School: A way to move the BSF industry forward
Bob Holtermans, The Netherlands

01:15 p.m. **Closing remarks** **Room C112**

VDI BEST YOUNG SCIENTIST'S PRESENTATION AWARD Sponsor: VDI – Magdeburger Bezirksverein

01:30 p.m. **Refreshments for farewell**

02:00 p.m. **Visit of LOEWE Center for Insect Biotechnology & Bioresources (optional)**

03:00 p.m. **End of conference**

Synergy between animal welfare & industrial production of insects: Opportunities created – not lost

Jeffery K. Tomberlin[1], Meghan Barrett[2,3], Shaphan Y. Chia[4], Bob Fischer[5]

[1] Department of Entomology, Texas A&M University, USA
[2] Department of Biology, California State University Dominguez Hills, USA
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Corresponding author: Jeffery.Tomberlin@ag.tamu.edu

Livestock production has experienced tremendous growth with regards to animal welfare. Such trends have expanded across practically all animals in mass production, including recent emphasis on the insects as food and feed sector. In many instances, altering methods to account for animal welfare can be challenging for the insects as food and feed sector – especially when one considers the historical relationship between humans and insects from a health and fitness perspective (e.g., insects as disease vectors or agricultural pests). The purpose of this presentation is to partially review animal welfare tools used for traditional livestock and compare the use of these tools for mass-produced insects. Key abiotic (e.g., light, temperature, larval and adult nutrition) and biotic factors (e.g., larval and adult density, pathogens) impacting the welfare of the black soldier fly in mass production will be discussed. Through this presentation, it will be demonstrated that numerous recommendations aimed at enhancing animal welfare for mass-produced insects may also enhance production, lower costs, and thus increase profit.

The black soldier fly gut microbiome and its contribution to the bioeconomy

Dorothee Tegtmeier[1], Patrick Klüber[1], Sabine Hurka[2], Andreas Vilcinskas[1,2]

[1] *Branch for Bioresources, Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Giessen, Germany*

[2] *Institute for Insect Biotechnology, Justus Liebig University Giessen, Giessen, Germany*

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Insects play an increasingly important role as an alternative source of protein for the sustainable production of food and feed. However, the economic competitiveness of farmed insects depends on the availability of inexpensive insect feed. Industrial side streams could play a low priced role in providing feed sources. Cottonseed press cake (CPC), empty fruit bunches (EFB; residues of the palm oil industry) or potato pulp (PP; byproduct of starch manufacture) are currently only inefficiently used, partly because of their low nutritional value. For example, EFB and PP are characterized by high fiber and low protein and lipid content. By contrast, CPC is rich in proteins and lipids, but unsuitable as feed for several animals, due to the presence of the anti-nutritional and insecticidal substance gossypol.

Our results showed that the larvae of the black soldier fly (*Hermetia illucens*) can be reared on CPC and PP at high yields when compared to EFB. Developmental time was much shorter on CPC when compared to PP. Since developmental parameters of larvae and flies were similar to the control group that received chicken feed, we conclude that *H. illucens* can tolerate gossypol present in CPC and might be capable of degrading this compound.

The degradation of such diverse substrates is mediated by an abundant gut microbiome. Therefore, we analyzed the microbiome of larvae via amplicon sequencing and isolated various bacteria and fungi using selective media. Our data show, that microbial communities in the gut adapt depending on the feed. In the CPC diet group, *Actinomycetaceae* and *Aspergillaceae* were significantly enriched, which can help in breaking down compounds such as gossypol. We found many isolates with cellulolytic activity only in larvae fed with the high fiber diets but not in larvae fed with chicken feed.

Due to the rapid larval development and the adaptation of the gut microbiome, CPC may be suitable as sustainable feed for the industrial rearing of *H. illucens*. Growth of larvae reared on the high fiber-diets could be improved by adding bacteria and fungi to the feed, which help in breaking down fibrous structures such as lignocellulose and pectin.

Biotechnology in food and feed applications with insects

Martin Rühl[1,2]

[1] *Institute of Food Chemistry and Food Biotechnology, Justus Liebig University Giessen, Giessen, Germany*

[2] *Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Giessen, Germany*

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Insect biotechnology concerning food and feed applications is a vast growing field in science and industry. This includes the usage of insect larvae as alternative protein source for human consumption as well as insect-based feed for farmed animals. Because insects can feed on side-streams that are indigestible for farmed animals, a sustainable production of animal proteins with insects is a promising way for both applications: food and feed. Furthermore, peptides and enzymes of insect origin can serve as food additives or processing aids to e.g. elongate shelf life of several foods or reduce unwanted food ingredients. In this regards, the lecture will give examples on biotechnological applications with insects that will have a share in a more sustainable production of food and feed in the nearer future.

Integrating social aspects in insect farming

Karol B. Barragán-Fonseca

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Science should respond to the social reality, helping to resolve the problems faced by humanity. However, scientific quality and social impact do not always go together. Identifying how to contribute to resolving social problems are the key and there will be always an idea, a species or a process that has the potential to be disruptive innovators to resolve a problem or to support a community. Insects have the potential to transform societies. Some insects' species may be produced locally on organic waste, providing protein as well as other nutrients for animal feed, thereby reducing dependence on high-cost imported protein sources and contributing to a circular economy. Indeed, insects may be used to replace conventional sources of protein in animal feed to improve socio-economic conditions of small farmers in general. It is clear that insect farming is growing fast rapidly in all continents and its benefits can be seen at the large, medium and small scale, and social aspects may be seen from a production scale perspective. There is a development in economics to not focus exclusively on gross domestic product growth and develop an economic vision that includes ecological and social aspects. In Colombia, a country currently in a post-conflict stage after over 60 years of war, we were able to help resolve a community problem: the high cost of fish feed and empower smallholder farmers through implementing small facilities for production of black soldier fly larvae to feed fish. This project of implementing the use of insects as feed by communities of former fighters and others affected by the armed conflict is called *Insects for Peace*, and is an example of how small-scale insect farming reintegrate former fighters into the local economy and thus to contribute to peace through science. This project has shown that even when large-scale transformations are not possible, small-scale initiatives can make significant contributions. Societal transformations – such as from war to peace - unleash social, economic, and political challenges that need to be dealt with by many committed actors through multi-, inter-, and trans-disciplinary perspectives.

Implementation of innovative technologies in the edible insect processing pathways

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With increasing demand of insect-based foods in western countries, many companies have started mass rearing systems; therefore, adequate insect processing are essential to ensure food safety and quality. Depending upon the nature of the initial material and the desired end product, the processing pathways vary and may include several unit operations already used in food processing. The EU H2020 funded MSCA project 'Eco-innovative technologies for sustainable exploitation of macromolecules from in-sects as alternative resource in bioeconomy (*in*TECH)' particularly focused on innovative food processing technologies (e.g. high pressure processing, pulsed electric field, microwave and ultrasound) to modify, complement or replace the conventional processing steps in insect processing. The project was successfully completed this year and explored the potential of these novel technologies to efficiently replace/reduce the chemical usage in extraction of the insect macromolecules to develop environmentally friendly process.

In the presentation, different case studies will be presented, where innovative technologies were implemented alone or in combinations. For example, 5 min treatment of ultrasound (100 W) in combination with microwave (220 W) yielded higher extracted water-soluble proteins than control 18 h overnight extraction. In summary, it became evident that these technologies could be implemented for versatile applications to improve the processing efficiency, safety and quality of insect-based products and contribute towards sustainable bioeconomy.

Insect production and processing in Europe – Recent developments and perspectives

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The European insect sector – an agricultural reality that is gradually growing into a reputable actor in terms of products' quality and sustainability - has the potential to become an important agri-food player, but also a strategic link of the EU's food and feed chains. Notably, insects have been recognised for their role in complementing both humans' and animals' diets, having an enormous potential to improve the circularity of the agri-food nexus. The benefits of insect farming go beyond that – the insect-based ingredients represent a local solution, improving circularity while also reconnecting regional agricultural supply chains.

This presentation will provide a general background of the recent developments in the field of insect farming, highlighting key trends in insects as feed, food and frass (insect dejecta), as well as relevant regulatory priorities for the sector in the upcoming future.

Insects as sustainable aquaculture feed

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In 2020, about 10% of the world's population had suffered from hunger. Therefore, the UN Sustainable Development Goal (SDG) 2 – Zero Hunger – remains a key focus of international development programs. However, SDG 2 often conflicts with other SDGs, such as SDG 6 (Clean Water), SDG 10 (Reduced Inequalities), SDG 13 (Climate Action), SDG 14 (Life below Water), and SDG 15 (Life on Land). These inherent conflicts between the economical, ecological and social pillars of sustainability are particularly evident in the fastest growing sector of food production – aquaculture. Shrimp aqua-culture, for example, is one of the largest driver of habitat loss in costal ecosystems, the extensive transport of feed and foot during the production process results in a large carbon footprint, and the frequent use of antibiotics and growth hormones may affect animal and human health. The arguably single largest problem is the shrimp feed, which is often based on fishmeal. It as a major driver of biodiversity loss, an important vector of marine diseases, and a large contributor to high CO_2 emissions.

Therefore, an alternative insect-based aquaculture feed could be an essential approach to improve the sustainability of global aquaculture. In my presentation, I will evaluate the opportunities and challenges of using insect feed in aquacultures. Specifically, I will address target insect species, insect feeds based on unused side-streams from agriculture, processing of insect feed to meet nutritional demands, the use of insect meal vs. insect larvae, high-tech and low-tech approaches in an insect-based circular economy, life cycle assessments, and implications of insect-based feeds for other aquaculture and food production systems. The talk will be rounded out by ad-dressing economic challenges in insect-based sustainable aquaculture (food price and quality, carbon footprint) and the need of ecological and genetic research in in-sects to pave the way for one of the most exciting transformations in global food production.

Lectures

Topic 1–4

Are black soldier fly trial results influenced by larvae handling?

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In the last years, the number of papers focusing on black soldier fly (BSF) has shown an exponential increase. The larvae manipulation is necessary for the determination of growth performance, bio-conversion efficiency and adult parameters, both in industrial and bench-top-sized containers. The aim of the present study is to evaluate the effect of the handling on larval stage duration (LD), final larva weight (LW), bio-conversion efficiency corrected for residue (BER), survival rate (SR) and fly's emergence rate (ER). Three treatments (6 replicate/treatment and 100 larvae/replicate) were tested as follow: no handling (NH), which remained untouched for the whole trial, soft handling (SH), that was not manipulated until the appearance of the first prepupae, and hard handling (HH), in which larvae were manipulated every 4 day until 5% of prepupae was reached. When the first prepupae was identified, SH and HH boxes were checked daily and the evaluation of the 5% of prepupae was carried out manually every day. Since no handling was carried out on the larvae of NH group, the data relating to the larval stage were not collected (environmental control). NH was a statistical control only for the ER, which was calculated at the death of the flies. Consequently, LD, LW, BER and SR data were analysed by T-test or Mann-Whitney U test, while ER by One-way ANOVA or Kruskal-Wallis tests (IBM SPSS Statistics V28.0.0, $p<0.05$). For all the parameters recorded and calculated in the larval stage, no differences were observed between SH and HH ($P > 0.05$). The ER was not affected by the larvae manipulation ($P>0.05$). In conclusion, neither soft nor hard manipulation, during the larval stage, have an effect on growth performance, bio-conversion efficiency and adult emergence.

Statistical comparison of insect growth parameters at different test sites

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Due to the upcoming insect sector, mealworms gained increased interest of researchers worldwide. This has led to numerous studies and publications on mealworm production recently performed. However, differences in rearing techniques and the statistical analysis hamper comparison among them.

The conditions in which insects are reared greatly affect the results of the experiments. This was demonstrated by testing a standardised rearing protocol with three different partners within the ValuSect project (Interreg NWE).

Then, different experiments with mealworms reared on side streams were conducted. It was shown that there were differences in the partners despite the fact that the experiments were carried out using the same protocol. To be able to compare parameters such as total final weight, larval final weight, ... the values were transformed by bringing the control treatments for every testing site to one level. To be able to statistically compare growth curves a biological, sigmoidal growth model was used. The results of the ValuSect experiments are used to demonstrate this transformations and the growth model.

Ozone treatment to control mite infestation in reared crickets (*Acheta domesticus*) oviposition substrate

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In Europe, the industry of insect farming as food and feed is exponentially growing and producers have to ensure the best farming practices.

Pests can represent a hazard during the production process of insect farming, infesting and damaging reared insects, insect feed and substrates, infrastructures, materials and equipment of the insect facility. For this reason, every insect facility must have an efficient pest control program and management.

Ozone (O_3) treatment has been applied in recent years as a reliable and sustainable sanitization medium. It offers the advantage of leaving no residues, due to a quick decomposition of its molecule.

Here we present the application of O_3 treatment to control mite infestation in farmed cricket (*Acheta domesticus*) oviposition substrate (soil), under laboratory conditions, used in an Italian insect facility (Italian Cricket farm). The objectives of this experiment was to determine the efficacy of O_3 as mite killer, while preserving cricket eggs hatchability.

The fumigation with O_3 at the concentration of 10 ppm was performed in two applications (day 1 and day 5) under environmental controlled conditions (T° and HR), to kill the newborn mites eventually hatched after the first treatment. Two different times of expo-sure were applied: 8 hours and 5 hours on 10 substrates each. Each substrate was sampled in different points, both on the surface and in the depth. Each substrate, obtained by coring, was sampled at four timepoints: 1) before 2) after treatment 1, 3) before 4) after treatment 2.

Two different mite species were found, *Acarus sirus* and *Lackerbaueria* spp., infesting the 95% of the substrate analyzed before O_3 treatment 1 (>100mites/gr). Two treatments of 8 hours each were needed to completely eliminate the A. sirus infestation. After cricket hatching, Lackerbaueria spp. specimen, more resistant to the O_3 treatment, were observed only on their surface. No negative effects were observed on crickets hatching.

This study demonstrate that O_3 has the potential to be an efficient and safe method to control mite infestation from *A. domesticus* oviposition substrate without negative impact on cricket hatching.

Can the use of brewing by-products modulate microbiota and mycobiota of black soldier fly larvae?

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Brewing by-products (such as brewer's spent grains [BSG] and brewer's spent yeast [BSY]) have been reported to influence black soldier fly (BSF) larval growth, bioconversion efficiency and microbiota, but no data are currently available on mycobiota changes. This study evaluated the effects of BSG-BSY-based diets on BSF larval microbiota and mycobiota. A total of 3000 6-day-old BSF larvae were allotted to 5 dietary treatments (6 replicate boxes/diet, 100 larvae/box, 0.9g/larva): i) BSY2.5 (97.5% of BSG + 2.5% of BSY), ii) BSY5 (95% of BSG + 5% of BSY), iii) BSY7.5 (92.5% of BSG + 7.5% of BSY), iv) BSY10 (90% of BSG + 10% of BSY), and v) control (GA, Gainesville diet). At the end of larval growth (5% of prepupae), 3 grams of larvae/replicate were sampled, frozen at -80°C and submitted to the 16S and 26S rRNA sequencing for the characterization of microbiota and mycobiota, respectively. Data were analysed by R software ($P<0.05$). Both the alpha and the beta diversity of the larval microbiota were not influenced by brewing by-products utilization ($P>0.05$). Porphyromonadaceae (recently considered as biomarker of BSF gut), *Sphingomonas* and *Bacillus* (commonly observed in BSF brewery waste rearing residues) were selectively identified in BSY5, BSY7.5 and BSY10 larvae, respectively, while *Ruminococcus* (a polysaccharide metabolizing bacteria) and *Myroides* (also commonly observed BSF brewery waste rearing residues) were characteristics of all the BSY-fed larvae only ($P<0.05$). Mycobiota was not influenced by brewing by-products utilization ($P>0.05$), but, independently of diet, co-occurrence and co-exclusion analysis showed that *Saccharomyces* cerevisiae and *Pichia* (both able to produce antimicrobial compounds) excluded and favoured, respectively, the presence of *Streptomyces* and *Fluviicola*, while *Clavispora lusitaniae* was associated with *Myroides* (both considered as opportunistic pathogens [$P<0.05$]). In conclusion, the use of BSG-BSY-based diets may modulate the microbiota of BSF larvae without affecting their mycobiota. A complex relationship between fungi and bacteria depending on their functional characteristics is also herein highlighted and deserves further investigations.

Bioconversion potential of agro-industrial by-products by *Tenebrio molitor* – long term results

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The larvae of the *Tenebrio Molitor* beetle could improve the quality of livestock feed by becoming a new high-quality protein feed source for animals and be a path to utilise and reduce the amount of agricultural and industrial residues.

This study aimed to compare the growth performance, feed conversion ratio (FCR) and efficiency of ingested feed (ECI) by yellow mealworms. The growth and development potential of the yellow mealworm has been evaluated by using chicken feed (CF) – control feed, rapeseed meal (RM), wheat bran (WB) and willowleaf sunflower pellets (WS). The feeds were mixed in different proportions (0, 25, 50 and 75% with CF) to obtain in-expensive and various feed sources; in total, 13 diets were obtained.

The diets were evaluated on 4-weeks old larvae until the first pupation occurred in each diet (excluding the first trial). The survival, larvae weight gain, and feed consumption were measured weekly. Based on obtained data, the FCR and ECI were calculated. All data were statistically tested by one-way ANOVA.

The results showed that the shorter larval stage (74.7 days) was found for insects fed on the WB100 diet. Diets influenced mealworm weight gain, fresh weight and dry weight of larvae. The final fresh larval weight was found for insects grown on WB diets (140.4 mg f.m.). However, the lowest FCR (1.53 to 1.59) was in the case of larvae fed on RM diets mixed with CF and the control diet (CF 100). In most cases, it was found that by-products mixed with CF improve the ECI of mealworms, thus contributing to an efficient bioconversion of by-products into edible sources of nutrients. The only exception was found for pure WS diet. In conclusion, tested by-products can be used to rear yellow mealworm larvae and contributes to the various list of substrates used by small- and large-scale insect producers.

The influence of *H. illucens* larvae on digested municipal sewage sludge microbial activity and functional diversity

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Hermetia illucens larvae are great candidate for environmental entomoremediator. Many researches showed that these larvae can eat and survive on many different substrates including plants biomass, waste food and sludges. Presented research focused on the use of *H. illucens* larvae to entomoremediate digested municipal sludge. This type of waste can be seen as onerous waste as is produced in large quantities and its use e.g. in agriculture or in soil recultivation is restricted due to heavy metal content and legislative regulations. Reduction of its amount as a result of feeding of the larvae is therefore entomoremediation of the sludge, understood not as its purification from contaminants, but as its complete removal for the environment.

The experiment was conducted in plastic boxes. The amount of fresh weight of the sludge was given in such way that the dose of the sludge was 500 mg dry weight on one larvae. Community Level Physiological Profiling (CLPP) was used to asses changes in functional diversity (catabolic traits) of microbial consortia in the sludge during feeding of the *H. illucens* larvae. Analysis of catalase (CAT) and dehydrogenase (DHA) activity as well as basal respiration have also been done. Results showed that biodiversity indexes lowered in variants with the sludge on which larvae were feeding. This was also reflected in basal respiration of the sludge, which was also the lowest in variant with the larvae. Enzymes activities were not significantly different form control level. During experiment catabolic traits of microbial consortia changed, which will be discussed during presentation.

The presented results were created as part of a project financed by the Poland National Science Centre no. 2019/35/D/NZ9/01835.

Nutritive value of different raw materials in yellow mealworms: optimizing *Tenebrio molitor* nutrition

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Feed efficiency is key to maximize mealworm's growth performance and the profitability of *Tenebrio molitor* L. production systems at commercial farm level. Data on the nutritional value of each raw material used in their complete diets is needed to design adequate diets that fit the complete requirements of the larvae at each stage in their growth development. The aim of this study was to determine the nutritional value of six raw materials (barley grain, hominy feed, wheat bran, corn gluten feed and soybean meal), using a digestibility procedure for mealworms previously developed. The raw materials were evaluated in 214.5 cm^2 trays (six trays per raw material). In each tray, larvae of 46.2 mg/larva on average were introduced, with an initial density of 0.23 g/cm^2. Each tray was supplied with 20 g/d of gel, as a source of water (97.5%) and micronutrients (1%). During the duration of the trial, the evaluated raw materials were administered weekly, collecting the feces once the feed had been completely ingested. The digestibility trial lasted 42 days. During this period, the initial and final weight of the larvae, the consumption of the raw material offered, and the production of feces were controlled. Raw materials and feces were analyzed for dry matter (DM), ashes, crude protein (CP), ether extract, acid detergent fiber and gross energy (GE), from which their coefficients of digestibility (d) were determined. The dGE values ranged from 76.3% for cereals to 37.5% for corn gluten feed, indicating good utilization of starch-rich products but low utilization of fibrous ones by mealworms. The dCP values were generally low, between 27.6% for corn gluten feed and 59.8% for corn, which indicates the need to search for more effective protein sources for mealworms. The nutritional value of the evaluated raw materials varied between 1639 and 3386 kcal of digestible energy and 48 and 242 g of digestible protein per kg of DM. These values are indispensable to design complete evaluated diets that can be used in the future to determine the nutritional requirements of the mealworm throughout their growth period.

Making insect farming simple and accessible to everyone, anywhere, by complete decoupling of BSF breeding from rearing & processing

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The insect protein industry is transitioning from technology development stage to scale-up and rapid growth and is expected to expand fast as demand for alternative protein source is on the rise.

While the first generation of players in the field were mostly A–Z, full cycle companies (developing all steps of the process including waste management, breeding, rearing, and processing, product development etc.), the market is currently maturing through segmentation, specialization, and new business model development.

Here, we present for the first time a completely decoupled production model, in which BSF breeding (reproduction) is performed at centralized hubs and transported to rearing & processing facilities in a form of pre-counted, ready-to-use packages of suspended neonates with extended shelf-life and superior performance.

FreezeM's proprietary technology enables our customers to build more efficient and cost-effective rearing only facilities, to increase their production capacity in a flexible and stable manner, and to easily expand to new sites without the need to establish a colony at each one. Most importantly, our solutions lower the barriers-to-entry for new players in the market which don't have the tools or knowledge of insect breeding.

We have validated the suspended animation technology by shipping the neonates from our breeding hub in Israel to a variety of partners and customers globally. The larvae showed >90% survival rate after international shipping and 14 days of storage, highlighting the strength of the technology, and paving the way for FreezeM's scale up and positioning as the "seed company" of the evolving insect industry.

New species of culturable bacteria from insects

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Current estimations of the total number of insect species are 5.5 million. Microorganisms associated with insects account for 1-10% of the insect´s biomass. Bacterial primary symbionts, which are essential for insects, have been studied intensively in the past decades. However much less research has been devoted to facultative symbionts, which are often inhabitants of the insect gut. In this work we cultured bacteria from different insects. Taxonomic identification of the colonies growing on agar plates was performed by amplification of the 16S rRNA gene using universal bacterial primers 27F and 1522R, followed by Sanger sequencing and comparison with curated ribosomal databases. The DNA of bacteria displaying sufficient novelty (% identity < 98.5%) on the 16S rRNA gene sequences, was sequenced using Illumina and Oxford Nanopore approaches. The resulting assembled genomes were compared with those of the type species of closest taxa. Pairwise genome relatedness indices (ANI, dDDH, ANI, POCP, AF) were calculated to establish the similarity to valid species. Whole-genome phylogenomic trees were constructed to examine the evolutionary relations of every candidate novel species. Using the polyhasic approach, which combines genomic and physiological data, the fol-lowing novel species have been published: *Entomobacter blattae* G55GPT from the hissing cockroach *Gromphadorhina portentosa*, *Agromyces archimandritae* G127ATT from the pepper roach *Archimandrita tesselata*, *Pseudocitrobacter corydidari* G163CMT from the Asian roach *Corydidarum magnifica*. The species *Vagococcus luciliae* G314FT from the fly *Luilia sericata* and *Aristophania vespae* DM15PDT from the wasp *Polistes dominula* are currently being described. Other novel species from the genera *Gordonia*, *Pseudomonas*, *Rosenbergiella*, *Pseudochrobactrum* and *Brevibacterium* will be reported soon. More than 500 unique bacterial isolates have been cultured from different insects. *Bombella apis* strains of the honey bee, which we have cultured have shown to release in the supernatant a potent inhibitor of the fungus *Beauveria bassiana*. Bacteria associated with insects are a source of biochemical novelty, which may provide useful biotechnological interventions in the future.

Bacterial babysitters: Decrypting the microbiota on the Black Soldier Fly's embryo surface and their origin

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The increasing world population leads to a growing demand for animal protein for food and feed, challenging also organic waste management and calling for sustainable solutions to the production of animal protein. The Black Soldier Fly (BSF) has proven its potential to substitute soybean and fishmeal protein and is known to thrive on a variety of waste organic matter. Therefore, the BSF is an ideal candidate for commercialization. Successful industrial rearing of BSF depends on a stable and fruitful reproduction. Studies have shown that conspecific embryos and microbes play an important role in the oviposition of BSF. A better understanding of the communication between microbes and gravid female BSF, which takes place in the regulation of the oviposition, can significantly increase the embryo production and mass harvesting of BSF larvae. In this study, we analyzed the microbiota on the surface of the embryo with the aim to understand if the microbiota would originate from before, during, or via active inoculation after oviposition. By applying 16S rRNA marker gene sequencing, we analyzed the microbiota derived from the larval hemolymph, the guts of larvae fed with sterilized and non-sterilized feed, the pupal cell pulp, the wash of the embryo-laying apparatus and the embryos oviposited directly after the wash, the ovarian embryos, the empty female abdomen, embryos of the fly cage with contact to adult BSF, and sterilized embryos. We demonstrated that bacterial communities differ among BSF life stages, showing a shift from dominance of *Enterobacteriaceae* during the larval stage to a dominance of *Burkholderiaceae* in the embryos. Decrypting the embryo's microbiota and its origin may be the first step to pinpoint the source of microbial volatile organic compounds involved in attracting conspecific gravid females and, thus, improve the understanding of oviposition choices.

Increasing the ω-3 fatty acids content of Black Soldier Fly Larvae through substrate fermentation

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Since the approval of the use of insects as a feed ingredient for aquaculture in the EU (legislation 2001/999 and 2017/893), more and more studies are looking into its potential to replace fish meal, which production has a detrimental impact on our oceans. Although the nutritional profile of BSFL is rich in proteins and saturated fatty acids, it lacks some other well sought after nutritional qualities, when using it as a replacement for fish meal. BSFL are relatively low on poly-unsaturated fatty acids (PUFAs) and specifically ω-3, which makes them less desirable as food supplements in the aquatic industry. The nutritional profile of the BSFL does depend on their feed and can therefore be artificially altered, but the sources of ω-3 rich products are often scarce and costly. In this study, microorganisms were used to enrich nutritionally low-value agricultural sidestreams with ω-3 fatty acids. Through fermentation with the oleaginous fungus *Mortierella alpina*, the fat content of wheat bran and DDGS based substrates was increased with over 500% (from 0.70 g/g DM to 5.30 g/g DM in wheat based substrates). Fermentation of the substrate led to significant increases in the ω-3 and ω-6 content of the substrate. Feeding the fermented substrate to the BSFL did not negatively impact their survival, however larval growth, final weight and fat content was reduced. This warrants further research to improve edibility of the fermented substrates. Nevertheless, analysis of the fatty acid profile of the BSFL fed on the fermented substrates did reveal similar increases in in the ω-3 and ω-6 content of the larvae. These results highlight the potential for microorganisms to enrich BSFL with certain desired nutritional elements through substrate manipulation. Further exploration of oleaginous microorganisms and their metabolic pathways can provide researchers insights for producing a more desirable BSFL as a food supplement.

Visualization and quantification of microplastics in the gut of black soldier fly larvae (*Hermetia illucens*)

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Plastics and microplastics are currently spread globally and can also be found in energy-rich residual streams derived from the food industry. To date, such streams are used for biogas production, while the residue is applied as organic fertilizer, potentially introducing microplastics into the environment.

An alternative approach to biogas production is utilizing black soldier fly larvae (BSFL) to upcycle these residual streams, as BSFL grow well on numerous substrates, turning them into valuable biomass like lipids, proteins, and chitin. In addition, previous research has proven that BSFL are not affected in terms of growth performance, survival rate, and substrate bioconversion efficiency by the presence of polyvinyl chloride microplastics. However, the possible ingestion and accumulation of the microplastics in their gut has not been documented yet.

In this study, the ingestion, accumulation, and excretion of microplastics by BSFL was investigated. BSFL were reared for 10 days on artificial biological waste streams containing different amounts (i.e. w = 0.01, 0.1, 0.5, 1.0 and 3.0 %) of polyethylene microplastics having a particle size of 53 to 63 μm. During the rearing phase, BSFL were daily sampled, and the microplastics in their gut were visualized and quantified through microscopical analysis. On days seven and ten, several larvae were starved for three days by transferring them into a clean rearing container to investigate the excretion of the microplastics.

Although the larvae were not affected in terms of growth performance, they clearly ingested the microplastics, as proven by their presence in the lumen of the larval gut. Notwithstanding the ingestion of up to 5000 particles in each larva, the larvae excreted them predominantly during the starvation process.

Higher larval density leads to lower survival and fat content, smaller size, longer development time and increased investment into immunity in the black soldier fly

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Larval density (i.e., crowding) is an important biotic factor but its impact on organisms has often been overlooked. Larval density is expected to correlate with immunity but investing highly in immunity has significant consequences on the fitness of larvae. This study explored the effects of larval density and its interactions with temperature on life history traits of economically important black soldier fly (BSF).

We reared BSF larvae at three separate larval densities (1, 5 and 10 larvae/cm²) and also at three distinctive temperature treatments (23, 27 and 30 ºC). A significant increase in prepupal mass, pupal mass, prepupal-to-pupal mass loss, survival, prepupal fat content, adult mass, adult longevity and a reduction in larval and pupal development time at low larval density was observed in our results. Temperature influenced significantly all studied traits except survival, prepupal fat content and adult longevity. BSF Larvae reared at high larval densities had significantly higher PO activity compared to larvae reared at low larval density.

We conclude that density and temperature and their interaction-related effects during larval development considerably affect BSF larval life-history traits. Higher immunity at high densities and temperatures suggests that larvae are better protected against pathogens, but this comes at a cost of remaining smaller and growing for a longer time. For successful rearing and effective production of BSF larvae, it is therefore essential to account for several environmental factors simultaneously.

Dose-response effects of starch in chicken excreta on *Musca domestica* larval performance and bioconversion efficiency

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Housefly (*Musca domestica* L.) larvae can convert animal manures into valuable protein and fat but little is known about their nutritional requirements. Larval performance and bioconversion might be limited by available energy and competition with microbiota. In this study we assessed the effects of differential starch content in unsterilized (UE) and heat sterilized chicken excreta (SE) on larval performance and bioconversion.

Gelatinized corn starch was added to excreta to construct substrates ranging from 0 to 50% starch based on substrate dry mass. Substrates were inoculated with housefly eggs (8/g wet substrate) and larvae harvested by floatation after 5 days.

Individual larval mass increased from 12±0.2 (UE) and 13±0.4 (SE) mg at 0% starch up to 18±0.4 (UE) and 18±0.5 (SE) mg at 15% starch and decreased to 5±0.4 (UE and SE) mg in 50% starch. Highest total larva wet yield in UE was 39±2.3 g at 10% starch, 43±2.8 g in SE at 15% starch, decreasing to a minimum of 3±1.5 g (UE) and 4±1.3 g (SE) in 50% starch. The decrease in yield after its maximum was faster in UE compared to SE. Larval survival was highest in 0% starch, 80±8% (UE) and 72±13 % (SE), de-creasing to 14±7% (UE) and 18±3% (SE) in 50% starch. Dry matter bioconversion in-creased from 4±0.1% (UE) and 4±0.3% (SE) in 0% starch to 6±0.2 in UE with 10% starch and 7±0.3 in SE with 15% starch, decreasing to 0.2±0.0% (UE) and 0.6±0.2% (SE) in 50% starch. Highest nitrogen bioconversion was 15±0.5% in UE with 10% starch and 15±0.5% in SE with 15% starch, decreasing for higher starch content. These optimal starch inclusions to UE and SE increased nitrogen bioconversion with 40 and 100% respectively, compared to pure excreta. Pronounced effects of starch on pH and ammonia content were also observed. The results indicate that adding easily digestible energy to chicken excreta can substantially increase larval yield and nitrogen bioconversion while reducing ammonia content in the substrate residue.

Development of a continuous reproduction system for the BSF

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Industrial scale black soldier fly (BSF) production requires high volumes of waste streams and BSF-eggs. Reproduction is often associated with high labor requirements, a poor harvest index and high downtime. InsectoCycle set out to design a system in which these problems for scaling up were tackled.

In high income countries, labour can have big impact on the costprice of egg production. Therefore we eliminated multiple steps in our breeding process, so no refeeding, sieving, dosing of pupae and attractant is needed.

We optimized our system on harvestable eggs or the harvest index. We took measures to eliminate mislaying of eggs by reducing the ambient humidity, providing a really smooth finished environment and a super attractive egg collector by re-using the exhaust air from the growing colony as an attractant.

Our egg-collector has also undergone many iterations and now services multiple goals, ventilation, egg collection and incubation. Furthermore it is integrated into the system so that it makes harvesting very easy.

In a batch system, downtime is very high due to the fact that flies take at least 3 days from emergence until the first eggs are laid. So that is why we choose to develop a continuous system that runs as long as possible.

With these principles we engineered and designed our solution, the InsectoCage.

Studies of *H. illucens* mating behavior under high density conditions

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The conditions experienced by insects raised in commercial production systems differ from those under which they evolved, including very high densities of adults in mating cages. After many generations of mass rearing, insects may adapt to high density by changing their mating behavior. Alternatively, behavioral studies may indicate a need to modify cage conditions in order to promote optimal fertile egg production.

A preliminary study of *H. illucens* adults enabled us to develop a standardized, modified scan sampling approach of videos to annotate mating behavior from unmarked groups of flies maintained under artificial lights in mating cages. The number, timing and duration of courtship and coupling behaviors, and what portion of courtship ended in successful mating, was recorded for the entire light period. Subsequently, we sought to investigate the effect of density on the number, duration and temporality of coupling events on the cage floor for the first days of the adult production cycle (control and high density; n=4-5 replicates/treatment, cage = experimental unit).

All couplings were preceded by courtship, but the vast majority of courtship did not end in successful coupling. Courtship lasts an average of 12 seconds and coupling an average of 33 minutes. The number and duration of couplings do not differ between control and high density cages. Two peaks in coupling events are observed during the light period, but density has no effect on the temporality of mating behavior.

The density levels studied, both much higher than those experienced under natural conditions, suggests that *H. illucens* may successfully mate even with increased competitive pressure for access to mates and cage surfaces upon which mating occurs. Although not explicitly tested, perhaps as in other mass-produced dipteran species, lek formation is no longer the strategy used to compete for mates. Rather, mating strategies may instead involve many courtship attempts and a temporal distribution of coupling over the entire light period.

The influence of dietary starch, fibre, and water on the nitrogen balance in black soldier fly larvae production

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Nitrogen is a constituent of proteins and a key element in environmental sustainability. Black soldier fly (BSF) larvae (*Hermetia illucens* L.) are a sustainable source of protein in a circular economy to reduce food waste. The nitrogen available in the rearing substrate of BSF larvae is either assimilated by the larvae, retained in frass, or released as gases. We hypothesized that the composition of the BSF larvae's diet can be steered to maximize nitrogen retention by the larvae. This has the potential to improve the efficiency of feed to protein conversion.

In this study we researched the effect of dietary starch, fibre, and water on the retention of nitrogen by the BSF larvae and nitrogen loss from the system. Additionally, we scored several indicators of the performance of larvae reared on these different diets.

We will analyse each of our added ingredients (and their interactions) as explanatory factors to try to explain the variation of the amount of nitrogen in BSF larvae and frass, and various indicators of larval performance. Our study will provide insights into the possibility of the modulation of the nitrogen balance in BSF production and a way to prevent excessive nitrogen loss.

Too hot, too cold: Scaling effects and standardised research for industry relevance

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Harnessing the power of insects to feed the world is becoming an important and increasingly more pertinent aspect of achieving global food and feed security. To be viable for industry, an enormous quantity of insect biomass needs to be consistently produced on a daily basis. Multiple companies have developed large-scale production facilities (>10,000-m^2) to meet the growing market demands for insect-based food and feeds. Research in this newly emerging biotechnology field, however, has mostly been limited to smaller scales, for both in-house R&D departments within the industry and for academic institutions. Trials conducted at the bench scale (rearing units <1-m^2) can manipulate environmental conditions to precise and narrow ranges of temperature and humidity, which is important for achieving repeatable results and highlighting differences among experimental treatments. However small-scale results under tightly controlled conditions may produce results that are not applicable and/or relevant to the reality at industrial scales. In this presentation we review data collected from in-house trials on black soldier fly rearing that highlights the importance of scale and environmental parameters in impacting insect health and overall performance. We discuss how scale impacts the conditions insects are exposed to at multiple life stages, ultimately affecting final performance and overall plant productivity. Considering scale when undertaking academic and industry research will prove vital for advancement of the insect industry. Through continued partnership and collaboration, we hope to refine strategies that will enable research of the highest quality, and research that is extremely relevant to both industry and academia alike.

Multifaceted approach to high fiber waste management using black soldier fly larvae

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With global food demands predicted to increase by 50% between 2020 and 2050, there will be a proportionate rise in the residual waste from food harvest which demands environmentally low impact strategies for management of such wastes. Black soldier fly (*Hermetia illucens* L.) larvae are being explored extensively as an economically viable solution for food waste management due to their ability to convert a wide range of low value substrates into high value animal proteins thereby simultaneously addressing the demand for animal feed. However, while several studies have assessed conventional waste substrates such as pre-consumer wastes and animal feeds, the residual waste from food harvest remains less explored. These wastes are high fiber substrates and have poor nitrogen to carbon ratio. Since black soldier fly larvae (BSFL) do not possess gut microbiota capable of breaking down lignocellulose effectively, the substrates need to be modified for consumption by BSFL. This study assessed the feasibility of different high fiber wastes, as feed, for black soldier fly larvae. The impact of fermentation, inoculum and a modified feeding regime coupled with nutritional additives on palatability of the substrates is investigated. The fermentation or inoculum alone, as done in this study, is not sufficient to convert the substrates to a feasible feed. However, supplementing substrates with nutritive additives, at different time points during the rearing cycle, to assist larvae in digesting the main feed showed a 10-fold increase in bioconversion compared to control. The findings demonstrate that high fiber wastes are not indigestible to BSFL and require a multifaceted approach to enhance their nutrient availability to larvae there-by improving the rate of bioconversion.

The influence of dietary fat on the yield, bioconversion efficiency, and body composition of black soldier fly larvae

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Black soldier fly (BSF) (*Hermetia illucens*) is increasingly considered as a sustainable source of protein, lipid, and fiber for animal feed. Nutrition is a core pillar in the BSF farming. Yet, however, our understanding of the influence of nutrients on the production of the insect is limited. Macronutrients including carbohydrates (C), proteins (P), and fats (F) are essential elements in animal growth and development. In BSF, research efforts have mainly concentrated on C and P. There is experimental evidence that the life history traits of BSF, such as development time and reproduction are influenced by P and C content of the diet. It has also been shown that the body fat content of the larvae is widely influenced by P and C while the larval body protein content is more independent of those macronutrients. F and C are critical energy sources in animal nutrition. In this study, we took an integrative experimental approach to understand how BSF larvae are influenced by F, C, and their interactions. We investigated the impact of these factors on the yield, bioconversion efficiency, body composition, and survival of BSF larvae using chemically semi-defined diets.

Our preliminary data analysis suggests that F and C content and F/C ratio widely influence larvae performance. There was a positive correlation between the C content of the diet and larval yield, larval body protein and fat contents, and bioconversion efficiency. F seems to enhance the positive impact of C when it is used at low levels. To some extent, F could replace C in the diet without negative influence on the larvae output. High F contents had negative impacts on the performance of the larvae. Our study will provide insights into the optimization of the nutritional composition of BSF rearing substrates for commercial production.

Development of an enzymatic treatment followed by tricanter centrifugation to produce insect meal with improved characteristics from *Hermetia illucens*

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The main goal of this work is to develop a method for the production of insect meals of *Hermetia illucens* lavae with low fat content while enhancing the protein digestibility, and demonstrate the process at industrial scale, work developed in the European Project SUStainable INsect CHAIN (SUSINCHAIN).

First, three proteases with activity ranges from acid to neutral pH and mid temperatures (<50ºC) were screened. After the centrifugation, three fractions were obtained: (i) floating oil fraction, (ii) aqueous fraction (mainly soluble proteins and hydrolyzed proteins), (iii) solid fraction (mainly chitin and insoluble proteins). Among the proteases tested, Prot1 was selected as the best protease because a clear lipid layer was observed, while the others showed only an emulsified layer. Next, conditions were optimized by combining Prot1 and lipases Lip1 and Lip2. A response surface methodology (RSM) was applied to optimize the fat extraction by varying enzyme doses and reaction time, resulting in 0.2% Prot1, 0.75% Lip1, 0.75 % Lip2 under agitation for 4 h at acid pH, mid temperature and followed by thermal treatment at 90ºC for 2 h and finally, centrifugation. This process was validated at lab scale obtaining insect meals with protein content of about 38% dw and 20% dw residual fat, representing 52.5% fat extraction yield. The total amino acid content obtained was about 30% dw, the most abundant being aspartic acid, glutamic acid, and alanine, similarly to the fresh larvae amino acid profile. The *in vitro* dry matter and protein digestibility in the insect meals obtained were approximately 87% dw.

The enzymatic treatment of *Hermetia illucens* larvae in a 100 L reactor followed by the tricanter centrifugation process was successfully demonstrated at industrial scale, providing high protein recovery and enhanced protein digestibility by over 20% with the addition of the enzymes. If fractionated, the aqueous fraction reached 100% protein digestibility and the solid fraction around 91%. In conclusion, the aqueous enzymatic treatment followed by tricanter centrifugation is a promising technology to reduce the fat content and enhance the *in vitro* protein digestibility.

Differences in process control starting with the killing of the larvae between *Hermetia illucens* and *Tenebrio molitor* in dry and wet processing – A detailed view of intermediates and end products

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Consistent product quality and achieving the maximum yield of a product along the process chain determine the profitability and success of a company. Fattening insects and processing the corresponding value-determining components such as protein and fat are among the new industrial sectors of insects. The main components of insects are protein, fat, chitin and water.

For processing, knowledge of separation technology from the food industry can be used. Basically, however, insect processing is divided into two established processes: wet and dry processing.

In these studies, the most common representatives, the species *Tenebrio molitor* and *Hermetia illucens*, were separated “wet” by centrifuging at different pH values, g-values and process temperatures. Large differences were found, especially with regard to the protein in the aqueous phase as well as in the fat content of the solid after centrifugation.

For the dry processing, investigations were carried out in the area of drying and behavior during pressing. And large differences were found between *Tenebrio molitor* and *Hermetia illucens* with regard to the input temperatures for the straining screw press.

In both processing strategies, the first step in the processing chain was inactivation of the larvae (killing). In the blanching process, there were large differences in the water absorption capacity of the insect species, which must be taken into account in the process control, among other things.

DTI – Insect industry prospects

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European feed sector requires new sustainable protein sources that have lower environmental and social impacts than conventional sources such as soy and fishmeal. In this regard, insect production, especially the Black Soldier fly (BSF), *Hermetia illucens*, production is increasing worldwide due to its larva ability to convert waste materials into products like high-quality protein, fat, and natural fertilizer (frass). The use of insect products in petfood and aquaculture is increasing and customer acceptance of using insects as a feed source has improved over the past year. Moreover, EU legislation has recently allowed the use of insects as a new source of protein in poultry and pig production, expanding business opportunities for insect producers. Commercial opportunities exist not only for insect-based protein and oil but also for other by-products such as wet extrusion or wet foods and low energy feeds. The insect industry is growing rapidly, and there is obvious momentum in terms of investment, production, and demand. The main challenge of insect producers is scaling up and achieving a production that can provide a stable supply to the market. Therefore, this nascent business requires a better understanding of BSF needs to develop technologies and methods that lead to cost-effective and well-optimized production. Danish Technological Institute (DTI) has established a research and development area with a focus on improving and optimizing insect breeding and developing insect products with health beneficial properties and nutrient profiles tailored to the target animal. Among the projects, WICE4Soil focuses on producing BSF on a pilot scale and assuring the quality and safety of the final products (larva and frass), the FlyScent project aims to centralize the BSF oviposition site using odour attractant, the RECIPIE project produces suspended neonates helping insect producers to reduce the operational costs and increase production capacity and the EntoPower focuses on heating and ventilation systems to reduce the total energy consumption during insect production.

Zinc twice: A trace element to benefit insects and consumers

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Optimal nutrition with adequate levels of trace elements ensures important structural, catalytic and regulatory functions; for the simplest unicellular to complex multicellular organism. Cellular quotas, i.e. the number of atoms per cell, for essential transition metals such as zinc are tightly controlled in the face of changing metal concentrations in the surrounding growth environment to maintain critical biological processes while limiting toxicity. Humans and livestock have specific requirements and toxicity problems depending on the zinc species and its interactions with other nutrients. The amount of this trace element that insects actually need to thrive in physiological or stress situations cannot really be predicted at present, as there is little knowledge about their zinc homeostasis nor reliable biomarkers to assess the zinc status of insects. Elucidating zinc tissue distribution as well as the expression and activity of proteins involved in the regulation of zinc homeostasis provides decisive advances when it comes to increasing zinc content by means of biofortification, which is important with regard to the use of insects in human and animal nutrition. In addition, trace element enrichment may affect immune functions of insects and their interactions with beneficial and/or pathogenic microbes, which both remain poorly investigated. This presentation aims to present analytical methods enabling quantification and visualization of trace element pools in insects exemplary on *Tenebrio molitor*, one of the most reared insect species and currently approved in the EU as Novel Food, as well as the western honeybee *Apis mellifera*, which is considered as one of the most economically valuable pollinator.

Taurine content in insects

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Taurine (2-aminoethane sulfonic acid) was first isolated from the bile of *Bos taurus* in the early 19th century. The major route for the biosynthesis of taurine is from methionine and cysteine via cysteinesulphinic acid decarboxylase followed by the oxidation of hypotaurine to taurine as the final step. Cysteinesulphinic acid decarboxylase levels are very low in cats, as well as in humans and other primates. Compared with conventional resources, insects can be used as proteinrich source with a low ecological footprint. Currently, the knowledge of taurine content is very limited and known only for a few insect species.

In our study taurine was determined by an electrophoretic method in the species suitable for large scale production – larvae of *Tenebrio molitor* and *Musca domestica*, larvae and pupae of *Alphitobius diaperinus*, adults of *Acheta domesticus*, *Blaberus craniifer*, *Blatta lateralis*, *Gryllus assimilis*, *Periplaneta americana*, *Schistocerca gregaria*, and *Hermetia illucens* prepupae. Taurine content in tested samples significantly differed and ranged from 18.4 to 382.7 mg/100 g DM. Among the samples, *Gryllus assimilis* and *Periplaneta americana* (382.7 ±32.4 and 369.8 ±12.7 mg/100 g DM) contained the highest values of taurine. On the contrary, the lowest taurine levels were detected in *Tenebrio molitor* (18.4 ±2.0 mg/100 g DM), and *Schistocerca gregaria* (20.4 ±2.2 mg/100 g DM). It was seen that some species have a potential to contain more taurine than others and there were also differences depending on the stage of insect in lesser mealworms. Their larvae contained almost three times more taurine than pupae.

In the past, the attention used to be mostly paid to the cats, which have limited ability to synthesize taurine. Nowadays, the main target of taurine-based research has shifted to the field of nutrition of commercially relevant species, particularly to marine fish aquaculture. Based on our results certain insects were proved to be the exclusive taurine source in feline diets, but none of the tested samples can provide optimal taurine level for fish.

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Nutritional analysis of seaweed flies *Fucellia maritima* (Haliday, 1838) reared on five different substrates

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As the world population grows to an estimate of 9.8 billion people by 2050, food production systems must grow accordingly. Aquaculture is an efficient food production system for producing animal protein. Nevertheless, it is highly dependent on ingredients such as Fish Meal (FM) and Fish Oil (FO), rich in polyunsaturated fatty acids (PUFA), causing a great pressure on natural fish stocks. Insect meals are considered alternative ingredients to FM, mostly due to their protein content, as terrestrial insect species lack PUFA to produce insect oil as an alternative to FO. However, coastal insect species seem to display PUFA in their composition.

In this work, we evaluated the lipids and protein content of adults and pupae of the seaweed fly *Fucellia maritima*, a species that feeds on algae and fish present in beach wrack. They were reared with five different substrates: Three algae (*Fucus sp*., *Ulva sp*. and the invasive *Agarophyton vermiculophyllum*), the invasive freshwater hyacinth (*Eichhornia crassipes*) and fish waste (codfish frames). Fifty adult flies were placed in cages containing these substrates, using five replicates per treatment. Flies were allowed to breed for 48 hours, lay eggs, and were then removed.

Results show that none of the individuals survived when fed with water hyacinth. The highest number of *F. maritima* was obtained with brown algae (581 pupae, mean 118.8±103.55 $p>0.05$). Fat content was higher in adult flies reared on codfish frames (13.79%±0.14 Dry weight basis, DW, $p<0.05$), however pupae had higher fat content when fed with *Ulva sp*. (11.54%±1.26, DW, $p<0.05$). Regarding protein content, this was higher for both flies and pupae fed with *Ulva* sp. (20.42%±0.35 DW and 21.66%±0.17 DW, respectively, $p<0.05$). The concentration of PUFA was higher in the pupae and flies fed with *Fucus sp*. (14.65 % and 17.86%, respectively, $p< 0.05$), followed by the codfish frames. A considerable higher amount of Eicosapentaenoic acid (EPA) (5.13% for pupae and 6.37% adult flies, $p<0.05$) and Docosahexaenoic acid (DHA) (0.87% for pupae and 0.56% for adult flies, $p<0.05$), was found for the ones fed with cod fish frames when compared with the other substrates.

These results clearly show that the nutritional profile of *Fucellia maritima* can be modulated with different feeding substrates, in line with findings already available for other insect species. However, the suitability of producing enough biomass is low if one is aiming to produce large numbers of specimens with a high content of omega-3 fatty acids.

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Microbial characterization of *Bombyx mori* and possible reutilization of chrysalides as food ingredient: circular economy approach in sericulture

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The sericulture in Italy has old origins but the competitiveness with the Asian markets and the use of pesticides have led to a decline of *Bombyx mori* (BM) farming in Italy. However, this sector seems to be characterized by future developments concerning the use of silk by-products in food industry, with consequent promotion of a circular production chain.

The present work aims to assess the use of BM as edible insect from a microbiological point of view through 2 approaches: 1) systematic review (SR); 2) experimental investigations on cocoons and dried chrysalides (DC) provided by Italian farmers.

The SR showed that, through culturing methods, the most investigated and detected bacteria were *Bacillus cereus* and *Pseudomonas fluorescens* in raw insects, and *B. cereus* and *Enterobacteriaceae* in insect-based food. The most detected genus in raw insects and silkworm-based food, by metagenomics analysis, were *Enterococcus* spp. (relative abundance from 5.00% to 70.10%) and *Bacillus* spp. (relative abundance >80%), respectively. *Listeria monocytogenes* and *Salmonella* spp. were never detected.

In the experimental investigation, *L. monocytogenes*, *Salmonella* spp. and *Escherichia coli* were absent in cocoons and DC. *B. cereus* and sulfite-reducing anaerobic bacteria were only reported in cocoons. *Enterobacteriaceae* and coliforms were present in both cocoons and DC, showing a higher contamination level in DC.

In conclusion, scientific evidence of microbiological risk due to the use of BM as a food is very limited. No significant risks were observed. Due to the presence of indicator bacteria and *B. cereus* in cocoons, a critical stage could be represented by the cutting process.

The oxidative stability of commercial *Hermetia illucens* meal

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Hermetia illucens meal (HI meal) is becoming a promising alternative to conventional proteins such as fishmeal (FM) and soymeal (SM) in pet food and feed formulations. One important quality aspect in these ingredients is fat oxidation since it influences the palatability of the feed and the animals' health consuming it. In general fat oxidation is influenced by multiple factors such as the total fat content, fatty acid composition, presence of antioxidants or catalysts, presence of oxygen and thermal processing conditions.

Therefore, in this study, the oxidative stability of commercial HI meal, SM and FM was evaluated by measuring the following; fatty acid profile, free fatty acid content, peroxide value, p-anisidine and electron spin resonance spectra of lipid. In this study, the HI used contained no added antioxidant while the FM and SM used did contain antioxidants. The antioxidant present in FM and SM is either added and or naturally present.

The results showed that HI meal and SM had a higher oxidative stability when compared to FM, since FM contained high levels of lipid free radicals and secondary products. The higher level of oxidation could be attributed to the FM unsaturated fatty acid content, the duration between harvest and processing as well as the temperature control before processing. On the other hand, both HI meal and SM had a similar oxidative stability. However in terms of sustainability, HI has an advantage of being local and sustainable when compared to SM which is usually imported. The oxidative stability of HI is mainly attributed to the short supply chain, the mild thermal processing, and its saturated fatty acid profile.

The influence of different side streams on the nutritional composition of the yellow mealworm (*Tenebrio molitor*)

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The yellow mealworm (*Tenebrio molitor*) is an interesting insect that has gained increased attention in recent years due to its potential applications in food and feed. In order to increase the sustainability of the rearing process and to improve circularity, mealworms can be reared using low-value organic side streams, however, this may impact yield and nutritional composition of the produced insects.

The effect of rearing mealworms on different side-streams and their nutritional composition was investigated. A selection of four organic wet side streams were used and partially fed to the mealworms, replacing the standard wet feed (agar).

The nutritional and chemical profile of the side streams and mealworms were determined by proximate analysis, mineral profile, fatty acid and amino acid profiles, and heavy metal concentration. For the substrates, a fibre profile was measured as well. As result a significant difference in fat percentage and fatty acid profile was found when mealworms were fed with a side stream that was very rich in carbohydrates. When providing side streams with high concentrations of minerals and metals, accumulation of heavy metals was determined in the mealworms. In the case of the macro- and minor minerals, some components appear to be accumulated.

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Evaluation of black soldier fly larvae proteins as substitute of poultry by-product meal in extruded dry diets for dogs

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Black soldier fly larvae (BSFL) proteins are rapidly gaining popularity as high value ingredients in pet food formulations. Some in vitro studies have indicated that BSFL proteins have strong antioxidant and antimicrobial properties. However, currently there are no in vivo reports to justify these findings. The current study evaluated the effect of two diets (diet with 29% BSFL protein and diet with 26% low ash poultry by-product meal- PBP) in adult dogs. These two diets were isonutritive extruded diets (25% crude protein, 12% fat, 3.5% crude fiber, 5% ash). During this study, a total of eight adult dogs (four dogs per diet) were fed with the formulated diets in a cross-over design with two periods of 60 days each period. Along the study, faces were collected for evaluation of dry matter digestibility and urine to evaluate pH and urine density. Additionally, blood was collected for biochemical, antioxidant, and immunological evaluation. Dogs showed good diet acceptance showing no refusal episodes. Stools were well-formed stools, having similar fecal scores for the two diets (3.7 and 3.9 for PBP and BSFL based diet respectively). Score scale goes from 1 to 5 scale, being 1 watery faeces and 5 hard and dry faces, 3 and 4 is considered ideal. No difference in dry matter digestibility was found among the two diets (81% and 80% digestibility for PBP and BSFL diets, respectively ($p>0.05$)). Additionally, similar levels of urinary pH (6.7 and 6.6 for PBP and BSFL diet respectively, $p=0.602$) and urinary density (1.023 and 1.021, $p=0.959$) were reported for both diets. Both limits are within the normal ranges. The preliminary data obtained so far showed promising results. In conclusion, BSFL seems a safe protein alternative to be used in extruded diets in adult dogs.

Effect of conservation, blanching and drying conditions on the safety and quality of *Tenebrio molitor* larvae

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The main goals of this study were to assess how different conservation, blanching and drying conditions influenced the safety and quality of *Tenebrio molitor* larvae. The different conditions were: conservation before and after blanching (refrigeration/4°C or freezing/-24°C), blanching techniques (water-immersion@100°C or steaming) and drying methods (electrical oven 80°C/7 h, microwave 800W/5 min or freeze-drying/24 h). In order to assess the impacts on the quality of *T. molitor* larvae, dry matter content, water activity and color were evaluated. Additionally, microbiological analysis (total viable count, *Enterobacteriaceae* – Entero- and bacterial endospores) were performed. Freezing samples before blanching led to better dry matter and water activity results for all drying methods, while also improving color – higher *L** and *b** values, both before and after drying. This effect on color was also observed for frozen samples after blanching. Immersion-blanching led to higher *L** and *b** values, both before and after drying, while also having better dry matter and water activity results for oven and freeze-drying. As for the drying methods, only oven-drying led to water activity values below 0.3. Regarding color, freeze-dried samples had the highest *L** values while the highest b* values were observed for the microwave-dried samples. Regarding microbiological analysis, both blanching treatments reduced the load for Entero and bacterial endospores communities. The application of drying methods had a significant effect on lowering the levels of total viable count and Entero. Comparing the samples after drying, there were differences for Entero, with immersion/microwave samples having the highest loads and steam/freeze-drying the lowest. For bacterial endospores, there were differences be-tween drying methods with oven and freeze-dried samples having higher loads, particularly for steam-blanched samples. Steam-blanching can be a viable alternative to immersion-blanching, especially for microwave and oven-drying. These drying methods can also present similar or better results than freeze-drying.

Detection of changes in physicochemical properties during storage of high pressure treated mealworm paste

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Insect pastes are highly perishable and prone to microbial and chemical changes upon storage due to the high moisture content, favourable pH and rich nutrient profile. High hydrostatic pressure (HHP) processing is an alternative method of food preservation capable of inactivating pathogenic and spoilage microorganisms. However, HHP is reported to have an impact on physicochemical quality attributes. This study focused on evaluating the effects of HHP blanching (600 Mpa; 5 min) and different modified atmosphere packaging (MAP) on the physicochemical of mealworm (*Tenebrio molitor*). The pH, colour, lipid oxidation and fluorescence analysis were carried out during four weeks of refrigerated (4°C) storage. Results showed that HPP treatment caused higher lipid oxidation, which could be minimised with no oxygen MAP condition. Further, parallel factor (PARAFAC) analysis indicated a shift and reduction of fluorescence peaks for treated samples compared to control, indicating unfolding, oxidation and degradation of proteins. However, additional analysis investigating a wider fluorescence region as well as using other more common techniques should be performed in order to validated such statement.

Hygienic status of black soldier fly based feeds during processing – A detailed view of intermediates and end products

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Each processing technology has a significant influence on the material caused by thermal and mechanical stresses due to processing. Currently two established industrial processes, wet and dry processing, are used to process insects. In practice, however, these processes are considered uncontrolled with regard to their effects on product hygiene. This therefore presents the critical point in the entire production chain and a limiting factor for the further development of insect-based products.

For this study, fresh and frozen black soldier fly larvae (BSFL) were processed in pilot plants for dry and wet processing to evaluate the hygiene status of BSFL along the product chain. To enable the evaluation of the hygienisation effect during processing, control points (CP) of intermediate and end products were defined within the process. In dry processing, the raw material, dried material and the press cake were chosen as CP. Correspondingly, the CPs in wet processing were assigned to the raw material, the step after killing the larvae by blanching, to the grinding step and the protein meal. The total bacteria count (TBC) and the routine analyses of enterobacteriaceae, salmonella, yeasts and fungi were chosen as microbiological markers to describe the hygiene status.

The analyses showed that directly after harvesting the BSFL had a TBC higher than 107 CFU/g. When using the drying process, a first reduction by three orders of magnitude is observed, which showed a similar effect as the blanching of the larvae at 100 °C for three minutes. On the other hand, the cell number of enterobacteriaceae was not reduced significantly through the drying process. There was also no degradation of TBC observed in the pressing process. It can be concluded that dry heat does not achieve a safe hygienic status of BSFL products. The wet processing of BSFL showed a degradation of two orders of magnitude in the killing step through blanching. The highest efficient impact on the hygienic status and the product quality was shown with a combined cell disruption step with heat and chloric acid in a concentration of 0.1 mol/l. This process step led to a reduction of the TBC from 105 to 102 CFU/g, which generated a hygienic safe product.

Feeding habits and acceptance of insect-based products by dog and cat owners – a survey

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Aiming to assess the feeding habits and willingness of dog (DO) and cat owners (CO) in feeding edible insect pet food (IPF) to their pets, a survey was conducted through an online platform. A total of 386 questionnaires were collected, comprising 77% (192/249) of women and 23% (57/249) of men. The majority (32.4%; 82/253) of the respondents were between 25-35 years old, followed by 45-55 years (24.5%; 62/253) and 35-45 years (15.4%; 39/253). Regarding the respondent's dietary habits, 53.4% (167/293) did not follow a specific diet, 16.6% (52/293), 5.4% (17/293), and 4.8% (15/293) were in a flexitarian, low-carbohydrate, and vegetarian diet, respectively. Most respondents (62%; 177/285) reported having never eaten insects before, however, 53% (94/177) were willing to try it. Of the DO, 62% (96/155), 11% (17/155) and 3.2% (5/155) fed dry food, raw meat and a home-cooked diet to their dogs, respectively. Whereas 67% (87/130), 12% (16/130) and 7% (9/130) of the CO fed dry food, wet food and a specific veterinarian diet to their cats, respectively. For both DO (51%; 75/146) and CO (59%; 75/127) the most important factor for purchasing a diet was the palatability. Other important factors were the veterinarian's recommendation (36% of DO [53/146]; 26% of CO [33/127]), "if the food seems to be healthy" (34% of DO [50/146]; 31% of CO [39/127]) and the product's cost (22% of DO [32/146]; 29% of CO [37/127]). CO showed a slightly higher acceptance of feeding IPF to their cats (mean 5±3.1) than DO (mean 4.7±2.6) on a 7-point Linkert scale. The acceptance increased for both CO (mean 5.4±2.7) and DO (mean 5.1±2.5) if they knew the product would be sustainable and with proven nutritional value. However, only a few CO (10%; 12/122) and DO (8%; 11/140) would be willing to pay more for IPF. When the owners were asked about the perceived benefits of IPF, the majority of the DO (48%; 74/152) and CO (61%; 80/131) believed it could be sustainable and environmental-friendly. As possible disadvantages, 32% (49/152) of DO and CO (42/130) believed that IPF could be too expensive, 23% of DO (35/152) and 21% (27/130) of CO were scared that not enough scientific research would have been done and 20% (31/152) of DO and 41% (53/130) of CO were scared that their pet would not like its taste.

Sensorial properties of raw and heated, fresh and spoiled Mediterranean crickets (*Gryllus bimaculatus*)

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A vital factor in evaluating foodstuffs for farmers, public health professionals and consumers are sensorial properties. Like for other foodstuffs, standards have to be established in order to tell fresh insects from spoiled ones. Only for some productive insects, scattered (and incomplete) data exists.

Panels of min. five persons (experts in sensorial analyses or laymen) in Cambodia, Thailand, and Germany were asked to describe colour and smell of raw and heated, and fresh and spoiled Mediterranean crickets (GB). When heated and fresh, taste and mouthfeel were also requested. Data from spoiled insects was recorded from accidental experiences.

While colour description was similar in all countries, smell rating differed. Thai panelists considered raw fresh GB to smell e.g. "peaty", Cambodian ones "pungent", and German ones "neutral, slightly chemical", improving after cooking. Taste was rated e.g. "neutral, somewhat sour" (Cambodia), "nutty and like crab meat" (Germany) and "neutral and slightly spicy" (Thailand). Smell and taste provide a specific, somewhat chemical flavor, which is hard to define, but typical for insects, although more pronounced in GB. "Insect" is proposed to coin this sensation. The mouthfeel is dominated by the combination of a hard exterior and a soft interior, but only German panelists used "slimy". The smell of spoiled crickets was generally regarded as "(highly) unpleasant" and "rancid", and accidental tasting was remembered to be sour, rancid, and like spoiled protein, with similar but more pronounced difference between the hard exterior and the soft interior.

The sensorial pattern of GB may be subsumed to "insect" plus basically neutral flavours when fresh, and unpleasantly rancid, sour and of decomposition when spoiled.

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Sensory analysis of edible insects in Czechia: does size really matter?

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This contribution follows our recently performed questionnaire survey focused on the acceptance of insects by Czech respondents, which revealed significant species preferences in the case of eating whole insects. Orthoptera were the top-rated insects, while cockroaches were the least popular choice. On the other hand, no significant preferences were found in the case of ground insects (insect meal). Therefore, we decided to verify this hypothesis using a panel of 16 trained assessors. Also, we hypothesized that the differences in acceptance may occur within the groups of insects. To identify whether the size of insects may affect their acceptance, we the assessors evaluated one small and one large species from the orders of Blattodea (*Blatta lateralis* and *Blaberus discoidalis*), Coleoptera (*Tenebrio molitor* and *Zophobas morio*), and Orhoptera (*Gryllus assimilis* and *Locusta migratoria*). To determine possible species-specific differences in products containing insect meal, the ground insects of all above-mentioned species were added to the chocolate crinkle cookies and bread (10% replacement of wheat flour). The hedonic ranking test of whole fried and salted insects evaluated by Friedman´s test showed the *T. molitor* as the best and *Z. morio* as the worst choice. In the case of white bread, the samples with no insect meal were rated as a significantly top choice. In case of cookies, no significant difference was detected among the samples. Conclusively, it has been proved, that the acceptance of whole insects is influenced by the species. On the other hand, no general effect of size on sensory acceptance was observed. It also turned out that 10% replacement of flour is probably too high in the case of white bread, while in the case of sweet bakers' confectionery, such replacement does not have any effect on the acceptance of crinkles.

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The influence of the product name on the acceptance of insect based food

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Despite the benefits of incorporating edible insects in human diets, such as health and sustainability, their consumption is still not common in western countries. Studies have examined factors affecting consumer behavior towards insect-based food, however successful implementation in markets is yet to occur. Disgust is suggested as one of the biggest obstacles for the acceptance of insect-based food. The product name has been shown to have a significant impact on the perception of a new product category and can therefore influence acceptance. However this has not yet been studied for Insect based foods. Depending on the words used in the product name the associations with the name can vary and thus change the perception of the product. This underlines the importance of framing innovative products.

This study examined the influence of different product names on consumer acceptance of insect-based foods. In an online survey (N=150), participants were asked about their perception of product names containing the word insect or worm. Furthermore, questions were asked about the perception of taste-related names such as grilled or Mediterranean, as well as fantasy names such as *Dschungelade* that only indicate the presence of insects. As an answer option, the subjects could choose between disgusting and delicious on a scale of 1–7. The names were also boxed into the three food categories 1) chocolate, 2) burger and 3) pasta to distinguish taste preferences. The focus of this survey is on the German market and addresses a specific target group of flexitarians.

The results of the study can serve as a guide to increase the acceptance of insect-based products through the use of a positively associated product name. The legal product name is to be distinguished from the product name as it is an additional aspect that has not been checked.

Economic viability of insect farms in the Netherlands

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In the past decades, a great deal of research into the production of insects has been done in various domains (e.g. technical aspects and potential applications of insects and related products), but empirical research into the economic viability of insect farms in Europe is limited. An understanding of the profitability of insect farms is crucial; not only for rearers to determine their position in the market, but also for starting entrepreneurs prior to their decision to start insect rearing. In addition, investors, insurance companies and creditors also need to have insights into the viability of the industry; in their case to make an informed decision in providing services. In this study, we analysed the costs and revenues for *T. molitor* farms in the Netherlands, distinguishing between the business models reproduction (n=2) and rearing (n=7). Revenues for *T. molitor* farms included those obtained from sales of fresh larvae and insect frass as well as other extension services. Regarding costs, fixed and variable costs were investigated, as well as investment costs. Results include data on operational inputs such as amount of substrate and utilities needed per tonne fresh larvae produce, and an overview of commonly used inventory and machines. Furthermore, averages (with ranges) of investigated costs, revenues and gross margins per tonne fresh larvae produce are presented. Results show that the main determining factors for profitability of *T. molitor* farms include labour and substrate costs, and sales prices. Furthermore, it stood out that costs and revenues vary greatly between individual farms. This may relate to the emerging nature of the sector and highlights room for improving cost-efficiency of production. This study provides first estimates that can be used in decision making on (credit) risk assessment for service providers and designing targeted interventions by policy makers. In addition, the results are a basis for further research into the most optimal and (cost) efficient form of insect production.

What motivates consumers to accept whole and processed mealworms in their diets? A cross-country study in Belgium, China, Italy, Mexico, and US

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There is a growing interest in the commercialization of edible insects as food, both in traditional and non-traditional insect eating countries. However, only few studies have conducted cross-country investigation on the acceptance of entomophagy. Thus, the aim of this study was to examine to which extent consumers are accepting to include a whole and visible mealworm or a powder mealworm in processed foods in their diet. We used an online survey with closed and open questions, and responses (=3,006) were collected across five countries, i.e., Belgium, China, Italy, Mexico, and US.

Countries with entomophagy tradition (Mexico and China) showed a higher acceptance level towards the inclusion of mealworms in their diet, regardless of the processing condition (whole or powder) of the insect. In all countries, gender was the main factor affecting acceptance level of mealworms in the diet. Our results indicated that men were more willing to consume mealworms than women. Whereas age affected acceptance level only in countries with very low acceptance (i.e., Belgium, Italy, and US), with younger people (below 42 years old) more open to accept mealworms. Curiosity is a recurrent motivator to produce a first taste of insect-based products. In addition, the reduction of insect visibility was another important factor to accept mealworms, especially in western countries. On the other hand, in all the countries, aversion, yuck factor, dislike, disgust, lack of interest were the most mention barriers to reject mealworms. Moreover, across countries, the acceptance level increased for processed mealworms compared to whole mealworms.

Zero Waste Protein Factories: Leveraging Biology to empower the industry for waste-to-protein conversion on-site

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A fifth of all the food that is produced in Europe becomes waste, equating to 88 million tonnes of food being lost on a yearly basis, with associated costs estimated at 143 billion euros. About 30% of food waste is being produced during processing or manufacturing. A typical food manufacturer company can easily produce 5,000 to 10,000 tons of organic byproducts every year. Many food companies have made the reduction of food waste one of their main sustainability goals for the near future. Insect farming could significantly contribute to the reduction of food waste and a circular economy, reintroducing nutrients from food waste directly into the feed chain. Also, the entrepreneurial appetite for integrating revenue-generating zero waste solutions such as insect farming is growing. However, implementation of insect solutions for waste upcycling on an industrial scale has been facing several challenges. Asides cost and sustainability concerns with transport and logistics, the biological and economic feasibility of insect farming on various byproducts remains a great unknown for industrial food processors.

This keynote demonstrates that, by diligent assessment of biological feasibility, “Zero Waste Protein Factories” utilizing Black Soldier Fly Larvae (*Hermetia illucens*) for “on-site waste-to-protein conversion” could be economically feasible, already at a small industrial scale. The assessment factors include the biological assessment of feed substrates and feed formulas, scale of the factories, as well overall economic feasibility. It highlights the potential for industry players to not only save costs on disposal of their by-products but turn it into revenues while saving emissions as compared to composting or biogas digestion.

Educational leadership chair in the production and primary processing of edible insects

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Food waste upcycling by mass rearing of edible insects is a rapidly emerging sector that is shaking up conventional agri-food production and regional waste management systems. Many issues are still to be anticipated to allow the sector to emerge. Industry, governments and researchers must use new knowledge and skills to identify high-potential insect species; identify and validate the potential of inputs; develop innovative techniques for rearing insects; process and stabilize insect products; integrate insect products into livestock feed; demonstrate the economic feasibility of different production models; and measure the social acceptability of these new products on the market. Until now, there were no universities in Canada that provide training in the production of in-sects for food and feed. The Chair's mission aims to develop training activities in the field of edible insect farming as well as to identify and put into practice solutions to combat food waste through edible insect production. The activities of the Chair will focus on education and training, knowledge transfer and, research and development to optimize production techniques, upcycle different types of organic residues and develop economic models in line with Québec's regional specificities. The presentation will provide an overview of the various objectives of the Chair and an update on their evolution. The creation of the chair was made possible by a unique program established at Université Laval and 14 partners who have provided a total of $635,000 in funding over a five-year period (Enterra, Recyc-Québec, Sanimax, Aspire, Telus, Équilibre Protéine d'Insectes, Hagen, Entosystems, Centre de développement bioalimentaire du Québec, Fonds Germain-Brisson, Jefo, Protix, Cégep de Victoriaville's Institut national d'agriculture biologique (INAB), and Corporation de développement économique Victoriaville et sa région).

Compliance of frass with legal requirements

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In 2021 a legal definition of frass was created by the European Union. 'Frass' is defined as a mixture of excrements derived from farmed insects, the feeding substrate, parts of farmed insects, dead eggs and with a content of dead farmed insects of not more than 5% in volume and not more than 3% in weight. Meanwhile the standards for the placing on the market of frass were set ie. frass should be treated at 70 degrees Celsius for one-hour and comply with the relevant microbiological standards.

In 2021 the Public Waste Agency of Flanders, Belgium (OVAM) funded a study to investigate if the produced frass meets these standards. Frass collected from professional producers and research institutions was used for this study.

After developing a draft protocol for identifying insects in the frass, the weight and volume percentage of insects in the frass samples was examined. This revealed that there were large differences between the different samples and that this depended mainly on the insect species from which the frass came.

The microbial load before and after heat treatment of 1h at 70°C was also examined. It was found that the effect of this heat treatment was also very dependent on the insect species from which the frass came.

The results of this research were also evaluated with respect to the legal requirements. This study showed that most of the samples complied with these requirements.

Insect School: A way to move the BSF industry forward

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The insect industry is known for its high potential, but it's also known as a closed industry for the pioneers who want to start. When everybody keeps thinking they are sitting on the "golden idea" then nobody will move. That also means the industry will not move forward. To make the industry move you need to offer something which triggers the people to move. The Insect School is set up with that goal. To create a meeting place where people can go to share knowledge and to obtain new knowledge. It's proven that a physical location has a very positive effect on cooperation's in an industry. At same time set-ting it up has been facing the same difficulties because nobody wants to make that first step. So this step is taken by 1 person in 2021 and from 30 June 2022 the practical location in America (don't be fooled, this is not the USA but a village called America located in The Netherlands!) is opened and accessible for all who have an interest in the BSF industry. From people who want to start, growers who want to experiment, feed trials, university research, technical developments, breeding, frass trials, etc. etc. Many universities are involved with the BSF industry and perhaps will say now but we already do our own research at our own university. Exactly! That we all should cherish and promote. The idea of the Insect School is actually the translation between the universities and commercialization. It's that inbetween step in the proof of concept on pilot scale before going large. From 1 till 20 tons of organic waste production rooms. That is good for commercial testing and showcasing investors your proof of concept. The physical location in America (Netherlands) is the practical centre. The online version www.insectschool.com is also being build and when IFW2022 takes place it will become online. It should be the information source for BSF farming. At same time training courses by universities and commercial companies can be shown so people are guided in their journey into BSF farming. This initiative might become for more insects in the future but first things first.

Venomics of the native myrmicine ants *Myrmica rubra* and *Myrmica ruginodis*

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Ants are one of the most diverse lineages of venomous animals. Their venoms have a great translational potential for biological pest control or medical application. Nevertheless, venoms of most species remain understudied so far and little is known about their complex chemical arsenals. Here, we applied a cutting-edge proteo-transcriptomic approach to disentangle the venom composition of two hitherto unexplored species (*Myrmica rubra* and *Myrmica ruginodis*). A rigorous bioinformatic filtering process of RNA-seq data linked to proteomics-based verification enabled us to identify an array of novel biomolecules with translational potential for the bioeconomy. In total, we identified 44 and 113 venom components in *M. rubra* and *M. ruginodis*, respectively. Both venoms are dominated by serine proteases and we provide the first report of phospholipase A1 in *M. ruginodis* venom. Both species contain homologs of highly algogenic EGF-like toxins previously known from Australian ants. Bioinformatic analysis revealed, that these toxins have undergone a diversification process in formicoid ants. They seemingly evolved towards similarity with known EGF hormones from vertebrates and insects, possibly facilitating predation or defense. Lastly, we retrieved a set of small linear peptides similar to insecticidal peptides isolated from other ant venoms. These have been synthesized and we provide first data on their bioactivity.

The fertilization potential of *Hermetia illucens* larvae post-breading residue

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Hermetia illucens (black soldier fly, Diptera: Stratiomyidae) larvae have a high survival rate on waste substrates, high protein and fat content in the body of the larvae, and short life cycle. Therefore, this insect is a good candidate for industrial scale production The main purpose of such breeding is to produce alternative feeds for animals and aquaculture, however, *H. illucens* can also be used for chitin extraction, biodiesel or biogas production, as well as for the extraction of antimicrobial proteins. *H. illucens* larvae can utilize and biorevalorize waste organic biomass such as agricultural waste (animal manure, plant biomass) and food related waste (out-of-date food, fruit and vegetable waste) and transform it into larval biomass. In addition, *H. illucens* breeding residues have the potential to be used as a replacement or additive for fertilizers.

The present study focused on testing whether the solid and liquid wastes remaining after *H. illucens* breeding on high-protein feed (pea and bean seed waste) have fertilizing properties and can be safely used for plant cultivation. Both substrate variants were initially flooded with water to obtain about 75% moisture content and allowed to stand for 24 h to swell. Approximately 1000 of 5-day-old larvae were used for the experiment and the substrate dose was 1 g FW of the wastes per larva. The breeding was conducted for 1 month at 25°C in containers that provided air flow and allowed to obtain liquid waste. Both variants were conducted in triplicate.

Preliminary results of the experiment indicate higher concentrations of nitrogen forms (NO_3- and NH_4+) in the solid waste in the variant of bean seeds than in the variant of pea seeds. For liquid waste, NO_3- concentrations were also significantly higher on beans, while NH_4+ concentrations were comparable on both variants. The parameters of larvae and pupae obtained in the experiment demonstrate good development and survival on the high-protein substrates, which may suggest a suitable food form for breeding *H. illucens*.

The chemical diversity in arachnid venom as a source of biomolecular innovation in medicine and agriculture

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Arachnids are ancient arthropods that evolved into insect hunting assassins. As such, they often rely on complex venoms to overpower their prey. These venoms are mostly composed by smaller and heavily cysteine crosslinked peptides that selectively and potently interfere with ion channels, thereby mounting neurochemical attacks on their victims. Due to these properties, arachnid venom peptides represent an outstandingly rich source for the discovery of novel drug- and pesticide leads. However, only a marginal fraction of arachnid venoms have been investigated so far owed to methodological difficulties when working with the miniscule amounts of starting material that is retrieved from most taxa. Only recently, the application of -omics and biotechnology approaches has enabled us to analyze and access the chemical diversity within arachnid venom systems. Here, we provide an overview about the different arachnid venom systems that have been studied in our lab over the last years, with particular emphasis on the rather atypical venom of wasp spiders (*Argiope bruennichi*). We highlight the importance of natural history, taxonomy and phylogenetic research as a guiding measure to rationalize and economize venom biodiscovery in hyperdiverse lineages. By applying the biotechnological toolkit, we were able to produce some venom components in laboratory scale and to test their bioactivity against pathogenic microorganisms plus pest- and vector insects. Our results support, that a plethora of the identified components has potential for further development into antiinfective agents and bioinsecticides.

Can further composting improve the fertilizer value of frass?

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The entomophagy and insect industries are not new to the world. Recently edible insects are highlighted as a future protein source for feed and food having high suitability. This has attracted huge attention from western countries. The International Platform of Insects for Food and Feed (IPIFF) predicts that Europe's insect protein production is expected to reach three million tons by 2030. The insect rearing industry generates three times as much waste (frass) as protein. This indicates that the development of a market for frass is as important for protein.

The black soldier fly (BSF) has gained increased importance as a primary candidate for animal feed and biowaste recycling. Hence BSF frass was investigated in this study. Although the BSF frass is a potential candidate to replace the traditional N to a great extent, frass on its own is a biologically unstable fertiliser. Both positive and negative effects of frass have been recorded in literature around the world.

Hence, this study investigates the effect of composting BSF frass to improve the fertiliser value of BSF frass. The frass was composted as a sole substrate and co-composted with wood chips for 40 days and matured for 80 days. The phytotoxic test reveals that fresh frass is more toxic than the composted frass, but the co-composted frass didn’t exhibit any toxic effects.

A glasshouse study with lettuce plant was conducted with fresh, composted, and co-composted frass at 5, 10, and 15% inclusion, and the lettuces were harvested on the 60th day. The plants treated with fresh and composted frass at 10 and 15% either didn’t germinate or survived till the 60th day. Whereas 50% of the plants survived at 15% inclusion of co-composted frass. Apart, tipburns were observed in the lettuces plants. The intensity of tipburn was maximum in the fresh frass, followed by composted frass and finally co-composted frass.

In conclusion, co-composting BSF frass with carbon-rich material would enhance the fertiliser value of the frass. BSF frass could be a potential fertiliser to replace the mineral fertiliser but it has to be processed before its application.

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Insects in the fight against antibiotic resistance: *in vitro* characterization of black soldier fly antimicrobial peptides

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The black soldier fly (*Hermetia Illucens*) has one of the largest antimicrobial peptide (AMP) repertoires ever recorded in insects. Exploration of the antimicrobial activity of these AMPs could lead to new antimicrobial therapies that help to combat the ongoing antimicrobial resistance crisis.

Our research aimed to upgrade the knowledge on the activity of black soldier fly AMPs and to gain insight in their potential as future therapeutics. A large library of synthetically produced black soldier fly AMPs was evaluated against a broad range of human pathogens, to test for antimicrobial activity, and a human cell line, to screen for cell toxicity. Further *in vitro* experiments were performed for two selected peptides (HC1 and HC10).

Potent activity in the low micromolar range was found for the family of cecropin AMPs. From these, HC1 and HC10 were selected to explore their *in vitro* activity against *Pseudomonas aeruginosa*, a clinically important Gram-negative pathogen with a high established resistance. Both AMPs have a low in vitro cell toxicity, are bactericidal and have a rapid onset of action with membrane-permeabilizing effects. In addition, they also show endotoxin-neutralizing properties.

These first promising in vitro results show that HC1 and HC10 could serve as starting points for the development of antipseudomonal drugs.

Poster Session

Unraveling the gut virome in Black Soldier Fly larvae (*Hermetia illucens*)

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Microbes in the gastrointestinal tract of traditional livestock exert important functions to modulate animal health and performance, e.g. the processing of dietary components. Given the importance of these interactions between the host and its microbiota and their influence on host phenotype, it is crucial to understand what is the 'best' microbiome from a production perspective, and which biotic and abiotic factors govern microbiome composition. With the success of industrial insect rearing, similar questions arise on their microbiome composition and how it is affected, especially for *Hermetia illucens*. Yet one biotic factor that remains uncharted terrain for all industrially reared insects is their virome. Studies in the human gut have shown its impact on the microbiome and gut health.

Therefore, shotgun viromics is used to map the virome in the gut of *H. illucens* larvae and explore whether and how its composition varies in function of diet and location in the gut. To enable this, we present here the selected method to sample virus-like particles from *H. illucens* gut samples and the method used for DNA extraction. In parallel, a set of phages is isolated and characterized from the gut virome that target key members of this insect's microbial gut community. More precisely, both gut and frass samples were screened against the following collection of isolates: *Enterococcus* sp. (n=50); *Morganella* sp. (n=7); *Providencia* sp. (n=20); *Klebsiella* sp. (n=34) and *Enterobacter* sp (n=13).

Once isolated and amplified, such phages can be used to answer what the role of phages will be in shaping the microbiome diversity.

Development of genetically improved Black Soldier Fly strains using targeted genomic mutagenesis

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Insects are promising and sustainable source of protein for livestock, essential for providing food supply needs of the growing world population. Black Soldier Fly (BSF) is considered the next “livestock insect” for domestication – it isn't harmful, grows rapidly and has a high conversion rate of various types of organic waste to larvae biomass.

FreezeM’s goal is to become the “seed company” of the evolving insect industry by providing high-quality BSF eggs and neonates to rearing farms and factories world-wide.

We aim to develop BSF strains with genetically improved features such as better food conversion rate, higher protein content and nutritional values that are optimized for different livestock species. Since the genetic toolbox for BSF is in its infancy, we set up a new protocol for directed genetic modification in BSF. We chose to focus on Cas9/CRISPR – a highly effective method for directed mutagenesis. Next, we chose microinjection as delivery tool of genome editing reagents into BSF embryos. In order to optimize the protocol, we determined the best developmental stage for injection, type of genome editing reagents and their concentration, eggs growth conditions post injection etc. We were able to establish a rapid and highly effective directed mutagenesis proto-col in which we deliver complex of Cas9 protein and gRNA via injection into BSF eggs shortly after egg laying. So far, we successfully generated more than 100 target specific mutations with high editing efficiency and survivability and reared a genetically modified line as proof of concept.

Along with our efforts to develop enhanced lines, we aim to develop additional molecular tools for BSF such as, targeted gene insertion using Cas9/CRISPR and HDR technology, genetically improved strains maintenance, efficient crosses between genetic lines and precise phenotypes characterization.

Amino acid profile of Jamaican field (*Gryllus assimilis/ locorojo*) and Mediterranean crickets (*Gryllus bimaculatus*) fed exclusively with chicken starter feed

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Although not developed for crickets on purpose, poultry feed is a common basic feed. Some advantages are an appropriate nutritional profile (protein), stable quality and relatively easy storage. Yet, the chemical composition of insects varies with the species and (much stronger than in vertebrates) the feedstuff, and each feedstuff produces individual profiles, e.g. amino acids (AA).

G. bimaculatus (GB) and *Gryllus assimili*s (GA) pinheads were placed in transparent boxes with egg carton and trays for water and commercial chicken starter feed (CSF; 16% protein). After 10 weeks, animals were killed (freezing), and AA were determined via acidic hydrolysis in crickets and their frass.

After 10 weeks, all GB completed their metamorphosis, while the GB population still contained nymphs, so the development of GA took longer. Signficant instar-related differences occurred in Tau, Tyr, and Met, species-related effects were assessed for Ser, Val, Cys, and Orn (GA > GB). Frass did not show species-related differences. CSF contained large (>1 g/100 g) amounts of Asp, Glu, and Leu, crickets of these and Ala, Val, Lys, Arg, and Pro. Frass yielded best in Asp, Glu, Ala, Leu, and Pro. Final feed consumption was up to 0.16 g/animal/week, mean weight 0.6 g, survival rate 10% in both species, showing that they weighed less than crickets fed CSF and vegetables.

Feeding GB exclusively with CSF resulted in a shorter life cycle duration; however, GA yielded a better amino acid profile in both animal and frass. Still, weight gain in both species was not as good as if raised with CSF and vegetable off-cuts.

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Container size affects life cycle duration and survival rate in Jamaican field crickets (*Gryllus assimilis/locorojo*)

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Population density is a crucial factor in any animal keeping, seeking the balance between squandering production space and risking overcrowding with subsequent animal health (production diseases) and animal welfare problems. Although *G. assimilis/locorojo* is a common commercially bred species worldwide, little has been published on population density.

Recently hatched crickets were placed in different transparent plastic boxes with egg cartons (small box: 260 cm², large box: 1475 cm²). All boxes contained 20 animals (in triplicates). Water, chicken feed and vegetable cut-offs were offered ad libitum. Each week, the total amount and individual weight of animals, along with the total biomass weight were assessed resp. calculated. Trial lasted until the animals of one box type became imagines.

After 15 weeks (i.e. much longer than usual 10 to 12), large box crickets completed metamorphosis while oldest small box animals were still in mid-nymph instars. Accordingly, large box crickets showed significantly higher individual (826 ± 73 vs. 325 ± 12 mg) weight, better weight development (60.000 vs. 35.000%), and a better survival rate (50 vs. <25%) than small box animals. Therefore, total biomass was also higher in large box animals (8160 vs. 870 mg). However, even in three triplicates, a high variability was observed in these lots, showing that space is but one of several influence factors.

There seems to be a minimal space requirement. This should be considered when assessing cricket density in terms of yield.

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The effect of feed fermentation and inoculation level on total biomass weight of *Hermetia illucens* larvae

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Fermentation process is a well-known technique of preservation. In animal nutrition this method is widely used for increasing digestibility, preservation and biostability. That could create opportunities for insect production sector, where often, agricultural plant-based waste is used as main feed component for insect diets.

The aim of this study was to determine the effect of full feed matrix fermentation on growth performance parameters of *Hermetia illucens* larvae. Furthermore, 5-DOLs larvae were fed with diets treated with, accordingly: 0% 0.1%; 1%; 10% of inoculant. The inoculant consisted of feed, previously prepared, and subjected to a natural fermentation process.

Monitoring of the fermentation process in the applied inoculant was conducted by daily measurement of the pH and Total titratable acidity. The diets` effect on the insects within the different concentrations were evaluated based on FCR performance.

Conducted study showed significant differences of growth performance of BSF between the groups. Diet containing 0.1% of inoculant showed optimum results regarding total larve biomass weight achieved at the end of rearing process, compared to the other inoculated groups and 18% higher biomass obtained than in the control. Groups containing 1 and 10% of feed inoculation achieved respectively 7.8% and 10.4% higher bio-mass weight in comparison to non-inoculated diet.

Those results clearly show beneficial effect of feed fermentation in BSF larve feeding. However, the level of inoculation had a big impact on the overall performance of fed insects.

Determination of microbiological safety of farmed and wild harvested edible insects from Kenya

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Food security is not accessible to everyone in Africa, since the availability of nutritious and safe food is yet to be achieved. Edible insects contributed to seasonal food diversity with animal-sourced protein. Locusts, grasshoppers, dung beetle larvae and termites, are commonly harvested from the wild in Kenya whereas black soldier fly farming is widespread. Edible insects are highly perishable and low cost traditional techniques like smoking, salting, frying, and sun drying are used for preservation since available infrastructure could not allow freezing and canning to increase its shelf life. With these traditional methods spoilage still occurred. Moreover, reports of increased diarrhea co-occurred in regions where insects swarmed. Data on the contamination of insects with human-gastrointestinal pathogenic bacteria like *Salmonella*, diarrheic *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* (s.l.) are rare as well as the distribution of antibiotic resistant bacteria. Thus, we aimed to analyze the occurrence of these pathogens in 29 samples (23 insect & 6 frass samples) from different regions and production types in Kenya. A questionnaire was used to collect information about the insect-source, persons involved in the collection, processing techniques and process flow description. Wild harvested and reared insects as well as frass were analyzed for the occurrence of the selected pathogenic and/or antibiotic resistant bacteria. First results revealed a total aerobic bacterial count ranging from 10^2 to 6.3×10^9 CFU / g (dried and powdered) insect and from $<10^2$ up to 10^{10} CFU / g frass. Coliform bacteria were detected in 9 of 23 insect and 2 of 6 frass samples. The 3 dung beetle samples contained the highest coliform load ($> 5 \times 10^4$ CFU / g). *B. cereus* (s.l.) species were detected in 20 of 23 insect samples with a maximum of 2×10^8 CFU / g in a cricket sample. Moreover, staphylococci were detected in 7 of 23 insect and 5 of 6 frass samples. The ongoing research will enlighten the contamination of edible insects with pathogenic and antibiotic resistant bacteria according to source and harvesting style. This knowledge would help understand safety issues surrounding edible insects.

The co-housing microbiome of black soldier fly larvae

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The use of insects as a resource for biomolecules (protein, fat, and chitin) has gained significant attention from science and industry. Although market forecasts regularly predict an outstanding growth of this novel branch of the bioeconomy, the general public is often not aware of or excluded from these developments. In most Western countries, the occasional encounters with food from insects are rather considered a gimmick than part of a dietary habit. This creates the need to raise awareness for the actual potential of using insects in feed and food and lead them out of their niche of exotic snacks.

Here, we present results from a combination of citizen science-based workshops on insect farming and microbiome research. Firstly, a reusable and economic rearing unit was developed that allowed citizen scientists to carry out and monitor black soldier fly feeding trials with household kitchen wastes. Secondly, bacterial and fungal communities in frass and larval gut samples from these home trials were analyzed and combined with data collected by the workshop participants, thus merging citizen science with basic research.

About 30 citizen scientists aged between 23 and 76 supervised and documented their own trial, resulting in a highly diverse pattern of organic wastes employed as rearing substrates. The distinctive environmental conditions prevailing during each home trial and their impact on the larvae's development were defined as co-housing effect. On average, 93% of the larvae survived the trials with 76% reaching prepupal or pupal stage within three weeks. While larvae and frass from the participants' trials were dominated by Ascomycota, Basidiomycota played a more important role in the chicken feed control trials. To allow citizen scientists to access their processed results and compare their rearing performance with other participants, an interactive online dashboard was developed (https://tklammsteiner.shinyapps.io/cohmila-app). By combining free hands-on workshops with insights into basic research, we were able to attract broad public interest in the peculiarities of insect farming. We are convinced that education is key to reduce prejudice and to mediate the extensive benefits that insects could provide as a resource.

Optimizing substrate for sustainable mass production of black soldier fly (*Hermetia illucens*) larvae utilizing side streams

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Various types of local side streams from food industry are currently utilized as substrate in black soldier fly larvae (BSFL) production. To meet the nutritional quality, essential for optimal larvae growth and performance, the substrate is typically prepared as a mix of different side streams. To control variation of nutritional quality of the substrate, the mix is often designed by standardizing carbohydrate:protein ratio. In practice, a sufficient homogeneity of the nutritional quality of substrate has been achieved by careful mix design. For larvae growth and well-being, it would also be important to control the content of certain amino acids in the mix design. In addition, other substrate features such as tendency to dry during the breeding or structural properties that effect the easiness of separation larvae from frass at harvest should be taken to account to achieve an efficient and robust process for BSFL mass production.

In our study, a test substrate is prepared by mixing plant-based materials such as kale, potato, tomato and breadcrumbs (which model side streams) with a balanced protein concentrate consisting of brewer's spent grain, feed yeast and synthetic amino acids, so that amino acid composition of the concentrate is comparable to that of commercial poultry feed, used as control.

Suitability of the test and control substrates for BSFL production are tested in feeding experiment with two ventilation treatments. A completely randomized design with four replications is performed in Luke's Insect Lab in Jokioinen, Finland. BSFL growth as well as substrate consumption and change in water content of the substrate is monitored during the experiment. At termination of the experiment, BSFL and frass are separated by sieving. BSFL are weighed, and success of sieving is rated. The results show the effect of substrate drying on BSFL growth and give an estimate on suitability of different side streams to BSFL mass-production.

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Identification of polystyrene degrading bacterial community isolated from *Tenebrio molitor* digestive system

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With growing population and rising standard of living the total annual plastic production in the world is close to 500 mt. Overall recycling rate (RR) of plastics is around 9%, but polystyrene which is produced in 15 mt per year amount has an RR of only about 1%. Although expanded polystyrene is one of the most used plastics in the world its recycling is very difficult, and expensive, mostly because of its high volume to mass ratio. By prior studies, polystyrene can take 30% of total landfill volume. These characteristics of this plastic require innovations in sustainable waste management, which is the main topic of our research.

It has been known for long that many insect species have a role as a degrading agent in the ecosystem, therefore many of them can be used to utilize organic wastes. Since 2015 it is proven that mealworms can fully consume polystyrene and digest it to a certain extent with the help of their gut microbiome (Yang 2015). Bacterial community living in the digestive system of insects fed with plastics might differ compared to those fed on natural feed.

With the aim of researching plastic degrading agents, we investigated community level changes in the digestive system of *Tenebrio molitor* fed with polystyrene (PS). Meal-worm larvae originated from the same parent community were separated into PS fed, and to regular wheat bran fed groups. During the one month feeding period all the external environmental factors were the same, except feed, regarding the separate groups. After the feeding period mealworms from both groups were dissected and the digestive systems were prepared and unfolded in sterile circumstances. Community DNA was isolated using the Zymo Fecal&Soil Miniprep kit to prepare the samples for Illumina 16S rDNA amplicon sequencing.

The bacterial communities associated with plastic degradation were described on different taxonomic levels. We investigated several genera abundant in both sample groups and investigated a few genera that become dominant in the PS fed group.

These outcomes will help us targeting bacterial genera in our further research with the aim of specifying PS degrading capabilities of certain bacterial strains originated form *Tenebrio molitor* digestive system.

Effects of live yeast *Saccharomyces cerevisiae* on growth, protein and fat content of the Black Soldier Fly *Hermetia illucens*

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Insects are becoming increasingly important over the last years as basis of food and feed products. The maggots of the Black Soldier Fly *Hermetia illucens* are an alternative for lipid and protein resources like fish oil and soy. Their production has a low environmental impact. To increase the yield of the maggots, the feed composition is a crucial aspect.

Here, we present the effect of living yeast cells (genus *Saccharomyces cerevisiae*) in the feed of reared *Hermetia illucens* maggots in an industrial setting. The analysis was conducted under normal production conditions at the “Madebymade” farm near Leipzig (Germany) and the weight-, protein- and fat content of the maggots was monitored. For this experiment, 8.5 million maggots and 6.080 kg feed were used. Three different yeast concentrations (group 1, 2 and 3, respectively) were tested, i.e. addition of a 0.1%, 0.2% and 0.4% *S. cerevisiae* share to the feed. A significantly higher growth was observed due to the addition of the yeast cells to the feed. On day 11, the weight of group 1 increased by 11.54%, the weight of group 2 by 13% and the weight of group 3 by 14.86% in comparison to the control group. In addition, the fat content in group 1 increased by 1.78%, in group 2 by 2.11% and in group 3 by 4.09% compared to the control. In contrast, the protein content showed no significant differences, reaching values between 40.28% and 40.80%.

Bioassay methods for optimizing Black Soldier fly (*Hermetia illucens*) rearing at industrial scale

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The black soldier fly, *Hermetia illucens*, has the potential to upcycle a variety of organic waste streams into high quality protein and oil for animal feed. Nutritional value of BSF larvae is discussed, as well as the effect of biotic and abiotic factors on both larval body composition and performance.

In order to make a true impact and replace existing components in animal feed, insects have to be produced at an industrial scale, all year long, and in a stable manner. FreezeM is developing novel technologies that will enable for the first-time to generate stocks of ready-to-use suspended neonates and frozen eggs – a solution corresponding to the agriculture seed production.

Our suspended neonates have a generalist ability to grow on various feedmix recipes, independent on the feed that was used in the reproduction process.

In order to test the rearing performance of larvae on large number of recipes, we have designed a bioassay system that simulates rearing on a small scale and provides insights on various feed compositions or additives in a high throughput manner. This method enables a wide range of applications such as: maximizing the size of the larvae, examining different food recipes, growth of small-scale transgenic lines, screening of substances in various concentrations to develop premium feed compositions and pre-mixes, and determining the density of the larvae and other abiotic conditions for optimal growth.

The insights gained from the bioassay were tested and validated at real rearing scenarios, demonstrating

Heating black soldier fly larval frass according to EU regulatory requirements significantly reduces *in vitro* antifungal activity against a range of phytopathogens

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Black soldier fly larvae (BSFL) have the potential to efficiently transform a wide range of organic waste into larval biomass with a high nutritional value. A secondary product obtained from this process is frass, which includes larval excrement, larval exoskeleton, and undigested diet. Increased research on frass is needed for its valorisation on an industrial scale. We have reported that frass demonstrates antifungal activity (AFA) against phytopathogens based on the antagonistic effect between the frass microbial community and phytopathogens. Our initial work employed conventional approaches to study antagonistic effects mainly examined the competition between antagonists and pathogens for food and space in freshly collected frass. However newly-established EU regulations requires heating of frass (70°C for 1 h) which may compromise this observed antifungal activity. In this study a fruit/vegetable/bakery waste-based diet mimicking a 6-month composite sampling from grocery store wastes supplemented with brewery waste (FVBB) was compared to a control Gainesville House Fly reference diet (GV) and fed to BSFL under standard conditions. A portion of the frass sample was heated in a water bath at 70°C for 1 hour. Mycelial radial inhibition of the fungi including *Pythium ultimum, Rhizoctonia solani, Phytophthora capsici, Fusarium oxysporum, Alternaria solani, Sclerotinia sclerotium,* and *Botrytis cinerea* were assessed. The inhibitory effects of secondary metabolites produced by the frass microbial community against phytopathogens re-veal AFA against every pathogen tested. The Minimal Inhibitory Concentration (MIC) for *B. cinerea, A. solani, P. ultimum* and *R. solani* was 1% (w:v), for *P. capsica, F. oxysporum* was 10^{-3}% (w:v) and for *S. sclerotium* was 10^{-5}% (w:v). As previously reported, the AFA was higher for frass derived from BSFL fed GV versus FVBB. We report that while residual AFA remains in heated frass, this activity is significantly lower in heat-treated frass, irrespective of feedstock origin.

Establishment of examination procedures for edible insects in official food monitoring

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The Novel Food Regulation (EU) 2015/2283 has provided the legal framework for the introduction of edible insect products onto the European market. To ensure the food safety of this products, the analytical methods of the official food control laboratories for the examination of edible insects need to be revised, adapted and expanded.

One method that has been increasingly used in microbiological laboratories in recent years is the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF-MS). As part of experiments in 2021, 138 microorganisms isolated from 22 insect samples were examined using MALDI-ToF-MS. Almost 70% of the organisms could be identified at the species level. Unidentified isolates were subsequently sequenced and entered into the MALDI-ToF-MS database.

One third of the identified isolates were strains of the Bacillus cereus group. To be able to differentiate this group on a species level a new primer pair targeting the pycA Gen was designed and tested. Compared to the previously targeted rpoB gene, species differentiation was improved with the newly constructed primers. However, unambiguous species identification was not possible. This may be due to the taxonomy of the group, which has not yet been finalized.

Another way to further characterize the Bacillus cereus group is to study its toxin-forming ability. In recent years, the emetic toxin cereulide, which is produced by some members of this group, has increasingly led to food intoxications. As part of this poster, the establishment of a real-time PCR for the detection of the ces gene, which is closely related to the ability of strains to produce cereulide, for the routine use in the CVUA Freiburg is presented.

Additionally, a protocol for the direct detection of the cereulide toxin using MALDI-ToF MS was developed and introduced into the routine of the CVUA Freiburg.

The poster also gives an outlook on open questions, which are to be clarified subsequently in the context of a doctoral thesis.

Cecal volatilome and microbiota profile of organic chickens supplemented with black soldier fly live larvae

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Insects have shown to be a potential nutritional replacement in poultry nutrition as substitute of traditional protein sources, with positive effects on gut microbiota. However, only few studies have investigated the effects of black soldier fly (BSF) live larvae provision on short-chain fatty acids (SCFAs) and microbiota composition in chicken's gut. Label naked neck (LNN, n. 240) chickens were reared in an organic production system from 21 to 82 days of age and randomly allocated to four experimental groups (10 birds/pen, 6 replicates/treatment) according to bird gender and larvae provision. Experimental groups were fed 10% supplementation of BSF live larvae, based on the expected daily feed in-take (DFI). At slaughter, samples of cecal digesta were collected from 60 animals (15 birds/treatment), frozen and stored at -80°C until to be analyzed by SPME-CG-MS and DNA sequencing techniques, respectively. Results showed that seven SCFAs were identified, with butyrate as the most abundant. Even if no significant differences were found between treatments, the cecal SCFAs concentration in insect-fed animals were noticed to be less variable than control group. Cecal microbiota analyses of birds fed BSF live larvae showed a higher incidence of *Coprobacillus*, *Synergistaceae* and *Christensenellaceae*, with the latter having the potential to degrade chitin's insect meal, a compound with immunoregulatory properties. In conclusion, results showed that even a dietary 10% supplementation of BSF live larvae can slightly improve microbiota profile and potentially, SCFAs production in LNN chickens. These results confirm what observed in recent studies on broilers, but with lower (5% of DFI) live larvae inclusion levels. Financial support for Poultrynsect project was provided by transnational funding bodies under the Joint SUSFOOD2/CORE Organic Call 2019.

Can black soldier fly live larvae supplementation modify gut histomorphology of organic chickens?

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The effects of insect meal on gut health have recently been reported in free-range and broiler chickens, but no data are available for organic intermediate growing chickens supplemented with black soldier fly (BSF) live larvae. Label naked neck chickens were reared from 21 to 82 days of age and randomly allotted into four groups (n= 240, 10 birds/pen, 6 replicates/treatment) according to bird gender (male-M and female-F) and larvae provision (Control-C and larvae-L). LM and LF groups received 10% supplementation of BSF live larvae, based on the average daily feed intake. At slaughter, samples of liver, spleen, bursa of Fabricius, and gut were collected from 60 animals (15 birds/treatment) and routinely processed for histomorphological examination. The following morphometrical parameters were evaluated on duodenum, jejunum, and ileum: villus height (Vh), villus width (Vw), crypt depth (Cd), Vh/Cd ratio, total absorptive area (TAA), mucosal, and muscular thickness. The observed histopathological findings were evaluated using a semi-quantitative scoring system (0: absent, 1: mild, 2: moderate, 3: severe). Morphometrically, Vh, TAA and mucosal thickness depended on sex, being greater in M than in F ($P<0.05$). Also, Vw was influenced by the interaction diet x sex, being greater in CM than in CF ($P=0.016$). Apart from Cd, all the evaluated morphometric indices depended on gut segment ($P<0.001$).

Histologically, gut showed absent to moderate multifocal lymphoplasmacytic enteritis. Liver showed mild lymphoplasmacytic inflammation and absent to moderate multifocal vacuolar degeneration. Bursa of Fabricius presented absent to mild cortical depletion, while spleen did not show any alterations. Although the severity of all the observed lesions was not influenced by diet ($P>0.05$), liver degeneration depended on sex, being higher in F than in M ($P=0.025$). In conclusion, results showed that BSF live larvae provision did not impair gut and general health of organic chickens, assuring a physiological, sex-dependent gut morphological asset. Financial support for Poultrynsect project was provided by transnational funding bodies under the Joint SUSFOOD2/CORE Organic Call 2019.

Ultrasound-assisted enzymatic extraction of protein from *Acheta domesticus*

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Separation of protein fraction from insect biomass is often desired for better nutritive and functional properties. This study investigated the effects of ultrasound-assisted enzymatic extraction on the yield, functional properties, antioxidant properties and molecular characteristics of the protein extracted from house crickets (*Acheta domesticus*). Different extraction methods including aqueous extraction (WE), enzymatic extraction (E) with bromelain, ultrasound-assisted extraction (US), extraction with ultrasound and enzymes (US+E), and ultrasound extraction followed by enzyme addition (US-E) were compared.

Results showed that US+E for of 1h and US-E (one hour each) could significantly increase the protein yield, reaching up to 41% and 38%, respectively. Further, process E achieved a protein yield of 30% and 45% after 1 and 5h respectively. US achieved a yield of 9% after 1h; whereas, WE yielded 7% 1h and 10% after 19h of extraction time. The extract was freeze-dried to obtain *Acheta domesticus* protein isolate (ADPI) and further analyzed for functional and physicochemical properties. ADPI had a high solubility of 84 to 90% at the pH values of 3, 5, and 7, and the pH did not affect the solubility. Only the ADPI-WE exhibited lower solubility of 50% at the pH values of 3 and 5, and 73% at pH 7. Ultrasonic treatment showed a positive effect; whereas, bromelain addition showed a detrimental effect on the emulsifying capacity of the proteins. The foaming capacity of ADPI-US+E was the highest at 231%. ADPI-U-E (15%), ADPI-E (12%), and ADPI-US (23%) showed low foaming capacity, whereas the ADPI-WE had no foaming properties. A strong positive effect on foaming capacity by the combination of ultrasonic and enzymatic extraction was observed. Further, the different extraction methods showed no influence on the zeta potential and antioxidant activity; however, some differences in particle size distribution could be detected. Therefore, a combination of ultrasound and proteolytic enzymes can improve protein extraction yield with tailored functional and physicochemical properties.

SensiBug – potential use of *Tenebrio molitor* in veterinary food for dogs with diet-dependent enteropathies

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Companion animals generate a total food requirement worth of EUR 21 billion in Europe. The number of cases of diet-dependent enteropathy in these animals is increasing. As part of the assessment of the possibility of using mealworms (*Tenebrio molitor*) in Poland, there is a need to develop dog food recipes with the use of mealworms protein and to conduct research on their impact on the health of pets. Such dog food, apart from high nutritional value, should be distinguished by its hypoallergenic nature, confirmed by reliable scientific research. The aim of the project is to develop recipes for dog food formula with the use of insect protein and to evaluate its influence on the symptoms of diet-dependent enteropathies. The pro-ecological aspect of the project is important because the planned activities will lead to the upcycling of by-products of the agrifood industry. Design innovation is part of 3 out of 5 Schumpeter cases, including: creating a new product, creating a new market and sourcing poorly used raw materials. The implemented formula is innovative with novel functionality. The novelty of the invention was rated as creative with imitation elements. The scale of the complexity of the changes was defined as related because the possibility of using mealworms by the companies producing dog foods will have a positive impact on the development of the insect rearing industry. The dimension of the changes created by the implementation was assessed as incremental with breakthrough elements and may also cause strategic reorientation, potentially influencing the shaping of the insect rearing sector. The final result of the project will be a recipe of an insect protein-based food for specialized dog nutrition.

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Infrared spectroscopy combined with multivariate, a novel technique to predict the insect protein added to raw doughs

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Infrared spectroscopy is a conventional technique for identifying chemical compounds. The chemical complexity of biological samples can be overcome by analysing spectral data using powerful supervised pattern recognition techniques. In previous research, we have proved the potential of using attenuated total reflected infrared spectroscopy combined with multivariate analysis to predict the amount of *Alphitobius diaperinus* and *Locusta migratoria* molturated added to raw salty dough and its 3D printed cooked version. The objective of the present research was to study the potential of using attenuated total reflectance Fourier Transform mid infrared spectroscopy (ATR-FTMIR) and Fourier Transform near infrared (NIR) spectroscopy combined with multivariate analysis to discriminate raw salty dough enriched with insect protein extracts. Several doughs were made using chickpea flour (26 to 56%), water (39%), olive oil (11%), curry, salt, and different quantities of protein extracts (*A. diaperinus* and *Hermetia illucens* up to 20%). For the ATR-FTMIR analysis, an amount of dough was placed onto the sample stage of a portable spectrometer equipped with a single bounce ATR diamond crystal accessory and spectra were collected from 4000 to 800 cm^{-1}. For the FTNIR spectra acquisition, the dough was placed on a 60-mm diameter glass petri dish and spectra were collected using portable FTNIR analyzer from 1300 to 2600 nm. Soft independent modelling of class analogy (SIMCA) and Partial least squares regression (PLSR) were used for the chemometric analysis. SIMCA models built up from MIR and NIR spectra, clearly discriminated insect by specie and amount of protein extract added. Quantitative prediction of protein extracts used to prepare the doughs showed good correlation (from 5 to 20%). This research has shown the potential of ATR-FTMIR and FTNIR spectroscopy combined with multivariate analysis to predict the amount of insect protein added into a complex food matrix.

Changes in the nutritional value of *Gryllus assimilis* and *Tenebrio molitor* depending on various culinary treatment

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The world population is currently rapidly growing and is estimated to reach 9 billion by 2050. Food demand is also expected to grow as the population grows. Therefore, searching for novel alternative food sources represents serious challenge for the future of humankind. One possible solution appears to be edible insects, which have little space and water requirements and reproduce rapidly.

The nutritional value of insects varies mainly depending on the species, sex and environmental factors. However, insects are a very rich source of protein.

Therefore, the protein content, fat content and amino acid composition of two species of edible insects, *Gryllus assimilis* and *Tenebrio molitor*, after various culinary treatments (without treatment, cooking, baking, drying and microwave heating) were determined. It was found that the content of individual nutrients does not change much depending on the treatment.

The protein content of *Tenebrio molitor* ranged from 29.8 to 32.2% and the fat content ranged from 54.9 to 56.7%. The most abundant amino acid was glutamic acid (6.17-6.51 g/100 g of sample), the most concentrated essential amino acid was leucine (4.03-4.30 g/100 g of sample) and the least tryptophan (0.64-0.69 g/100 g sample).

The protein content of *Gryllus assimilis* ranged from 63.7 to 67.7% and the fat content ranged from 18.1 to 23.5%. In both samples, methionine was identified as the limiting amino acid. The most abundant amino acid was glutamic acid (6.50-7.06 g/100 g of sample), the most concentrated essential amino acid was leucine (4.35-4.60 g/100 g of sample) and the least tryptophan (0.65-0.71 g/100 g sample).

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Insect lecithin extraction and potential application in oil-in-water emulsion

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The increase in the world population demands the inclusion of new food sources. Although edible insects represent a feasible alternative of protein and fats, consumer acceptance of these products remains low. Incorporating insects as an ingredient in food products might be a solution. Therefore, in this study, we investigate the extraction of insect lecithin and study its emulsion capacity in an o/w emulsion.

Lecithin was extracted from two insects, yellow mealworm (YMW) and black soldier fly larva (BSFL). The effect of starting material was studied (fresh vs dry). In addition, two extraction methods Palacios & Wang (2005) and ETHEX (Mirja et al., 2020) were compared and ultrasonic treatment was adopted to improve extraction yield. Furthermore, the emulsion capacity of these insect lecithins was explored. Emulsions (o/w) were prepared with ultrasound probe and its stability (by Lumisizer), particle size and surface charge were determined. The microstructure of these emulsions was observed by microscope and Cryo-SEM.

The extraction methods had s significant effect on lecithin yield but the effect differ between the studied species. ETHEX (Mirja et al., 2020) had lower extraction yield for YMW when compared with Palacios & Wang (2005). However, the reverse applied for BSFL. Similarly, ultrasonic treatment showed a different effect between the studied insects. For YMW, ultrasound helped to increase extraction yield in most samples. While for BSFL, it had no significant influence on Palacios & Wang (2005), and even negative effect on ETHEX (Mirja et al., 2020). Meanwhile, elimination of moisture in the matrix decreased lecithin yield in both YMW and BSFL samples. Therefore, the best extraction option might be Palacios & Wang (2005) with ultrasound for fresh YMW, while ETHEX (Mirja et al., 2020) for fresh BSFL.

Regarding emulsion capacity, for both YMW and BSFL, the emulsion made by lecithin extracted from dry material by ETHEX (Mirja et al., 2020) was most stable. A network of lipids was observed in both YMW and BSFL samples. Both YMW and BSFL lecithins have potential to be emulsifier alternatives of soy lecithin.

Prediction of insect lipids composition using infrared spectroscopic combined with multivariate analysis

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Insect lipids can contribute to human nutrition by supplying energy and essential fatty acids. Insect lipid fractionation, ingredient characterization, and bioavailability are required in view of the increase in insect production and demand of insect ingredients worldwide. Insect lipid crude is constituted of several types of lipids e.g., triacylglycerols, phospholipids, sterols, and glycolipids. Infrared (IR) spectroscopy is a conventional technique for the identification of chemical compounds, the spectrum of each compound is unique and is used as a “fingerprint” for its identification. When IR spectroscopy is combined with data analysis it becomes a rapid diagnostic technique.

Progress in miniaturization of vibrational spectroscopy components (micro-electro-mechanical systems (MEMS), optical components, wavelength selectors, and detectors) has allowed the development of portable or hand-held systems. These equipment are simple to use, require minimal or no sample preparation, for these reasons it increases operating speed.

The present study describes a new approach to predict the amount of lipids and the composition of the lipids present in commercial partially defatted edible *Tenebrio molitor* and *Alphitobius diaperinus* powders using portable near infrared and mid infrared spectroscopy combined with multivariate analysis.

Fatty acid composition analyzed as fatty acid methyl esters were analyzed using GC-MS. For FT-MIR analysis, were placed onto ATR diamond crystal and spectra were collected in the mid-infrared region (4000-800 cm^{-1}) using a portable spectrometer. For NIR analysis, insect powders were inside a polyethylene bag and spectra were collected (7750 – 3750 cm^{-1}). Multivariate analysis and data preprocessing was performed with Pirouette and Partial least squares regression (PLSR) model to predict quantify the amount and composition of lipids present in the insect powder.

This research has proved the potential of infrared spectroscopy coupled with multivariate analysis as a powerful method for the prediction of lipid amount and composition in insect powders.

Fatty acid composition of Black Soldier Fly Larvae reared on comparable diets of different origin

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Black soldier fly larvae (BSFL) growth depends on the nutrient composition of the diet. Diets with similar nutrient composition but different component composition may result in differences in growth and body composition. We analysed BSFL fed with chicken feed (C) or the Gainesville diet (GD) of different origin and respective frass to investigate whether subtle differences in diet composition may affect crude fat (CF) and fatty acid (FA) composition in BSFL. On day (d) 5 BSFL were transferred from local C (LC) to the experimental diets: local GD (LGD; CF 3.5%; 8 MJ metabolisable energy (ME)/kg dry matter (DM)), GD from Italy (IGD; CF 3.5%; 9 MJ ME/kg DM), LC (CF 2.4%; 12.7 MJ ME/kg DM), C from Belgium (BC; CF 4.9%; 11.6 MJ ME/kg DM). 150 BSFL were spread on 114 g of substrate (70% water:30% feed). The larvae were reared at 27.5°C and 70% humidity between d 5 and 8. From d 9 to 16 they were individually ventilated with 40 l humidified ambient air/h. Nutrient composition was determined in samples pooled per diet group. Fatty acids were analysed by gas chromatography-flame ionization detection. Body weight (BW) data was analysed with PROG GLM followed by Tukey test ($P<0.05$; n=6/diet). At harvest, BW was highest in the C groups (LC 198 mg, BC 212 mg), and higher than in the LGD (106 mg) and the IGD (122 mg) groups. The CF content was higher in the C (30%) compared to the GD groups larvae (22%). The FA pattern of the 2 feeds (LC v. BC; LGD v. IGD) and the BSFL fed on it did not differ distinctly. The C frass (1.3% CF) had 43% saturated FA (SFA) and 33% polyunsaturated FA (PUFA) compared to the GD frass (1.4% CF; 64% SFA, 17% PUFA). Cis-9,trans 11 conjugated linoleic acid was enriched in GD larvae vs. C larvae 32 fold, in GD frass vs. C frass (5 fold) and in GD frass vs. GD diet (8 fold). Vaccenic acid (C18:1cis-11) was enriched in frass compared to feed (GD 4 fold, C 3 fold). Differences in FA of frass are thought to be due to differences in microbiota.

Evaluation of the biochemical composition of the black soldier fly larvae (*Hermetia illucens* (L.)) and blow fly maggot harvested from poultry waste in Abeokuta, Ogun state, Nigeria

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Black soldier fly larvae (*Hermetia illucens* (L.)) are notable insect species that have been reported to have significant importance in nutrient recycling of waste and also as an essential ingredient in animal feed. Therefore, this study was carried out to evaluate the biochemical composition of the black soldier fly larvae and blow fly maggots naturally occurring in poultry waste in Abeokuta. The proximate composition of larvae samples were determined using Association of Analytical Chemists (A.O.A.C) method. Amino acids profile in larvae samples were determined using high Performance Liquid Chromatography. Vitamins (A, B, C, D, K) were analyzed by Spectrophotometric methods and gut digestive enzymes activities (lipase, proteinease, amylase, trypase, peptase, peptidase, lactase, maltase and invertase) of samples were assessed by standard methods. Minerals concentration (Ca, Na, Fe, Zn, P, Mn, and Mg) were analyzed by the Flame photometry and Atomic Absorption Spectrophotometry. Fatty acids profile was done using the Gas Chromatography. Data obtained were subjected to one way Analysis of Variance (ANOVA) using SPSS version 20. The crude protein, fat content, carbohydrate and energy values of the 6th instar of the black soldier fly were significantly higher ($p < 0.05$) than the 5th instar of black soldier fly and the blow fly larvae. Similarly, lipase and proteinease activities of the 6th instar of black soldier fly were significantly higher ($p < 0.05$) than the 5th instar of black soldier fly and blow fly larvae. Blow fly larvae had a significantly higher ($p < 0.05$) values of amylase activity. Significantly higher ($p < 0.05$) concentrations of vitamins B, C, D and E were recorded in the 6th instar of the black soldier fly larvae. Na, K, P and Zn concentrations were significantly higher ($p < 0.05$) in the 6th instar larvae of the black soldier fly. Amino acids and fatty acids concentrations were not significantly different ($p > 0.05$) in the 5th and 6th instar of the black soldier fly larvae and blow fly larvae. The evaluation of the biochemical composition has showed that the 6th instar (pre-pupae) of the black soldier fly have the highest concentration of fats, proteins, minerals and vitamins. Hence, may be considered beneficial as an alternative form of protein and feed in animal diets.

Utilization of biomass from house crickets into valuable ingredients

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House crickets are expected to play a significant role in the future agri-food systems due to their nutritional value, easy rearing process and already existing acceptance as food and feed. Their introduction to the food industry would often require the utilization of isolated fraction from the crickets that are rich in protein, fat, chitin or bioactive com-pounds. The isolation of these fractions from the crickets was performed by implementing emerging food processes like high pressure (200-500 MPa, 10 min) and ultrasound (25-50% amplitude, 5-10 min) as pretreatment for a single-step separation of fat and phenolics with a hexane/methanol solvent (1:1). Afterwards a deep eutectic solvent (betaine/urea, 1:2, 80°C) was used to further separate proteins and chitin. The process pathway was sufficient in separating the fractions. The fat yield was equal to 12.61±1.91%, without any effect of the pretreatments, while the phenolic content ranging between 528 and 732 mg GAE/100 g cricket meal, with a 37% increase of yield after the ultrasound pretreatment. Furthermore, ultrasound enhanced the ferric iron reducing power and the radical scavenging capacity of the phenolic fraction by 57% by 10%, respectively. Proteins and chitin were successfully separated using the deep eutectic solvent from both untreated and ultrasound pretreated samples. The protein fraction had a protein content of 90.32±6.38% and the chitin fraction had a chitin content of 77.44±4.41%. High pressure processing showed no significant effect on any step of the process pathway. It was therefore concluded that the suggested process is appropriate to utilize the cricket biomass with an enhancement of the quality of the phenolic fraction after ultrasound treatment.

Allergenic potential of cricket powder and the effect of *Yarrowia lipolytica* RO25

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There is still considerable uncertainty as to what extent primary sensitization to insects occurs that can lead to allergic reactions. Similarly, there is a need to clarify possible cross-sensitivities to arthropod species. However, it can be stated that only a few available food technology processes, such as fermentation and hydrolysis, are able to achieve a significant reduction in food allergenicity.

Thus, the principal aim of this research was to evaluate the allergenic potential of *Acheta domesticus*, to be used as innovative ingredient (i.e whole insect frozen, dried, powder) in food formulation. Furthermore, the ability of *Yarrowia lipolytica* RO25 to produce a cricket powder-based hydrolyzed with a reduced allergenic potential was evaluated.

The hydrolysed cricket powder was obtained by mixing commercial cricket powder with water (1:3 w:w) and *Y. lipolytica* RO25 inoculated at about 6 log CFU/ml. The identity of the relevant allergens (IgE Western blot), the relative IgE binding capacity (IgE ELI-SA) and finally the allergenicity (allergenic potency) in the cell test were determined on all the samples.

The preliminary results, regarding the samples of *Acheta domesticus* processed in different way and obtained by sera from sensitized donors or allergic to shrimp, showed that the allergenic power of the samples was clearly comparable to that of the total protein of shrimp. In particular, the cricket powder, which is the raw material for further technological transformation, clearly showed allergenic potential.

Finally, the results obtained from the analysis of cricket powder hydrolyzed with *Y. lipolytica* RO25, showed some effect of hydrolysis on allergenicity when compared with the non-hydrolysed samples.

These promising results suggest that the use of cricket powder-based hydrolysate to produce sourdough for breadmaking could be considered safe from an allergenic point of view.

Hazards and risks associated with the use of honey bee drone brood as food

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Honey bee drone brood as food according to Regulation (EU) 2015/2283 is a seasonal product obtained from an open system with the environment. This results in differences in extraction, use, associated hazards, and risks of consumption compared to other edible insect species in the approval process as novel food. The results of previous investigations have been summarized to provide an overview of possible microbiological hazards, allergenicity, and pesticide exposure in particular.

In current publications on microbiological investigations of drone brood, no amounts of microorganisms hazardous to the consumers could be detected. However, the presence of low levels of microorganisms (e.g. *Bacillus cereus*, coagulase-positive staphylococci, Enterobacteriaceae, *Escherichia coli*) underlines the need to comply with the general hygiene and control measures.

In addition to allergic reactions caused by cross-reactions in people that are allergic to crustaceans and dust mites, allergies may also triggered by remaining honey components such as glandular secretions, propolis components in the wax, nectar as well as pollen and should therefore be labeled.

Since honey bees feed their drones e.g. with honey and pollen, in addition to possible allergic reactions, there could also be an entry and accumulation of pesticides in drone brood. In this context, the results of own investigations for pesticide residues carried out in 2019 and 2020 are presented, in which 37 drone brood samples were analyzed by liquid chromatography-mass spectrometry. The samples were examined for the presence of 292 different substances (fungicides, insecticides, acaricides and herbicides) but no pesticide residues were detectable.

The statements of the publications and our results of the pesticide investigations provide a basis for both producers and official food control authorities for the use, safe marketing, and planning of investigations of these edible insect species.

Improvement of the nutritional value of Jamaican field cricket (*Gryllus assimillis*) as a result of the addition of carrots to the feed

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In this study, the effect of carrot addition to the feed on the composition of five groups (0, 3, 7, 14, and 60 days of adding carrot to the feed) of Jamaican field cricket tissue was investigated. First, metabolomic analysis based on ultra-performance liquid chromatography with high-resolution tandem mass spectrometric detection was performed. Using multivariate statistical analysis, it was possible to distinguish the groups of the Jamaican field crickets according to the composition of the feed.

In combination with target screening, the markers of the addition of carrots to the feed were found. Crickets fed by carrots contained more β-carotene (Vitamin A precursor, antioxidant) and its degradation products such as β-apo-14'carotenal and β-apo-12'carotenal. An increase in phytoene as a lycopene precursor was also detected.

Trends in lipid profile were also observed. The concentration of triacylglycerols with unsaturated fatty acids was higher in samples with the carrots in the feed. On the other hand, the concentration of saturated triacylglycerols was higher in samples of the control group. Changes in phospholipids and lysophospholipids profile were detected as well. There was a decrease in lysophospholipids in carrot-fed crickets. Various trends were observed across the studied samples for phospholipids. For example, phosphatidylcholine, phosphatidylserine, and phosphatidylinositol with 32 carbons and 2 double bonds were found to be on higher levels in carrot-fed crickets. Contrary to that, the content of phosphatidylglycerols, PC 34:2, and PC 34:1 in cricket tissue was lower after serving carrots to the studied crickets.

This study comprehensively describes cricket biomass changes after changing the feed composition, especially in terms of transfer of bioactive compounds and micronutrients, which are still relatively unexplored aspects of insect rearing. The results can contribute to the future optimization of edible insect breeding and the production of high nutritional quality products.

Insects for food and feed: microbiological and molecular methods for Salmonella detection verified according to ISO 16140-3:2021 standard

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Insects have been proposed as a high quality, efficient and sustainable alternative protein source for human and animal nutrition. In this perspective, their safety as food and feed becomes a relevant issue and can only be assessed with methods whose analytical performance is verified in the user laboratories. Currently, data on this is rather scarce and performance of food microbiology ISO standards for the detection and enumeration of food and feed pathogens in insect products is not yet available.

The aim of this study is to verify that the reference method ISO 6579-1:2017/Amd1:2020 for the detection of *Salmonella* spp. and the alternative validated PCR method iQ-Check Salmonella II, Biorad (NF Validation: BRD 07/06 – 07/04) perform according to the method's specifications on insects and insect products.

The verification was conducted according to the protocol and criteria included in ISO 16140-3:2021. Specifically, the protocol for the verification of food categories not included in the primary validation study was followed.

Two insect species were selected: *Tenebrio molitor*, approved in the EU for human consumption, and *Hermetia illucens*, which is largely used for animal feed. Two items were tested for each insect species: dried whole and powdered larvae.

The estimated LO_{D50} value (eLO_{D50}) was calculated for the four food items by inoculating the test portions with defined numbers of *Salmonella Typhimurium* ATCC 14028.

The eLOD50 value resulted 1.5 cfu/test portion for both dried whole and powdered larvae of *T. molitor*, 0.7 cfu/test portion and 0.5 cfu/test portion for dried whole and powdered larvae of *H. illucens*, respectively. The results obtained meet the requirements of ISO 16140-3: 2021.

When performed in the IZSLER laboratories, the ISO 6579 method and the iQ-Check Salmonella II PCR Detection Kit generate reliable results on insect and insect-based products; the methods were found to be fit for Salmonella safety criterion determination, as required by EU Regulation 2073/2005.

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The effect of technological treatment on microbiological changes of edible insect

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The placing on the market of dried, frozen and powder yellow mealworm (*Tenebrio molitor* larva) as a novel food under Regulation (EU) 2015/2283 has been recently authorized by the European Commission. Insects are consumed with their gastrointestinal tract and therefore their microbial risk should be considered. The aim of this study was to monitor microbiological and nutritional changes of yellow mealworm larvae during their heat treatment, namely cooking, roasting, drying and microwave heating. The effect of killing (boiling or freezing) was also monitored. From microbiological aspects *Bacillus cereus*, detection of Clostridium and total bacterial counts were determined.

Culture methods according to ISO standards for individual categories of bacteria were used for the detection of microorganisms. Killing by boiling alone had a significant effect on the decline of all microbiological indicators, in contrast to insects killed by frost. In the case of further processing, the best microbiological results were recorded in the insects, which were subsequently roasted. However, the smallest overall loss of microorganisms was observed in the microwave treatment, but it was still acceptable. To destroy the Clostridium, it was enough to kill the samples by boiling. In the case of *Bacillus cereus*, there was a significant decrease when killed by boiling, but the overall destruction of *Bacillus cereus* was only after the namely cooking.

Overall, it can be stated that if the insects are killed by boiling before culinary preparation, they provide sufficient microbiological quality regardless of further processing.

Determination of chitin in insect based products

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Insects are used as an alternative sustainable protein-rich compound in fish, pet, pig and poultry diets. Insect meals have a considerably good nutritional composition with regard to amino acids, minerals or fatty acids for feeding animals. However, the significant difference between insect meals and common protein sources is the content of chitin.

Chitin is an amino polysaccharide present in yeast cells, exoskeletons of insects and arthropod cuticles and is one of the most abundant biopolymers. In general, it is a homopolymeric chain of β-(1–4)-linked N-acetyl-D-glucosamine units, which has a linear fiber structure with a high amount of hydrogen bonds between the single chitin molecules. The polymer properties like solubility, bioavailability and reactivity are similar to cellulose. In the classical nutritional score analysis (Weende analysis) chitin is determined in the fiber fraction together with many other polymeric compounds. In addition, chitin also influences the determination of crude protein in the product. The nitrogen contained in chitin, which makes up approximately 13% of the chitin mass, is detected as protein in the analysis and therefore deludes the crude protein content in a faulty higher range. Furthermore, β-glucans in particular have been identified as active immunostimulating agents.

To reliably analysis the chitin content with simple methods is still a goal for production control. There are some easy manageable analysis techniques, which are based on classical chemical methods such as determination of fiber content, nitrogen content and photometry methods that can be used to determine chitin. All these methods can be modified to obtain the chitin content in the best way. One example of these modifications is the determination of fiber content after enzymatic hydrolysis to remove cellulose and starch. Suitable is also the determination of nitrogen in fiber residue. So all in all, the common methods without modification are not able to assess the chitin content separately without a prior isolation of chitin in an insect meal. In this poster we will show the problems and solutions for chitin analysis by classical chemical analysis methods.

Effect of tyrosine-supplementation on browning and emulsifying properties of protein fractions of yellow mealworm (*Tenebrio molitor*)

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Some insect species and their larvae are characterized not only by good nutritional value but also by promising technofunctional properties (e.g. foaming, gelling or emulsifying properties). Our research aims at the exploitation of insect proteins as technofunctional food ingredients. Corresponding studies concerning use of processed insects contribute to an increasing consumer acceptance.

A method adapted from literature established to extract proteins from soldier fly larvae, based on Osborne fractionation for grain proteins, could be optimized for *T. molitor* larvae, using the ground, freeze-dried and defatted insects. During fractionation, a browning reaction occurs especially in the aqueous as well as in the alkaline fractions, which is due to the reaction of phenoloxidases. Considering this background, the aim of our work was to investigate the influence of a tyrosine-supplemented diet (0.2 or 1.03% Tyr) on browning and emulsification properties of mealworm proteins. Furthermore, the influence of different premortal fasting periods (1 or 5 days) on above mentioned parameters was investigated, since an influence on phenoloxidase activity is suspected. Results are so far only available for the aqueous-derived Osborne fraction.

Enhanced browning was observed for the combination of tyrosine supplementation and prolonged fasting time. Without supplementation, the least browning response appeared (difference approximately 36%, determined by photometry at the same protein concentration).

To characterize the emulsifying properties, oil-in-water emulsions were prepared from aqueous solutions of the freeze-dried protein fractions and canola oil (3:1, v/v). Turbidity measurements of the emulsions showed that there is a negative correlation between the stabilized interface and browning, with maximum browning causing a reduction by the factor of 4 in stabilization.

Thus, it was revealed that supplementation of *T. molitor* with tyrosine enhances the browning that occurs in the aqueous isolates, and the enhanced browning decreases the emulsification capacity.

Effects of the incorporation of extruded mealworms on the nutritional and sensory properties of wheat bread

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Due to a growing population and a future food shortage, the exploitation of insects as an alternative food source is aimed by numerous research projects. However, in western countries, insects are often not accepted as food due to cultural reservations, especially if they are still visible. To overcome these limitations, insects are to be incorporated into conventional foods. The aim of this project was to investigate the impact of enriching wheat flour with insects on its baking properties and the nutritional properties of the bread made from it. The techno-functional properties of mealworms should be improved by means of extrusion.

In this study, freeze-dried and blanched mealworms (larvae of *Tenebrio molitor*) were used. The mealworms were extruded with two different extrusion parameters (different thermo-mechanical stresses), then mixed with wheat flour (20% mealworm content) and grinded. Breads were made from these flours. Flours with non-extruded mealworms and pure wheat flours served as controls. The entire process chain (freezing, blanching, drying, extrusion, grinding, baking) was investigated regarding protein digestibility, trace element accessibility and microbiological safety. The breads were evaluated for their sensory and flavor characteristics.

The protein digestibility of the pure mealworms increased through the extrusion process, compared to untreated mealworms. The addition of mealworms resulted in darker breads, which had an insect-like taste. A previous extrusion of the mealworms led to a more homogeneous pore distribution of the crumb. The levels of iron, calcium and zinc in bread increased by appr. 60%, 25% and 200%, respectively, when insects were added, regardless of whether they had been previously extruded.

This study will help to understand extrusion-induced changes in insects. This knowledge is important if insects are to be used as an ingredient in complex food systems.

SMARTinFOOD: Insect-based food sources to supplement nutrient deficiencies in vulnerable areas

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The food demand is estimated to increase by 70% by 2050 to feed a global population of over 9 billion according to the United Nations Food and Agricultural Organization (FAO). Worldwide, 0.8 billion people suffer from chronic hunger and more than 2 billion are deficient in relevant nutrients such as proteins, essential vitamins, and minerals. In Africa, about 60 million children under five are suffering from stunted growth and malnourishment while at least 10 million others are classified as overweight. Nigeria is placed among the countries displaying commitment to reduce hunger and improve health nutrition. However, it is one of the five large low-middle income countries where more than half of children under age 5 are either stunted. In South Africa, although regarded as food secure, 20% of households were estimated to have inadequate access to food and 6.8 million people experienced hunger in 2017.

The integration of a variety of new and alternative protein sources are needed to be exploited to develop and ensure more sustainable, resilient food and feed supply chains. SMARTinFOOD aims to address the circular bioeconomy and sustainable food and feed systems by researching technologies based on insect farming as the main vector for the valorization of agricultural waste. Insects require much smaller space, less water and less feed. Mealworms and crickets will be reared using different substrates previously identified in Africa and Thailand, respectively, and selected based on the characterization and availability. Next, the insect biomass will be processed into insect meals and formulated into appealing food products in Africa and feed in Thailand. The proposal SMARTinFOOD was awarded by WAITRO, the World Association of Industrial and Technological Research Organizations under the Framework of Food Security and Sustainable Agriculture to contribute to the UN Sustainable Development Goal 2 – Zero Hunger.

A multitrophic culture system for the production of black soldier fly larvae (*Hermetia illucens*)

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A multitrophic system involving mealworm larvae (MWL) and black soldier fly larvae (BSFL) was developed to transform kitchen waste into usable biomass.

MWL, fed mainly on kitchen waste, reached an average prepupal length of 2.4cm, fresh weight of 0.12g and protein and lipid content (dry matter) of 44.2% and 16.5% respectively, with an average specific growth rate (SGR) of 2.2%/day and a feed conversion ratio (FCR) of 7.9. The frass generated by the MWL was collected and subsequently fed to the BSFL for an average period of 21 days. On average each MWL, weighing about 0.13g, produced 0.14g of frass over an average period of 97 days.

Different batches of BSFL, fed on a variety of feeds, including the MWL frass, kitchen waste and oats, had an average prepupal length of 1.3cm, fresh weight of 0.16g and protein and lipid contents (dry matter) of 41.4% and 26.3% respectively, with an average SGR and FCR of 4.3%/day and 8.9 respectively. BSFL fed MWL frass only obtained some of highest SGR values and the best FCR, with one group achieving 7.5%/day and 2.9 respectively. The amount of frass generated by the MWL, a total of 646g, was capable of sustaining 253.0g of prepupal stage BSFL.

This preliminary investigation has demonstrated that a multi-trophic production system using kitchen waste-fed MWL frass to feed BSFL is feasible. Further studies should be carried out to see if the multitrophic concept can be exploited as a production approach.

Effect of acidulants on post-mortem off-color in BSF larvae (*Hermetia illucens*)

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Industrial insect farming has experienced rapid growth over the last decade, with the increasing number of new entrants globally. The surging demand for different forms of insect-based ingredients comes with logistical challenges. One of these challenges is the rapid darkening of fresh insects after the deactivation step. Although it is a harmless reaction and not necessarily linked to microbial spoilage, the off-color formation could lower the commercial value of the final product.

The post-mortem black pigment formation occurs rapidly after the deactivation of BSF larval biomass. It is mainly due to an oxidation reaction of phenolic compounds in the presence of transition metals (e.g., iron and copper), enzymes (phenol oxidase), and oxygen. It is also dependent on environmental storage conditions such as pH and temperature. The experiment aimed to assess the delay of black pigment formation of freshly deactivated BSFL biomass for at least seven days. Deactivated larvae were marinated in a water-based solution with the inclusion of citric acid and ascorbic acid and stored in a fridge at 4°C.

Results demonstrated that BSF larvae maintained the initial sensory characteristics after seven days of suspension in the water-based solution compared to the non-treated BSF larvae. The experiment showed the feasibility of preservation method for fresh insect larvae. However, there is still a need for studies on feasible, innocuous, and efficient processes to prevent or inhibit off-color formation in deactivated BSF larvae.

Increasing yields in maize production by application of insect frass and pupae shells of BSF

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International policy agendas, increasing prices, and environmental concerns of agricultural fertilizers has led to interdisciplinary research for new and innovative fertilizing products. Insect-based fertilizers represent promising alternatives to conventional fertilizing products while supporting sustainable value chains and boosting circular economy.

To assess the potential of insect-based fertilizers to substitute or replace conventional agricultural fertilizers, we tested different fertilizer combinations on the area of a commercially used maize field. Plants of each category were analyzed in terms of their height and weight, cobs weight, and grain weight.

Within our research, the application of insect-based fertilizers is linked with a significant growth of plant weight and cobs weight. This difference is particularly clear when insect frass is applied. The analysis of grain weight reveals, that the increasing yield is based on a higher weight of grain of maize. The associated increase in land use efficiency is an important contribution for the achievement of political objectives, because it allows farmers to introduce natural protection measures while maintaining the old yield level.

Our study confirms the high quality of insect-based fertilizers in central European climate conditions. Moreover, insect-based fertilizers have a huge potential to play a decisive role in the transformation of our agricultural value chains towards more sustainability, independence, and social goodwill.

Optimisation of heat treatment for black soldier fly (*Hermetia illucens*) and yellow mealworm (*Tenebrio molitor*) frass: compliance with EU regulation microbiological safety

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Industrial scale insect production generates large quantities of “residue” or “frass” consisting of larval faeces, unconsumed substrate and moulted skins. To assure the economic sustainability of insect rearing, this sidestream should be valorised. Evidence exists in literature it may be used as soil improver, fertilizer and health promotor in plant cultivation. Regulation (EU) 2021/1925 defines insect frass and sets standards for placing on the market of processed frass to allow its use as organic fertiliser. The regulation requires a heat treatment for 1 h at 70°C as the reference, after which the product needs to meet specific criteria for *Salmonella* and *Escherichia coli* or *Enterococcaceae*.

Yet, questions remain whether the selected time and temperature (e.g. 1h and 70°C) is the optimal combination to meet the microbiological criteria. Therefore, a number of heat treatments with different time-temperature combinations were executed on the frass from black soldier fly (BSFF) and yellow mealworm (TMF) rearing. To ensure correct application, the time-temperature profile of the coldest sample point was monitored. *Enterobacteriaceae* counts (indicator for *Salmonella* and *E. coli*) were determined before and after each heat treatment. All counts were below the detection limit of 1 log cfu/g for all tested heat treatments of BSFF, meeting the legal EU criteria. Despite comparable levels of *Enterobacteriaceae* for fresh BSFF and TMF, the reference treatment nor prolonged heating at 70°C reduced counts of TMF sufficiently. Only an elevated temperature of 80°C for 10 minutes was sufficient to reduce levels below detection limit (1 log cfu/g). A possible explanation for this could be the observed difference between both frass types in moisture content and water activity, respectively 43% and 0.92 for BSFF and 12% and 0.63 for TMF. Ongoing experiments will now explore a possible correlation between these physicochemical properties of frass and the reduction of its microbial load. Finally, the most effective treatments will be validated using frass inoculated with *Salmonella*.

Optimized production platform for the insect metalloproteinase inhibitor (IMPI)

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The increased use of antibiotics has led to a multidrug-resistance in several pathogens, resulting in a considerable challenge for the treatment of patients. An alternative therapeutic approach could be based on the use of antimicrobial peptides (AMPs). These small proteins and peptides are part of the innate immune system of many organisms and serve as a protective mechanism against pathogens by effectively preventing or inhibiting the reproduction and growth of bacteria, viruses, and fungi.

Here, we demonstrate the development of an economic platform technology for the expression of AMPs in bacteria, using the insect metalloproteinase inhibitor (IMPI) from *Galleria mellonella* as a model AMP. IMPI is a more complex AMP, which cannot be chemically synthesized, making it an excellent model.

The upstream is based on an optimized expression in Rosetta-gami, an *E. coli* strain, which allows improved disulfide bond formation and an enhanced expression of eukaryotic proteins with codons rarely used in *E. coli*. The expression took place in a fusion protein, containing, among others, a small C-terminal tag consisting of 4 amino acids (E-P-E-A), which supports both DSP and process analytics. A major advantage of this fusion tag is its small size. We were able to show that the activity of IMPI was not affected by the tag, so that it can be concluded that the tag can remain on the product and is, thus, available throughout the process for product monitoring. Furthermore, the C-tag influenced the expression behavior of IMPI in such a way that significantly higher yields were achieved in the USP.

In summary, we demonstrated a promising platform technology for the entire production process for different AMPs.

Toxins from insects as a source for valuable new therapeutics and insecticides

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Venomous insects and other arthropods produce a variety of bioactive compounds, which act very selective against physiological targets, including ion channels, receptors or enzymes. The tremendous diversity of venomous animals provides an indispensable biologic resource. Drugs developed based on venom components have the potential to play a key role in combating cardiovascular diseases, chronic pain, bacterial infections, or may represent ecofriendly insecticides.

Our group investigates insect venoms as a source for valuable new proteins by combining traditional approaches with actual 'omics' based techniques. We predict new candidates for potential applications in medicine or plant protection and produce these candidates in order to facilitate their biological profiling and the screening of their potential. We apply both solid phase synthesis for linear toxins and heterologous expression for complex toxins. The challenge for the recombinant production of those toxins is their small size, their high amount of disulfide bridges and their potential toxicity against the host organism. Therefore, we are investigating different production strategies in order to find a suitable strategy for our toxins. We produce them as inactive fusion proteins in *E. coli* followed by activation as well as in cell free expression systems. Subsequently, we screen the purified toxins regarding their bioactivity.

Insect toxins that show promising activities have the potential to be developed to novel therapeutic agents and bioinsecticides with a high degree of potency and selectivity.

Insect-based biopolymers for developing functional coating: effect on the quality of fresh-cut apples

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Edible biopolymers, such as polysaccharides, proteins and lipids, are biodegradable substance, made by renewable raw materials. When applied as coating on the surface of fruits and vegetables, they are able to extend shelf-life. House crickets, a largely studied insect species for being used as a new food source, is a protein rich material, with a protein extraction yield in aqueous solution of 20–40% and a purity of the extracted protein of approximately 60–75%. In this work an insect-based edible coating was developed by blending protein extracted by house cricket (PE) with chitosan (CH), one of the most used polysaccharides used for fruits and vegetables coating. The objective of the work was to evaluate the impact of the coating on the shelf life of fresh-cut apples. Two different edible coatings were obtained by mixing commercial low molecular weight CH with PE and dissolved in two different acid solution: 1% of acetic acid (AA) and plasma activated water (PAW). Developed coatings were applied on fresh-cut apples by dipping. Respiration rate ($RRCO_2$) and transpiration rate (TR) were measured at 60, 76, 86 and 96% of RH and 5°C of temperature. Chemical-physical and nutritional properties of coated and uncoated samples were evaluated during 13 days at 5°C. Results showed that coatings preserved the physiological properties of the fresh-cut apples reducing RRCO2 and TR, while chemical-physical properties were not affected. Antioxidant capacity and total polyphenol content recorded at the end of the storage period were significantly higher (+20% for both) when coating was applied. Such results suggest a great potential applicability of insect protein for developing edible coating. Further studies should be conducted in order to promote their large-scale use for preserving quality of fruits and vegetables, enhancing their shelf life.

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