Exploring the potential of house crickets (*Acheta domesticus*) in future agri-food systems

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Marios Psarianos, Potsdam 2023

Preface

This thesis is based on reformatted work, which has been published in the following peer reviewed publications and book chapters:

1. Ojha, S., Bußler, S., **Psarianos, M**., Rossi, G., & Schlüter, O. K. (2021). Edible insect processing pathways and implementation of emerging technologies. *Journal of Insects as Food and Feed*, *7*(5), 877-900.
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5. **Psarianos, M**., Fricke A., Altuntas H., Baldermann S., Schreiner M., Schlüter O. Effect of different illumination conditions on the production of house crickets *(final preparation)*
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7. **Psarianos M**, Ojha S and Schlüter OK (2023) Evaluating an emerging technology-based biorefinery for edible house crickets. Front. Nutr. 10:1185612
8. **Psarianos M**., Marzban N., Schneider R., Ojha S., Schlüter O. Methylene blue adsorption and bioactivity of chitosan produced from house crickets with novel methods *(under review)*
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10. **Psarianos, M.,** Baliota, G., Rumbos, C. I., Athanassiou, C. G., Ojha, S., & Schlüter, O. K. (2024). Insect processing for chitin production. In Insects as Food and Food Ingredients (pp. 129-143). Academic Press.
11. Rossi, G., **Psarianos, M**., Schlüter, O. K., & Ojha, S. (2024). Other applications of insects in the agrifood sector. In Insects as Food and Food Ingredients (pp. 215-235). Academic Press.

Abstract

Edible insects have been identified as an important resource for future applications in the food sector, due to their low environmental impact, high nutritional value and contribution to circular economy. House crickets are a species that shares these qualities, while being already consumed as food and feed in many parts of the world. Therefore, they are a considerable candidate to answer the need for resilience and sustainability of future agri-food systems. This need for sustainability can be addressed, also, with a variety of innovations in the food chains. One of them is innovative farming methods, which can reduce the use of farmland and facilitate the recycling of resources. Another one is the application of innovative food processes, with low environmental impact and high efficiency. The present work aimed to apply these innovations in food chains to house crickets and evaluate their efficiency and effect on the quality of the product.

The first step was to explore the possibility of introduction of house crickets to indoor farming systems that can host various organisms under the same facilities. For that purpose, artificial illumination was identified as a key factor. Therefore, led emitting diodes (LEDs) coupled with narrowband ultraviolet-b (UV-B) radiation at 285 nm was applied on a range of conditions (50-150 μmol $m^{-2}s^{-1}$) to the rearing procedure of house crickets. The artificial light system was found appropriate for application in the rearing of house crickets, due to their normal development. The narrowband UV-B radiation was found to have a positive effect on the crickets, because it improved their survival rate, but also increased their content in proteins and chitin. Furthermore, house crickets showed potential for being reared together with other organisms due to the rich mineral profile of the waste of their rearing process.

At a post-harvest stage, non-thermal processing was applied to facilitate the production of house cricket-based flour. In particular, pulsed electric fields (PEF) processing was applied as pre-treatment to fresh insects because it can disrupt cell matrices and facilitate conventional processes with a simultaneous quality improvement. The effect of PEF on the drying rate and quality of crickets was explored. Additionally, electrohydrodynamic drying (EHDD) was evaluated for replacing conventional drying of crickets, due to its low energy requirements. PEF was found to accelerate the conventional drying rate of house crickets, thus reducing energy consumption of the process, and to have a positive impact on the quality of cricket flour. PEF processing led to an increased antioxidant activity of the cricket flour, as well as an improved functionality and chemical quality. EHDD was not efficient in replacing the conventional drying process. However, when the two drying methods were combined, the duration of conventional drying can be significantly reduced, thus reducing energy consumption more than 50%.

The positive effect of PEF on the house crickets, in terms of enhancement of the product quality and the efficiency conventional processing led to the exploration of the effect of emerging food processes on extraction of intracellular compounds from the crickets. When PEF processing was applied in a range of conditions (4.9 - 49.1 kJ/kg), even by applying the mildest treatment (4.9 kJ/kg), the solid-liquid extraction yield of fat and proteins was significantly improved, while chitin yield was unaffected. This finding led to the further exploration of emerging food processes, e.g. ultrasound (US) and high pressure (HP), as a suitable pretreatment for the extraction of valuable compounds from crickets. HP and US were applied at different conditions to facilitate the valorization of house cricket flour into valuable ingredients via sequential extraction steps. Additionally, deep eutectic solvents (DES) were applied in this valorization process since they are considered green solvents. House crickets were appropriate for valorization into food ingredients (fat, phenolics, proteins and chitin) that were successfully recovered. US was found to increase the extraction yield of phenolic compounds, while the DES composed of betaine and urea and a molar ratio of 1:2 was suitable for separating proteins from chitin.

The recovery of chitin was an important finding, because chitin can be used to produce chitosan, which is a molecule with various applications. However, the chitin extraction method requires hazardous materials. Different alternative methods were tested for chitin extraction from house crickets, e.g. DES, enzymatic treatment, fermentation and microwave treatment. An alternative processing pathway was designed that implemented fermentation with *Lactococcus lactis* and enzymatic digestion with bromelain for isolation a chitin-rich fraction. This fraction was used to produce chitosan at a high yield (>85%), which was then evaluated for its possible applications. This chitosan was found to have a high antioxidant activity, antimicrobial activity against bacteria that are commonly found in foods and a high capacity for purifying wastewater from ionic dyes.

The present work highlighted the suitability of innovative methods for food production to house crickets for the purpose of generating products with improved quality and lower environmental impact and provided a significant contribution in including house crickets in future sustainable and resilient agri-food systems.

Zusammenfassung

Essbare Insekten sind aufgrund ihrer geringen Umweltauswirkungen, ihres hohen Nährwerts und ihres Beitrags zur Kreislaufwirtschaft als wichtige Lebensmittelquelle für zukünftige Anwendungen identifiziert worden. Heimchen sind eine Insektenart, die diese Eigenschaften hat und in vielen Teilen der Welt bereits als Lebens- und Futtermittel konsumiert wird. Deswegen sind sie ein geeigneter Kandidat, um dem Bedarf an Widerstandsfähigkeit und Nachhaltigkeit der zukünftigen Agrar-Lebensmittelsysteme gerecht zu werden. Diesem Bedürfnis nach Nachhaltigkeit kann auch mit einer Vielzahl von Innovationen in den Lebensmittelketten entsprochen werden. Eine davon sind innovative Anbaumethoden, die den Verbrauch von Land verringern und das Recycling von Ressourcen erleichtern können. Eine andere ist die Anwendung innovativer Lebensmittelprozesse, die geringe Umweltauswirkungen und hohe Effizienz aufweisen. Ziel der vorliegenden Arbeit war es, diese Innovationen in der Lebensmittelkette auf Heimchen anzuwenden und ihre Effizienz und Auswirkungen auf die Qualität des Produkts zu bewerten.

Der erste Schritt bestand darin, die Möglichkeit der Einführung von Heimchen in Indoor-Anzuchtsystemen zu untersuchen, die verschiedene Organismen in denselben Einrichtungen wachsen können. Für diesen Zweck wurde die künstliche Beleuchtung als ein Schlüsselfaktor ermittelt. Daher wurden Leuchtdioden (LEDs) in Verbindung mit narrowband Ultraviolett-b-Bestrahlung (UV-B) bei 285 nm unter verschiedenen Bedingungen (50-150 µmol $m^{-2}s^{-1}$) bei der Aufzucht von Heimchen eingesetzt. Das Kunstlichtsystem erwies sich als geeignet für die Aufzucht von Heimchen, da sie sich normal entwickeln. Die narrowband UV-B-Bestrahlung wirkte sich positiv auf die Grillen aus, da sie ihre Überlebensrate verbesserte, aber auch ihren Gehalt an Proteinen und Chitin erhöhte. Darüber hinaus zeigte sich, dass die Heimchen aufgrund des reichhaltigen Mineralienprofils der Abfälle ihres Anzuchtprozesses zusammen mit anderen Organismen gewachsen werden können.

Nach der Ernte wurde eine nicht-thermische Behandlung angewendet, um die Herstellung von Mehl aus Heimchen zu erleichtern. Insbesondere wurde die Behandlung mit gepulsten elektrischen Feldern (PEF) als Vorbehandlung frischer Insekten eingesetzt, da sie die Zellmatrix aufbrechen und konventionelle Prozesse bei gleichzeitiger Qualitätsverbesserung erleichtern kann. Die Wirkung von PEF auf die Trocknungsgeschwindigkeit und die Qualität von Grillen wurde untersucht. Zusätzlich wurde die elektrohydrodynamische Trocknung (EHDD) als Ersatz für die konventionelle Trocknung von Grillen ausgewertet, da sie nur wenig Energie benötigt. Es wurde festgestellt, dass PEF die konventionelle Trocknungsrate von Heimchen beschleunigt und daher den Energieverbrauch des Prozesses reduziert und sich positiv auf die Qualität des Heimchenmehls auswirkt. Die PEF-Verarbeitung führte zu einer

erhöhten antioxidativen Aktivität des Grillenmehls sowie zu einer verbesserten Funktionalität und chemischen Qualität. Die EHDD konnte das konventionelle Trocknungsverfahren nicht ersetzen. Durch die Kombination der beiden Trocknungsmethoden kann jedoch die Dauer der konventionellen Trocknung erheblich verkürzt werden, wodurch der Energieverbrauch um mehr als 50 % gesenkt wird.

Die positive Auswirkung von PEF auf die Heimchen in Bezug auf die Verbesserung der Produktqualität und die Effizienz der konventionellen Behandlung führte zur Erforschung der Auswirkung neuer Lebensmittelprozesse auf die Extraktion von intrazellulären Nährstoffen aus den Heimchen. Bei der PEF-Behandlung unter verschiedenen Bedingungen (4.9 – 49.1 kJ/kg) wurde selbst bei der mildesten Behandlung (4,9 kJ/kg) die Ausbeute von Fett und Proteinen bei der Fest-Flüssig-Extraktion erheblich verbessert, aber die Chitinausbeute blieb die gleiche. Diese Ergebnisse führten zur weiteren Erforschung neuer Lebensmittelverfahren, z. B. Ultraschall (US) und Hochdruck (HP), als geeignete Vorbehandlung für die Extraktion wertvoller Nährstoffen aus Grillen. HP und US wurden unter verschiedenen Bedingungen eingesetzt, um die Verwendung von Heimchenmehl zu wertvollen Inhaltsstoffen durch aufeinander folgende Extraktionsschritte zu erleichtern. Zusätzlich wurden in diesem Verwendungsprozess deep eutectic solvents (DES) benutzt, da sie als umweltfreundliche Lösungsmittel gelten. Heimchen eignen sich für die Verwendung zu Lebensmittelinhaltsstoffen (Fett, Phenole, Proteine und Chitin), die erfolgreich zurückgewonnen werden konnten. Es wurde festgestellt, dass US die Extraktionsausbeute an Phenolen erhöht, während das DES aus Betain und Harnstoff und einem Molverhältnis von 1:2 für die Abtrennung von Proteinen aus Chitin geeignet war.

Die Isolierung von Chitin war ein wichtiges Ergebnis, da Chitin zur Herstellung von Chitosan verwendet werden kann, einem Molekül mit verschiedenen Anwendungen. Die Chitin-Extraktionsmethode erfordert jedoch gefährliche Stoffe. Es wurden verschiedene alternative Methoden zur Chitinextraktion aus Heimchen getestet, z. B. DES, enzymatische Behandlung, Fermentation und Mikrowellenbehandlung. Es wurde ein alternativer Behandlungsweg entwickelt, der die Fermentation mit *Lactococcus lactis* und die enzymatische Behandlung mit Bromelain zur Isolierung einer chitinreichen Fraktion vorsah. Diese Fraktion wurde zur Herstellung von Chitosan mit einer hohen Ausbeute (>85 %) verwendet, das anschließend auf seine möglichen Anwendungen hin untersucht wurde. Es wurde festgestellt, dass dieses Chitosan eine hohe antioxidative Aktivität, eine antimikrobielle Aktivität gegen Bakterien, die oft in Lebensmitteln vorkommen, und eine hohe Kapazität zur Reinigung von Abwasser von ionischen Farbstoffen aufweist.

Die vorliegende Arbeit unterstreicht die Eignung innovativer Methoden der Lebensmittelproduktion für Heimchen, um Produkte mit verbesserter Qualität und geringerer

Umweltbelastung zu erzeugen, und leistet einen wichtigen Beitrag zur Anwendung von Heimchen in künftige nachhaltige und widerstandsfähige Agrarnahrungssysteme.

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List of abbreviations

Abbreviation	Description
DES	Deep eutectic solvents
EHD	Electrohydrodynamic drying
FAO	Food and agriculture organization
FCR	Feed conversion ratio
HBA	Hydrogen bond acceptors
HBD	Hydrogen bond donors
HP	High pressure
LED	Light emitting diode
MW	Microwave
PAR	Photosynthetically active radiation
PEF	Pulsed electric fields
UPA	Urban and peri-urban agriculture
US	Ultrasound
UV	Ultraviolet

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1. Introduction

1.1 Resilient and sustainable food systems

A sustainable food system is a food system that has a positive or neutral environmental impact (environmental sustainability), is beneficial to society (societal sustainability) and is profitable (economic sustainability) (Nguyen). A resilient food system is one that is considered prepared to withstand and recover from shocks, disruptions and the various possible futures (Tendall, Joerin et al. 2015, Moynihan, Avraam et al. 2022). Agri-food systems are facing a series of challenges, which create a need for sustainability and resilience in the future agri-food systems (Bhavani and Gopinath 2020, Boyacı-Gündüz, Ibrahim et al. 2021).

The main challenge is climate change and global warming, with the world heading towards a 2-3 ^{o}C of temperature increase, where even a 1.5 ^{o}C could lead to multiple tipping points around the globe (Armstrong McKay, Staal et al. 2022). East Asia in particular is expected to suffer more due the consequences of global warming of 1.5 and 2 ^{o}C above pre-industrial levels, in compare to the global mean (You, Jiang et al. 2022). Furthermore, the quality of the water and the soil are being degraded due to climate change (Gomiero 2016, Srivastav, Dhyani et al. 2021), leading to problems like land scarcity (Gomiero 2016). However, agriculture is, also, responsible for greenhouse gas emissions, like CO_2 and CH_4, due to crop and livestock production and land use at a percentage of approximately 20%, with a low decrease from 24% to 17% over the period between 2000 and 2018 (FAO:Faostat analytical brief 19) (Tubiello, Conchedda et al. 2021). Livestock production alone is responsible for a 14.5% of total greenhouse gas emissions (Gerber, Steinfeld et al. 2013, Toro-Mujica and González-Ronquillo 2021).

Hunger is another major problem of future agri-food systems. It has been estimated that in 2021 the number of people that faced hunger was between 702 and 828 million (an average off 768 million). This number is an additional 46 million people compared to 2020 and 150 million people more, compared to 2019, before to the Covid-19 global pandemic (UNICEF 2022). World hunger comes in addition to overpopulation, with the global population being expected to reach almost 10 billion people by 2050 (FAO 2017, Boretti and Rosa 2019) and climate change, which makes the feeding of higher populations even more difficult (Godfray, Beddington et al. 2010). Furthermore, the Covid-19 pandemic created many challenges due to disruption of food chains and its effect on livelihoods (Bhavani and Gopinath 2020, FAO 2020), considering that agriculture (crops, livestock, aquaculture, fisheries and forests) is the main source of income, employment and food for the poor, according to the Food and Agriculture Organization (FAO) (Nguyen).

The shock that the food systems went through due to the Covid-19 pandemic and climate change pose the need for resilience and sustainability in the future agri-food systems (Bhavani and Gopinath 2020, Boyacı-Gündüz, Ibrahim et al. 2021). FAO focuses its future strategic framework for 2022-2031 on climate change, together with elimination poverty and hunger (Carron, Brand et al.). The European Commission released the Communication on the European Green Deal with the aim of climate neutrality and economic growth and the ambition of net-zero greenhouse gas emissions in 2050 and a 55% reduction of greenhouse gas emissions by 2030 (Sands and Suttles 2022).

There are multiple proposed strategies to secure sustainability and resilience of agri-food systems. In this chapter, some of them are discussed; innovative agriculture and edible insets as future food.

1.1.1 Innovative agriculture: vertical and integrated farming

Vertical farming

Urban and peri-urban agriculture (UPA) refers to the cultivation, processing and distribution of food in and around cities (Bailkey and Nasr 1999). UPA offers several advantages due to its productivity, financial benefits and sustainability. Apart from generating income, UPA can help provide food and jobs in city areas, contribute to city ecology by reducing farmland, and secure urban sanitation (Baudoin and Drescher 2008, Bruinsma 2017). UPA includes several practices, e.g. vertical farming, where the combination of some of these practices can be applied as well (Specht, Zoll et al. 2019, Aschenbruck, Esterhuizen et al. 2020).

Indoor vertical farming is the cultivation of crops in vertically stacked layers under controlled environmental conditions (Birkby 2016). There are different categories of vertical farming, such as stacked horizontal farming surfaces with or without level rotation, multi-floor towers with each level isolated from the surroundings, balconies for crop production, cylindrical growth units or green walls (Beacham, Vickers et al. 2019). Vertical farming is suggested as a sustainable food distribution method in urban areas, depending on the location and the design (Al-Chalabi 2015), providing several environmental and social benefits (Kalantari, Tahir et al. 2018).

Vertical farming can be energy conserving, even by facing challenges such as providing light (Kalantari, Tahir et al. 2018). Natural light, as well as artificial, can be utilized as a lighting source (Germer, Sauerborn et al. 2011), with the artificial lighting being provided by light emitting diodes (LEDs) (Banerjee and Adenäuer 2014). LEDs can be turned on and off, based on the requirements of the organisms of the system, thus conserving energy (Perez 2014, Thomaier, Specht et al. 2015). Photosynthetically active radiation (PAR) for biomass conversion is an important parameter for the energy considerations (Despommier 2014, Perez

2014). Apart from reducing energy consumption, LEDs can facilitate plant growth (Agarwal and Gupta 2016) and increase the yield of valuable compounds, as shown for tomatoes (Dannehl, Schwend et al. 2021). LEDs can, also, be combined with artificial ultraviolet (UV) irradiation, which can trigger the production of plant metabolites (Schreiner, Krumbein et al. 2009) and positively affect their composition, e.g. phenolic content (Escobar-Bravo, Klinkhamer et al. 2017), thus producing healthier food. Further advantages of vertical farming is the reduction of farmland, since cultivation would be performed indoors (Kalantari, Tahir et al. 2018), and water demand by water recycling (Despommier 2010). Additionally, vertical farming is a production method that is resilient to climate change, since the organisms grown with indoor cultivation are protected from the environmental and seasonal conditions and possible natural disasters (Kalantari, Tahir et al. 2018).

Integrated farming

Integrated farming refers to the farming system for production of both crops and livestock on one farm (Soni, Katoch et al. 2014, Walia and Kaur 2020), e.g. the combination of aquaculture and hydroculture in order to produce fish and vegetables (aquaponics) (Love, Fry et al. 2015), aiming to reduce resources and recycle wastewater (Cohen, Malone et al. 2018). Among livestock, cattle is the one most commonly integrated, although other animals such as pigs, poultry, ducks, llamas, sheep, goats and rabbits (Hilimire 2011). Integrated agriculture includes different categories (Hendrickson, Hanson et al. 2008):

- Spatially separated: animals and crops are kept in separate parts of the farm, as for instance
- Rotational: animals and crops are grown in the same space, but not at the same time, as for instance
- Fully combined: animals grow underneath or between crops, as for instance

Integrated farming has many benefits (Martin, Moraine et al. 2016). One major benefit is the improvement of soil quality due to fertilization with manure or planting of pasture (Hilimire 2011). A properly managed manure can provide the soil with organic matter, macronutrients and minerals, reducing the requirement for input of external fertilizer (Russelle, Entz et al. 2007), as reported for cattle (Acosta-Martinez, Zobeck et al. 2004), sheep and broilers (Lowy 2009). Improved soil quality can lead, also, to an increased yield, as shown for the integration of cattle on corn production (Maughan, Flores et al. 2009), whereas pasture is pesticide-free and bees can exist around it (Sjödin, Bengtsson et al. 2008). Other advantages of integrated farming are the efficient land use and the pest and weed management of animals, like chicken (Hilimire 2011).

Integrated farming has, also, social benefits via the organizational coordination of farmers. These benefits are related to work management, social learning and social acceptance of agricultural activities (Martin, Moraine et al. 2016). A synergistical organization of work can reduce workload during busy periods, as for instance in Sweden where some farmers share equipment, tasks and workforce to improve efficiency (Andersson, Larsén et al. 2005). Additionally, integrating farming among different farmers can deepen collaborative work, as for instance in Denmark where livestock farmers that were selling manure to other producers formed additional collaborations with their manure partner (Asai, Langer et al. 2014, Asai, Langer et al. 2014). Finally, the sharing of knowledge via by complementarity and synergy can improve troubleshooting due to increased options and collective problem solving (Martin, Moraine et al. 2016).

1.1.2 Edible insects as food for the future

Diversity in protein sources is a major determinant of resilience in an agri-food system. Edible insects are expected to contribute to this diversity together with various protein sources, e.g. legume, algae and microbes (Kahiluoto 2020, Tzachor, Richards et al. 2021, Marrero and Mattei 2022). The consumption of insects by humans as food is called entomophagy and it is estimated that approximately 1900 insects species are edible, while approximately 2 billion people practice entomophagy worldwide (Van Huis, Van Itterbeeck et al. 2013). Entomophagy is reported to offer a variety of advantages in environmental, nutritional and social terms (Adegboye, Bawa et al. 2021, Lumanlan, Williams et al. 2022).

The major advantage of edible insects is their potential for application in circular economy (Figure 1) (Ojha, Bußler et al. 2020). A sustainable food cycle would include production, processing distribution and consumption of food, combined with waste management (Wunderlich and Martinez 2018). Edible insects are relevant to the management of waste (Ojha, Bußler et al. 2020). They show potential of bioconversion of waste to food products or biodiesel, as shown for black soldier fly (Surendra, Olivier et al. 2016, Jagtap, Garcia-Garcia et al. 2021), as well as other insects such as *Acheta domesticus, Alphitobius diaperinus,* Cockroaches*, Musca domestica and Tenebrio molitor* (Żuk-Gołaszewska, Gałęcki et al. 2022). Furthermore, insect consumption is suggested as a means for pest control (Yhoung-Aree, Puwastien et al. 1997). However, insect production generates its own waste, which includes frass, exuviae and uneaten feed, which is disposed on agricultural fields, due to its potential as fertilizer, as shown for black soldier fly (Salomone, Saija et al. 2017, Jucker, Lupi et al. 2020) and mealworm (Poveda, Jiménez-Gómez et al. 2019). This is a further indication of the potential of insects closing the cycle of circular economy (Ojha, Bußler et al. 2020).

Figure 1:Graphical depiction of a circular economy based on edible insects

One further advantage of edible insects is their low environmental impact, in compare to conventional livestock, such as pigs, poultry and cattle (Lumanlan, Williams et al. 2022). For instance, the life cycle assessment of the production of insects such as mealworm (Oonincx and De Boer 2012), black soldier fly (Smetana, Schmitt et al. 2019) and crickets (Halloran, Hanboonsong et al. 2017) have shown their low environmental impact. Furthermore, different insect species are suggested to require fewer resources, such as farmland and water and produce lower greenhouse gas and ammonia emissions in compare to conventional livestock (Oonincx, Van Itterbeeck et al. 2010, Oonincx and De Boer 2012, Van Huis 2013, Dossey, Morales-Ramos et al. 2016, Lumanlan, Williams et al. 2022).

The nutritional value of edible insects is another major advantage for their utilization in the food sector (Rumpold and Schlüter 2013). They contain a high fat and protein content with essential amino acids and minerals like calcium, iron and zinc (Adegboye, Bawa et al. 2021). The edible amount of some insect species, such as the house crickets, is reported to be higher than the one of conventional livestock (Van Huis 2013). Additionally, species like house crickets (Bawa, Songsermpong et al. 2020), mealworms and caterpillars (Rumpold and Schlüter 2013) are reported to contain higher amount of nutrients than conventional livestock, like energy, proteins, fat, calcium and zinc (Adegboye, Bawa et al. 2021). Insects are, also, a source of biologically active compounds, such as phenolic compounds (Nino, Reddivari et al. 2021), taurine and vitamins (Oonincx and Finke 2021) and bioactive peptides (da Silva Lucas, de Oliveira et al. 2020). Finally, insects contain chitin, which can be transformed into chitosan that is a biomolecule with antioxidant and antimicrobial properties (Mohan, Ganesan et al. 2020).

Entomophagy can, also, have a positive social impact (Adegboye, Bawa et al. 2021). Insects can be cultivated or collected from the wild. The technical skills and investment funds that are needed for producing insects, by either harvesting from the wild or rearing, are few. This makes insect production appropriate for vulnerable social groups, such as women and landless people in rural areas, as well as indigenous people (Van Huis 2013, Gasca-Álvarez and Costa-Neto 2022). Entomophagy is being practiced in countries of Latin America (Costa-Neto 2015) and South Africa (Hlongwane, Slotow et al. 2020) and there is an already existing indigenous knowledge about insect production among communities in these areas (Selaledi, Hassan et al. 2021). Insect consumption can, also, improve the diet of households and improve the quality of street food due to the insects' nutritional value (Halloran, Roos et al. 2017). Finally, insects can be used to improve the nutrition of populations with a consequential economic growth that can allow resources to be spent on social services (Adegboye, Bawa et al. 2021).

1.2 House crickets in the agri-food systems

House crickets (*Acheta domesticus*) are cold-blooded, nocturnal insects that belong to the order of *Orthoptera* and the family of *Gryllidae* (crickets). House crickets are omnivorous, have a length of 2-3 cm and a weight that can reach 1 g in adulthood. They reproduce asexually by laying eggs in moist environments and they need 8-12 weeks to reach maturity (Stärk 1959, Bellmann 2006, Orinda, Oloo et al. 2021). The body of the cricket is separated in three main parts: head, thorax and abdomen, which include body parts with different functions (Figure 2).

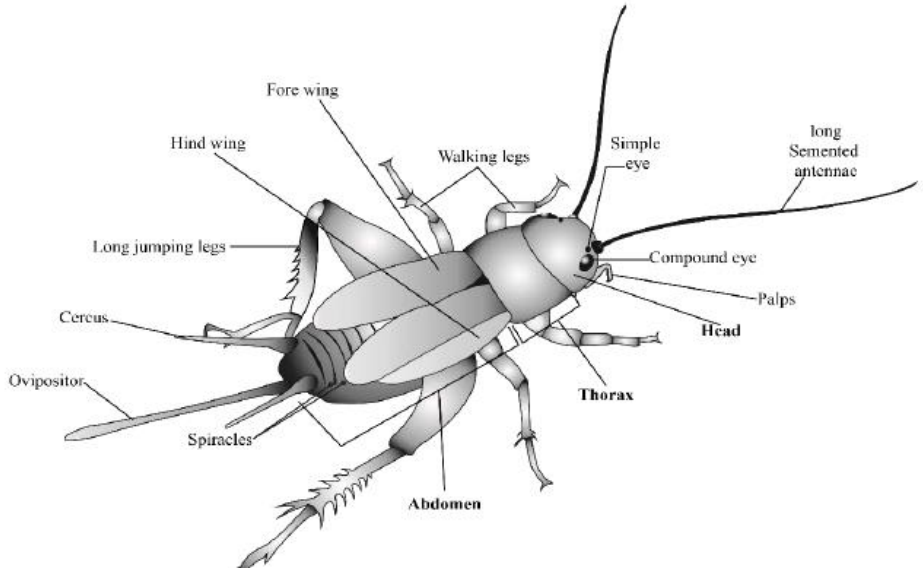

Figure 2: Anatomy of crickets, Picture: (Orinda, Oloo et al. 2021).

House crickets are hemimetabolous insects, meaning that they undergo an incomplete metamorphosis without a pupal stage. They hatch looking like adult crickets without wings. The life cycle stages of the house cricket include egg, nymph and adult (Figure 3). Egg hatching can last up to 2 weeks, with female crickets laying hundreds of eggs per day.

Temperatures between 28 and 35 ^{o}C are optimum for egg hatching and cricket rearing, which last longer under lower temperatures (Orinda, Oloo et al. 2021).

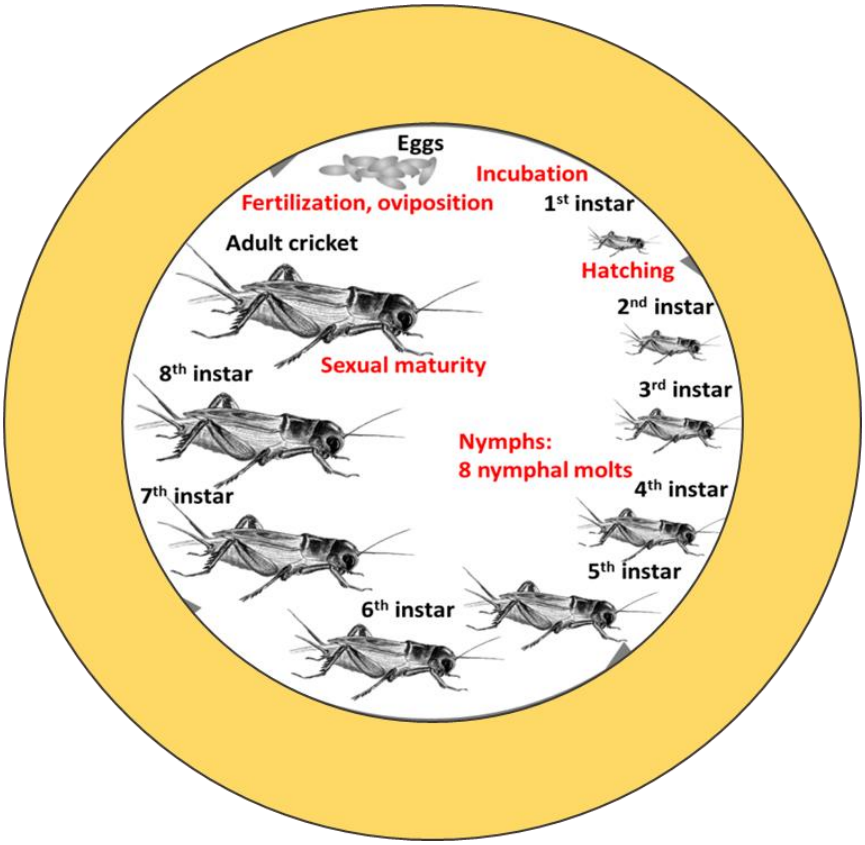

Figure 3: Life cycle of the house cricket

House crickets are edible insects and are strong candidates for inclusion in the future agri-food systems. The various aspects of implementation in the food sector are discussed below.

1.2.1 Environmental impact and nutritional value of house crickets

The main advantages of house crickets, which make them suitable for inclusion in the food sector, are their high nutritional value and their low environmental impact in compare to conventional livestock (poultry, pigs and cattle).

Their advantages over conventional livestock are identified in different aspects of their production. House crickets contain a high amount that can be utilized into food and feed (approx. 80% of them is edible), while for conventional livestock the edible amount is lower. In specific, it was estimated as 55% for pig and poultry and 40% for cattle (Nakagaki and Defoliart 1991, Van Huis 2013). Furthermore, house crickets are reported to have a higher production efficiency than conventional livestock. This efficiency is commonly estimated via the feed conversion ratio (FCR), which corresponds to the amount of feed that is provided to an animal over a lifetime divided by the gained weight of that animal. Lower values of FCR indicate higher efficiency (Fry, Mailloux et al. 2018). The FCR of house crickets can vary depending on the rearing conditions (Bawa, Songsermpong et al. 2020), but has been reported by several

studies to be lower than 2 (Veenenbos and Oonincx 2017, Bawa, Songsermpong et al. 2020, Sorjonen, Karhapää et al. 2022). Regarding conventional livestock, FCR is reported to range between 6 and 10 for cattle, 2.7 and 5 for pigs and 1.7 and 2 for chicken (Fry, Mailloux et al. 2018).

Furthermore, house crickets have lower resource requirements than conventional livestock. Regarding farmland, 2.5 cm^2 of surface is required for one cricket (Orinda, Oloo et al. 2021). This would correspond to 3.125 m^2 for 1 kg of protein, considering 0.4 g the weight of an adult house cricket (Clifford and Woodring 1990, Collavo, Glew et al. 2005) and 20% of protein in fresh weight basis (Finke 2002). However, for conventional livestock products, including cattle, pig, poultry, milk and eggs, the farmland requirements are higher than 50 m^2 for 1 kg of protein (De Vries and De Boer 2010). Regarding water requirements, house crickets require low water consumption, since they can get water from their feed (Van Huis 2013). Finally, regarding greenhouse gas emissions and ammonia emissions, house crickets are reported to produce more x1000 times lower emissions in compare to conventional livestock (Oonincx, Van Itterbeeck et al. 2010, Van Huis, Van Itterbeeck et al. 2013).

Regarding their nutritional value, house crickets have been identified as a source of various high value nutritional compounds (Oonincx and Finke 2021). They contain a high protein content that can reach the value of 70% in dry basis and a fat content that can reach the value of 22% in dry basis (Rumpold and Schlüter 2013). House crickets contain both essential and non-essential amino acids and their amino acid profile is unaffected by the their diet and life stage (Finke 2002, Oonincx and Finke 2021). Furthermore, house crickets have a rich fatty acid profile with both saturated and non-saturated fatty acids (Tzompa-Sosa, Yi et al. 2014), with the fatty acid profile being consistent for different life stages (Finke 2002). However, house crickets are able to synthesize, also, linoleic acid and linolenic acid (Borgeson, Kurtti et al. 1991), which is not possible for other insects (Oonincx and Finke 2021). Finally, several minerals are identified in house crickets, such as calcium, phosphorus, magnesium, sodium, potassium, chloride, iron, zinc, copper, selenium and manganese (Finke 2002, Rumpold and Schlüter 2013).

Edible insects are, also, a source of biologically active molecules (da Silva Lucas, de Oliveira et al. 2020), with house crickets being no exception (Oonincx and Finke 2021). The enzymatic treatment of house crickets has been shown to generate antioxidant peptides (Messina, Gaglio et al. 2019), while a diuretic peptide has been isolated from house crickets, as well (Coast, Wheeler et al. 1990). House crickets, similarly to other insects, contain, also, phenolic compounds (Nino, Reddivari et al. 2021, Nino, Reddivari et al. 2021), with the major phenolic compounds being 4-hydroxybenzoic acid, syringic acid, p-coumaric acid and ferulic acid (Nino, Reddivari et al. 2021). Flavonoids have been identified in house crickets as well, with apigenin

being the major one (Nino, Reddivari et al. 2021). Additionally, house crickets contain a significant amount of chitin (approx. 6% in dry basis), which can be deacetylated into chitosan that has antimicrobial properties (Malm and Liceaga 2021). Finally, house crickets contain vitamin A, C, E, B1, B2, B3, B5, B7 and B12 (Finke 2002, Rumpold and Schlüter 2013, Oonincx and Finke 2021) and are reported to synthesize vitamin D under UV-B exposure (Oonincx, Van Keulen et al. 2018).

1.2.2 Farming of house crickets

The first step in farming house crickets is the selection of the appropriate materials and conditions. Materials have to be affordable and the caging system should be spacious and convenient for the farmer, but also away from pesticides, secured from predators and away from food prone areas. Crickets are cultivated in rearing containers, e.g. boxes or buckets (Hanboonsong and Durst 2020, Orinda, Oloo et al. 2021). The rearing conditions are important as well. In order to grow normally, house crickets require a temperature of 28-35 ^{o}C, a relative humidity of 60-65%, photoperiod of 8 h and an available surface of 2.5 cm^2 per cricket (Fernandez-Cassi, Supeanu et al. 2019, Orinda, Oloo et al. 2021). They, also, require shelter, which usually is egg carton, and ventilation and a water source (McCluney 2008, Orinda, Oloo et al. 2021) (Figure 4). Providing the favorable rearing conditions is important for ensuring the normal growth of the crickets. For instance, a lower temperature can reduce growth rate and food consumption (Roe, Clifford et al. 1980). Furthermore, overpopulation should be avoided because it can compromise the rearing due to cannibalism or diseases (Orinda, Oloo et al. 2021).

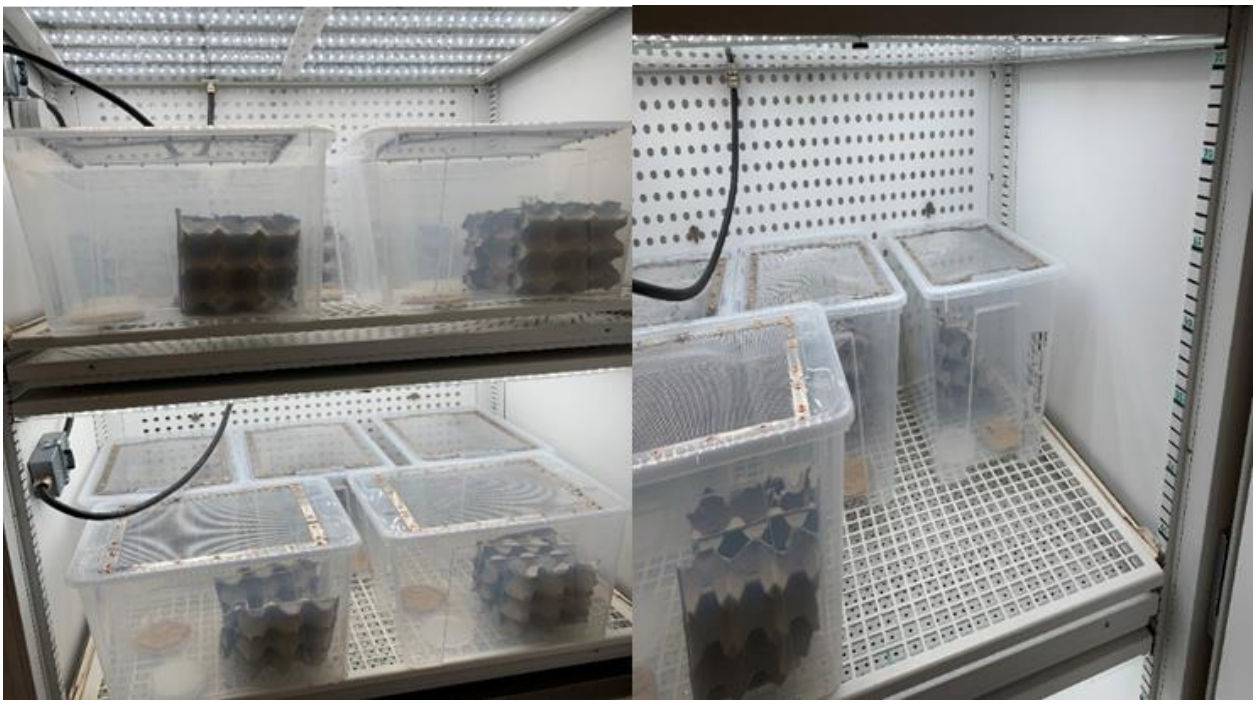

Figure 4: Rearing system of house crickets that was used during the experimental procedures of the present thesis (facilities at the Leibniz Institute of Vegetable and Ornamental Crops (IGZ)).

A commonly used rearing system is the pen system, with pens being constructed with cement and bricks, which allows minimal maintenance, mass production and safety from predators. However, it requires a lot of space, high costs and more land use (Orinda, Oloo et al. 2021).

In order for crickets to reproduce, an egg laying substrate should be provided. This substrate has to be sterilized and kept moist and covered with a mesh to avoid egg being eaten by male crickets. During the rearing cycle, hygiene has to be ensured to avoid contamination, diseases, inbreeding and pathogens. Attraction of predators, e.g. spiders, has to be considered, while the provided shelter has to be replaced (Mellberg and Wirtanen 2018, Hanboonsong and Durst 2020, Orinda, Oloo et al. 2021). All materials that are used in the cricket rearing should be decontaminated between rearing cycles (Hanboonsong and Durst 2020). House crickets can show a normal growth performance in different housing facilities (Orinda, Mosi et al. 2017), meaning that there is room for improvisation on the rearing system with more sustainable or efficient materials (Ngonga, Gor et al. 2021). The rearing system should be monitored regularly to report the growth performance and possible hazards (Hanboonsong and Durst 2020).

A rearing system can be continuous, with an egg laying substrate (e.g. soil) being placed permanently on the bottom of the rearing container or batch, with the egg laying substrate being placed in a separate container and replaced regularly (Orinda, Oloo et al. 2021).

House crickets require a nutritious feed with a protein content of 20% and the presence of minerals and carbohydrates (McFarlane 1964, Nakagaki and Defoliart 1991, Hanboonsong and Durst 2020). Nowadays, there is available commercial cricket feed, which can, however, be replaced by commercial chicken or fish feed. After the 15th days of age of the crickets, the feed can be updated with the inclusion of vegetables (Hanboonsong and Durst 2020).

Until recently, cricket farming was not as common as harvesting insects from the wild. However, in the last decades, cricket farming for food and feed production has become an emerging market. In Asian countries, it is more common to find small livestock farms, whereas several funding projects supported the establishment of cricket farming. On the contrary, in western countries cricket farming takes place on a more large industrial level, focusing on optimization of rearing and reduction of labor (Reverberi 2020).

1.2.3 House crickets as food and feed

Edible crickets are among the species that are commonly consumed in different parts of the world (Table 1) according to Magara et al. (Magara, Niassy et al. 2021). They can be consumed as fresh, frozen, dried, fermented, powder, but they can, also, be used as food ingredient or as basis for extraction of their nutritional compounds (Ojha, Bußler et al. 2021). House crickets

have been used as food ingredients in a variety of products, such as sourdough (Rossi, Parrotta et al. 2021), bread and cookies (Bawa, Songsermpong et al. 2020), extruded products (Igual, García-Segovia et al. 2020), snacks (Ribeiro, Cunha et al. 2021) and cereal bars (Ribeiro, Lima et al. 2019).

Table 1: Countries of the world, where edible crickets are consumed (Magara, Niassy et al. 2021)

America	United States of America
	Mexico
	Colombia
	Brazil
Africa	Kenya
	Zambia
	Guinea Bissau
	Sierre Leone
	Guinée
	Liberia
	Tongo
	Benin
	Nigeria
	Democratic Republic of Congo
	South Sudan
	Uganda
	Zimbabwe
	Niger
	Angola
	Tanzania
	Botswana
	South Africa
	Mali
	Congo/Congo Brazzaville
	Ghana
	Burkina Faso
	Cameroon
	Madagascar
	Central African Republic
	Papua New Guinea

Oceania	New Zealand
Europe	Netherlands
	Switzerland
	Belgium
	Poland
Asia	Thailand
	Philippines
	India
	Vietnam
	Indonesia
	Laos People's Democratic Republic
	South Korea
	Malaysia
	Cambodia
	Japan
	China
	Sabah
	Myanmar

Regarding EU in particular, house crickets were accepted as novel food pursuant to Regulation (EU) 2015/2283. They can be used as frozen or dried formulations, as well as defatted powder (EFSA Panel on Nutrition, Allergens et al. 2021, EFSA Panel on Nutrition, Allergens et al. 2022), while their risk profile as food has been evaluated (SLU, Sciences et al. 2018, Fernandez-Cassi, Supeanu et al. 2019).

House crickets are prone to microbial contamination (Fernandez-Cassi, Supeanu et al. 2019). Pathogenic bacterial such as *Enterobacteriaceae, Lactobacilaceae, Pseudomonaceae, Ruminococcaceae, Lachnospiraceae, Bacteroides, Clostridiaceae, Staphylococcaceae, Rikenellaceae, Bacillaceae, Porphyromonadaceae* have been identified in whole insects (Garofalo, Osimani et al. 2017, Vandeweyer, Crauwels et al. 2017), whole dried insects (Osimani, Garofalo et al. 2017) and cricket powder (Garofalo, Osimani et al. 2017, Osimani, Garofalo et al. 2017). Furthermore, yeasts and molds have been identified in house crickets, with a consequential content of mycotoxins, including several aflatoxins (Fernandez-Cassi, Supeanu et al. 2019, EFSA Panel on Nutrition, Allergens et al. 2021). Other chemical hazards of house crickets include dioxins and heavy metals, such as lead, cadmium arsenic and mercury. However, apart from the microbial growth, lipid oxidation is one further parameter that affects the self-life of house crickets (EFSA Panel on Nutrition, Allergens et al. 2022).

Furthermore, house crickets can be contaminated by parasites, anti-nutrients (SLU, Sciences et al. 2018, Fernandez-Cassi, Supeanu et al. 2019) and viruses, such as the Acheta domesticus densovirus (Liu, Li et al. 2011, Szelei, Woodring et al. 2011).

Finally, crickets can be a source of allergies, even though allergy on crickets based on food consumption is rare (Pener 2016). An allergy due to cross-reactivity of arthropods has, also, been suggested (Panzani and Ariano 2001), while allergenic reactions to entomophagy have been reported (Chomchai, Laoraksa et al. 2020, Ribeiro, Sousa-Pinto et al. 2021). However, due to a similar protein homology between crickets and crustaceans, which are known for causing allergies, an allergic reaction against crickets can be expected (Fernandez-Cassi, Supeanu et al. 2019). Crickets were among species that were consumed during a histamine poising due to entomophagy in Thailand (Chomchai and Chomchai 2018). Regarding house crickets particularly there are concerns regarding their allergenicity (De Marchi, Mainente et al. 2021), although the knowledge on the topic is scarce.

In order to ensure the safety of cricket (and insect in general)-based products, food processing pathways can applied to affect their properties (Ojha, Bußler et al. 2021).

1.3 Edible insect processing pathways

The purpose of insect processing is to ensure the quality and safety of the product and at the same time address consumer preferences (Ojha, Bußler et al. 2021). The processing pathways that are applied to edible insects are dependent on the species, possible hazards and desired form of the final product (whole, insect meal or extract) (Van der Fels 2015).

Prior to application of conventional food processing, the insects are harvested by separating them from their rearing substrate. Inactivation by freezing, blanching or asphyxiation usually follows harvesting, as well as washing and in some cases removal of legs and wings (Rumpold and Schlüter 2013). Also, in some cases, insects are starved prior to inactivation, since starvation can positively affect their characteristics (Garofalo, Milanović et al. 2019). The above mentioned processing steps apply to *Acheta domestica*, which are reported to go through starvation (Fernandez-Cassi, Supeanu et al. 2019). Furthermore, all inactivation methods can be applicable to house crickets, since none of them showed strong disadvantages related to the quality of the final product (Singh, Cullere et al. 2020). Harvesting, inactivation, washing and starvation are considered as pre-processing (Ojha, Bußler et al. 2021).

The production of whole insects is implemented to produce a number of products, which are usually chilled (≤5 ^{o}C), frozen (≤-18 ^{o}C) or dried and then packaged and stored. However, cold storage or freezing is not sufficient since they can only decelerate or delay microbial and chemical degradation (Kamau, Mutungi et al. 2020). A decontamination process is applied after pre-processing and prior to packaging such as blanching, cooking, steaming,

margination, smoking or drying. In the case of freeze-dried insects, pre-processing is followed by freezing and freeze-drying (Ojha, Bußler et al. 2021).

In some cases, insects need to be transformed into a homogenous material, which is achieved via milling. Milling can be applied to fresh or dried insects, in order to produce a paste or powder, respectively (Dossey, Tatum et al. 2016). Insect paste produced via wet milling is microbially unstable due to the high moisture content, as in the case of mealworms (De Smet, Lenaerts et al. 2019), when dry milling results in a dry powder with higher stability that can be stored in room temperature (Klunder, Wolkers-Rooijackers et al. 2012). Insect meal can be defatted or whole fat and defatting can be performed prior or post milling (Ojha, Bußler et al. 2021). The purpose of defatting of foods is to alter physicochemical properties of the final product (Son, Lee et al. 2019) or extend self-life (Temba, Njobeh et al. 2017). Fat is extracted with a variety of methods like chemical or mechanical, as shown for mealworms (Azagoh, Ducept et al. 2016, Son, Lee et al. 2019) or physical (Arsiwalla and Aarts 2015).

The previously mentioned processes find application, also, in house crickets, while influencing product characteristics in various ways. *Acheta domestica* can be stored at room temperature for a long time, when dried, due to their low moisture content and water activity (Kamau, Mutungi et al. 2018). However, a thermal treatment can be crucial in ensuring microbiological safety (Fröhling, Bußler et al. 2020). The reduction of total viable count, yeasts and molds of house crickets can be achieved by a combination of a thermal treatment like boiling and toasting with conventional oven drying (Nyangena, Mutungi et al. 2020). However, the drying method of house crickets should be chosen carefully, considering the overall quality and desired characteristics of the final product. For instance, solar drying cannot ensure microbial safety (Nyangena, Mutungi et al. 2020). Freeze-drying can cause a higher lipid oxidation than oven drying (Khatun, Claes et al. 2021), but ensure higher antioxidant activity and better functional properties of cricket flour (Lucas-González, Fernández-López et al. 2019). Microwave-drying is more efficient that oven drying in ensuring microbial safety and improving composition due to higher mineral and vitamin B2 content (Bawa, Songsermpong et al. 2020).

Edible insects can be further utilized in the food sector, by extracting their valuable compounds for industrial or household use. The extraction yield of the nutritional compounds is dependent on the species and the extraction method. The main fraction that are usually extracted are fat, proteins and chitin (Ojha, Bußler et al. 2021). In this way, one can address the disapproval of western consumers towards visible edible insects (Chen, Feng et al. 2009) and the need to create tailored and functional foods to address the malnutrition of a growing elderly population (Fernandes, Araújo et al. 2021).

Commonly used methods for chemical extraction of fat from house crickets are solid-liquid extraction, Soxhlet extraction, Folch extraction, aqueous extraction and extraction with

supercritical CO_2. Usually crude fat is recovered by removal of the solvent via evaporation (Tzompa-Sosa, Yi et al. 2014, Ramos-Bueno, González-Fernández et al. 2016, Laroche, Perreault et al. 2019). Protein extraction is performed with aqueous solvents with alkaline pH (Laroche, Perreault et al. 2019), since house cricket proteins have higher solubility in pH values between 10 and 12 (Udomsil, Imsoonthornruksa et al. 2019). Protein extraction from house crickets with water has been reported to have a low yield (Yi, Lakemond et al. 2013, Ndiritu, Kinyuru et al. 2017). Nevertheless, chemical solvents can negatively influence the functional properties such as emulsifying and foaming capacity (Ndiritu, Kinyuru et al. 2017). Proteins can be recovered with isoelectric precipitation (Laroche, Perreault et al. 2019). Chitin extraction from house crickets is performed by sequential removal of soluble fractions (delipidation, deproteinization and demineralization) and recovery of the insoluble chitin (Malm and Liceaga 2021), as in the case of various edible insects (Mohan, Ganesan et al. 2020).

Emerging food processing technologies, such as non-thermal processing, are applicable to edible insects and specifically house crickets (Ojha, Bußler et al. 2021). These processes have the ability to enhance or replace conventional food processes and affect food functionality. The fundamentals of the major of these processes and their applicability on edible insects and house crickets, in specific, are discussed in the following section.

1.4 Implementation of emerging technologies

1.4.1 Pulsed electric fields (PEF)

Pulsed electric fields (PEF) is a novel non-thermal food process. It refers to the repetitive application of high voltage (0.1-30 kV/cm) and short duration pulses (ms-µs) to a food product that is placed between two electrodes. The application of the electric field increases the transmembrane potential of the cell membranes of the product leading to the generation of pores in the cell membrane. This phenomenon is called electroporation. The applied field intensity has to surpass a critical value, for electroporation to take place and if it is too high then electroporation can be irreversible (Raso-Pueyo) (Figure 5).

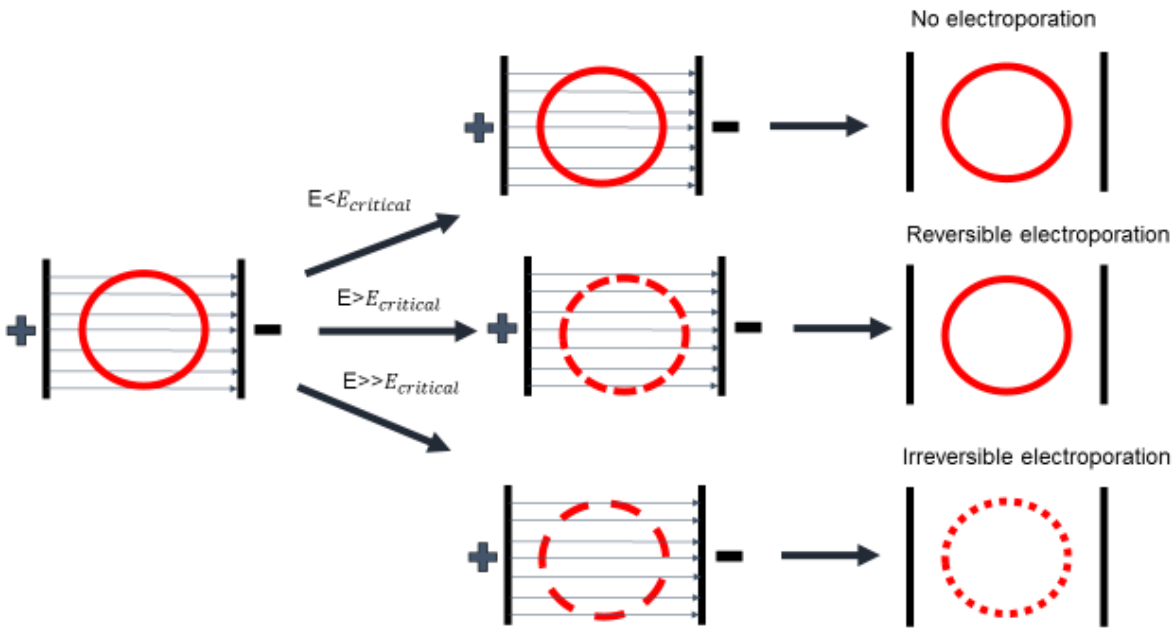

Figure 5: Graphical depiction of electroporation after application of PEF processing. Dashed lines represent cell membranes with the generated pores.

The mechanism of electroporation has been explained as a reduction in cell membrane thickness and thus pore formation due to attraction of oppositely charged ions in both sides of the non-conductive cell membrane (Teissie, Golzio et al. 2005). Another described mechanism is the reorientation of the membrane's lipid molecules, which generate hydrophilic pores with a difference in potential. This current produces Joule heating on the lipid bilayer. These phenomena rearrange the lipid bilayer, thus enlarging existing pores and creating new hydrophobic pores and stable hydrophilic pores. During the application of PEF, the electric current penetrates the pores and denatures all the protein bodies of the cell modifying their functionality (Saulis 2010).

The parameters that affect the efficiency of PEF application are (Toepfl, Heinz et al. 2006):

- the electric field intensity (kV/cm), which as mentioned has to surpass a critical value for electroporation to take place (Equation 1):

  E (kV/cm) = $\frac{V}{d}$ (Equation 1)

  where V (kV) is the applied voltage and d (cm) is the distance between the electrodes,
- the treatment time,
- the frequency, which refers to number of pulses per second,
- the pulse shape, which can be rectangular or exponential decay,
- the treatment medium characteristics, like pH, ionic strength, water activity, composition and conductivity.

Furthermore, the type of cell is important, since larger cells have higher transmembrane potential and require lower intensity for electroporation to take place (Knorr, Angersbach et al. 2001). Finally, the efficiency of the treatment depends on the specific energy (kJ/kg), which considers all above mentioned parameters and is described by Equation 2 (Raso, Frey et al. 2016):

$W_{specific} = \frac{n}{m} \cdot \int_0^\infty V(t) \cdot I(t) dt$ (Equation 2)

where n is the number of pulses, m (kg) is the mass of the sample and V(s) and I(s) are the voltage and current at time t(s), respectively.

PEF processing finds a variety of applications in the food sector (Barba, Parniakov et al. 2015). Cell disruption due to electroporation contributes to the quality and safety of food matrices via microbial inactivation and enhancement of conventional food processes as a result of an enhancement of transport phenomena between the intracellular and extracellular environment of the treated food material (Buchmann and Mathys 2019, Nowosad, Sujka et al. 2021). For instance, PEF processing has been applied for microbial inactivation, as in the case of milk (Sharma, Bremer et al. 2014) or blueberries (Jin, Yu et al. 2017).

Regarding enhancement of conventional processes, PEF treatment can reduce freezing time, as e.g. for apples (Parniakov, Bals et al. 2016) and increase freezing tolerance as shown for spinach leaves (Demir, Dymek et al. 2018). Additionally, PEF processing can significantly reduce drying time of foods, like basil leaves (Telfser and Galindo 2019) and carrot (Wiktor, Nowacka et al. 2016). Increasing the extraction yield of intracellular compounds of foods is, also, one of the main application of PEF processing (Barba, Parniakov et al. 2015). For instance, PEF has been shown to improve extractability of phenolic compounds for different materials, such as onions (Liu, Zeng et al. 2018) and olive pomace (Andreou, Psarianos et al. 2020). Additionally it can improve extraction of plant oils (Guderjan, Töpfl et al. 2005, Veneziani, Esposto et al. 2019), but also proteins for materials such as mussels (Zhou, He et al. 2017) or microalgae (Buchmann, Brändle et al. 2019). In some cases, PEF treatment was reported to reduce enzymatic activity, as for instance polyphenoloxidase activity of peach (Giner, Ortega et al. 2002) and apple juice (Riener, Noci et al. 2008). Finally, PEF has potential in reducing formation of food processing contaminants (Barba, Parniakov et al. 2015), like pesticides in apple juice (Chen, Zeng et al. 2009, Zhang, Hou et al. 2012).

Regarding edible insects, the application of PEF is limited. PEF has been shown to reduce drying time of *Hermetia illucens larvae* (Shorstkii, Comiotto Alles et al. 2022). Furthermore it has been applied for the bio-refinery of *Hermetia illucens larvae* and increased the amino acid content of the extracted fat, without affecting the fat yield (Alles, Smetana et al. 2020). Finally

PEF has been shown to cause cell disruption of Tenebrio molitor, enhancing the pressing extraction of fat (Smetana, Mhemdi et al. 2020).

1.4.2 Electrohydrodynamic drying (EHDD)

Electrohydrodynamic drying (EHDD) is a non-thermal drying technology that is based on convection of foods, making it particularly useful for fruits and vegetables. EHDD dehydrates foods by a direct use of electricity. The mechanism of EHDD refers to the generation of a high voltage difference (10-40 kV) between an emitter (a set of metal wires or a needle) and a collector electrode (a plate or metallic mesh). This voltage difference causes a discharge and therefore leads to the local ionization of air at the emitter electrode. The flow of the ionized air due to Coulomb forces, leads to elastic and non-elastic collisions with neutral air particles causing a secondary airflow (ionic wind) towards the collector electrode that has opposite polarity, which then dehydrates the food that is placed on the collector electrode via convection. (Singh, Orsat et al. 2012, Onwude, Iranshahi et al. 2021). The minimum voltage that is required for the discharge depends on a number of parameters, like curvature, distance between emitter and collector, space between emitters, ambient temperature and humidity (Mujumdar and Xiao 2019). Several water transport mechanisms are contributing to the dehydration during EHDD, as described for plant products. However, the dominant is convection to air and partially electroporation (Iranshahi, Onwude et al. 2022).

The efficiency of EHDD is evaluated by a number of performance indicators (Onwude, Iranshahi et al. 2021):

- Drying time (h)
- Drying rate calculated as:

  DR (kg H_2O/kg·s) = $\frac{dM_w}{M_d \cdot dt}$ (Equation 3)

  where M_w (kg) is the mass of the water in the sample at time t (h) and M_d is the mass of the dry matter of the sample (Berk 2018)
- Specific energy consumption calculated as:

  W (kJ/kg) = $\frac{P \cdot \Delta t}{M_w}$ (Equation 4) (Singh and Kumar 2013)

  where P (W) is the input power and Δt (h) is the time that the dryer operates
- Nutritional and quality characteristics of the product
- Scalability calculated as:

  Scalability (kg/h) = $\frac{M_d}{\Delta t}$ (Equation 5) (Walters, Bhatnagar et al. 2014)
- Cost index calculated as:

  CI (cost/kg) = $\frac{cost}{M_d}$ (Equation 6) (Tippayawong, Tantakitti et al. 2008)
- Greenhouse gas emissions calculated as:

GHGE (kg CO_2 equivalent/kg) = $\frac{M_{CO_2\ equivalent}}{M_d}$ (Equation 7)

where $M_{CO_2-equivalent}$ (kg) is the mass of the equivalent CO_2 (Tippayawong, Tantakitti et al. 2008)

The main advantages of EHDD is the low energy consumption combined with high scalability allowing it to be applied industrially (Martynenko, Bashkir et al. 2021, Onwude, Iranshahi et al. 2021, Onwude, Iranshahi et al. 2021). The specific energy consumption can be affected by a wide variety of factors such as (Martynenko, Bashkir et al. 2021):

- Electrical characteristics: voltage, current, electrode gap, polarity
- Emitter electrode characteristics: material, diameter, type (needle or wire), arrangement, spacing
- Collector electrode characteristics: material, type (plate or mesh), wires diameter
- Airflow characteristics: speed, direction distribution
- Environmental conditions: temperature, pressure, humidity
- Food properties: water content, thickness, porosity

EHDD has been applied to several food materials, e.g. apple slices (Martynenko and Zheng 2016), carrot slices (Ding, Lu et al. 2015), sea cucumber (Bai, Qu et al. 2013) and potato (Yu, Bai et al. 2018). However, the investigation of its application on foods is scarce. Furthermore, regarding edible insects, EHDD has never been applied.

1.4.3 High pressure (HP)

High pressure (HP) refers to the treatment of food materials with high hydrostatic pressure (100-1000 MPa) at controlled time and temperature conditions (Martínez-Monteagudo and Balasubramaniam 2016). HP can be applied to liquid and solid materials, both packaged and non-packaged (Balasubramaniam, Martinez-Monteagudo et al. 2015). During HP processing a food is placed in a sealed pressure vessel. The pressure transmission medium, which is usually water, is pumped into the container. Once the pressure inside the container has reached the desired level, pumping is stopped without further energy input to maintain the pressure during the treatment (Figure 6).

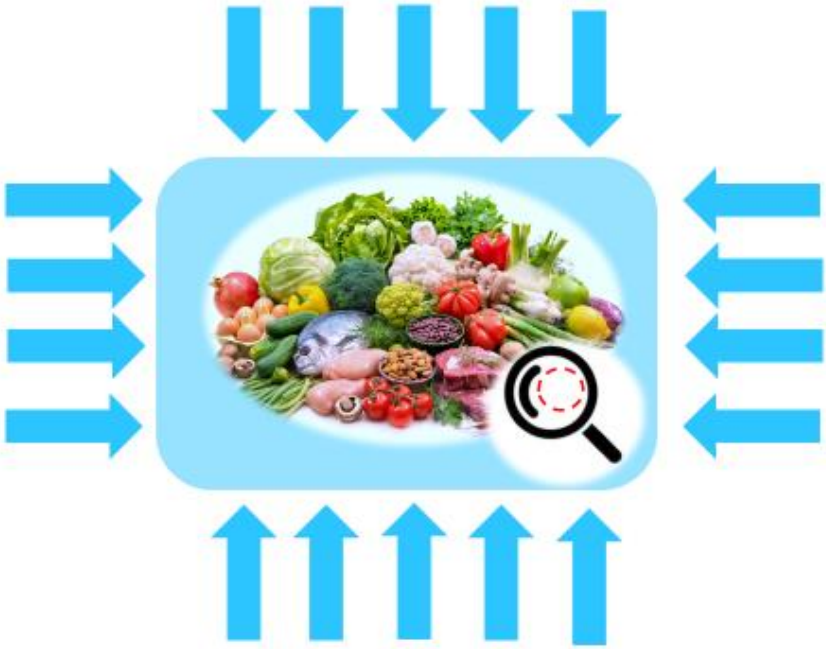

Figure 6: Graphical depiction of HP treatment of foods. Dashed red lines represent the disrupted cell membranes.

The main principles of HP processing are the following (Balasubramaniam, Martinez-Monteagudo et al. 2015, Balasubramaniam, Barbosa-Cánovas et al. 2016):

- Isostatic principle: the application of pressure is uniform and applied instantaneously and homogeneously in all directions regardless of the shape and size of the food. When pressure is not applied anymore, the food will return to its original state and shape. This is why non-porous foods with a high water content do not show alterations macroscopically due to HP. Due to the different compressibility of fluids (water and air) under pressure, foods containing air may change significantly in shape and size after HP treatment
- Le Chatelier's principle: This principle refers to the changes in equilibrium because of the application of pressure. Any process in which a decrease in volume occurs (increase in pressure and vice versa), the equilibrium shifts in a direction that tends to reduce the change caused (volume change). HP can affect interatomic distances, affecting distance-dependent bonds. For example, hydrogen bonds and van der Waals forces are distance-dependent and are influenced by pressure.
- Principle of microscopic ordering: at a constant temperature, an increase in pressure leads to an increase in the degrees of the ordering of molecules of a given substance. If the molar volume of an intermediate state (activated complex) differs from that of the reacting components, the reaction rate can be increased or decreased by changing the pressure, depending on whether the intermediate state is less or more bulky. This principle accounts for the effect of pressure in chemical and biochemical reactions.

Pressure is a thermodynamic parameter and changes in the pressure level leads to changes in the temperature and volume (Balasubramaniam, Barbosa-Cánovas et al. 2016, Martínez-Monteagudo and Balasubramaniam 2016). From a thermodynamic point of view this correlation of parameters can be described by Equations 8 & 9 (Balasubramaniam, Martinez-Monteagudo et al. 2015):

$G = H - T \cdot S$ (Equation 8)

where G is the free Gibb's energy, H is the enthalpy and S is the entropy

$H = U + p \cdot V$ (Equation 9)

where H is the enthalpy, U is the internal energy, p is the pressure and V is the volume.

Consequently, Equation 10 arises:

$d(\Delta G) = \Delta V \cdot dp - \Delta S \cdot dT$ (Equation 10)

The specific energy of the HP treatment can be estimated using Equation 11 (Toepfl, Mathys et al. 2006):

$W_{specific} = -\int p(v)\, dv$ (Equation 11)

where P (MPa) is the applied pressure and v (m^3/kg) is the specific volume.

The main factors affecting the efficiency of the HP treatment are the applied pressure, the temperature and the treatment time. The treatment time corresponds to the time of the pressure increase to the targeted value (with simultaneous temperature increase due to adiabatic heating), the duration that the food material will stay in the applied pressure environment and the time required for pressure reduction (with simultaneous temperature decrease). (Farkas and Hoover 2000).

One of the main applications of HP is pasteurization of foods via microorganism inactivation due to cell membrane disruption (Huang, Lung et al. 2014, Balasubramaniam, Barbosa-Cánovas et al. 2016). Cell membrane proteins are denatured and phospholipids become smaller, reducing the cell's ability to absorb amino acids. The high application of pressure causes mechanical stress on the cell membrane of the microorganisms, resulting in disruption of the cell membrane and therefore in cytoplasmic components leaking out of the cell, disrupting its vital functions and ultimately leading to its death. The resulting changes in cell morphology of microorganisms are reversible at low pressures, but irreversible at higher pressures where microbial death is due to rupture of the cell membrane (Balasubramaniam, Martinez-Monteagudo et al. 2015, Balasubramaniam, Barbosa-Cánovas et al. 2016). HP has been applied for the purpose of microbial inactivation in a variety of foods, e.g. mango juice

(Hiremath and Ramaswamy 2012), ham (Tassou, Galiatsatou et al. 2007) and fish slurry (Ramaswamy, Zaman et al. 2008).

Furthermore, HP can affect the activity of enzymes due to the rupture of non-covalent bonds and rearrangement and reforming in the surrounding solvent molecules (Balasubramaniam, Barbosa-Cánovas et al. 2016), leading to both enzyme activation (Eisenmenger and Reyes-De-Corcuera 2009) or inactivation (Chakraborty, Kaushik et al. 2014), as shown for plant proteases (Katsaros, Katapodis et al. 2009). Finally, HP has been shown to enhance the extraction of intracellular compounds from foods as a result of cell disruption, as shown for olive oil (Andreou, Dimopoulos et al. 2017) and phenolics from citrus peels (Casquete, Castro et al. 2014).

Regarding edible insects, HP has been applied to *Hermetia illucens larvae* for microbial inactivation (Kashiri, Marin et al. 2018, Campbell, Ortuño et al. 2020), as well as to *Tenebrio molitor larvae* (Rumpold, Fröhling et al. 2014). Furthermore, it has been shown to improve enzymatic hydrolysis of *Tenebrio molitor larvae* powder and reduce allergenicity (Boukil, Perreault et al. 2020), while it is observed to reduce enzymatic browning (Tonneijck-Srpová, Venturini et al. 2019). Furthermore, HP can improve the functional properties of proteins from *Protaetia brevitarsis seulensis (*Kim, Yong et al. 2021) and *Tenebrio molitor* (Boukil, Marciniak et al. 2022) cause alterations to hydrolysates obtained from *Gryllodes sigillatus* and *Tenebrio molitor* (Dion-Poulin, Laroche et al. 2020). Regarding house crickets specifically, HP can inactivate microorganisms on insect protein gels (Marin, Ibañez et al. 2020) and increase the phenolic content and antioxidant activity of the oil from house crickets and *Tenebrio molitor* but with a decrease in oil yield and oil oxidative stability (Ugur, Bolat et al. 2021). Finally, HP enhanced some of the functional properties, like antioxidant activity, protein solubility and oil binding capacity of defatted powder from house crickets and *Tenebrio molitor* (Bolat, Ugur et al. 2021). However, knowledge on the effect of HP on house crickets has room for further advancement.

1.4.4 Ultrasound (US)

Ultrasound (US) refers to sound waves at a frequency higher than the capacity of the human ear. Regarding food processing, the applied frequencies range between 20-1000 kHz. US is produced from ultrasonic transducers, which convert electrical energy to vibrational sound energy. US is generated through a medium causing a series of compressions and expansions at specific areas in the medium. The result of these pressure alterations is the formation of bubbles, which expand and then implode in the ultrasonically irradiated medium, generating a local pressure of 1000 atm and local heating up to 5000 K (Figure 7). This phenomenon is called acoustic cavitation (Abela, Sango et al. 2014, Ojha, Tiwari et al. 2018).

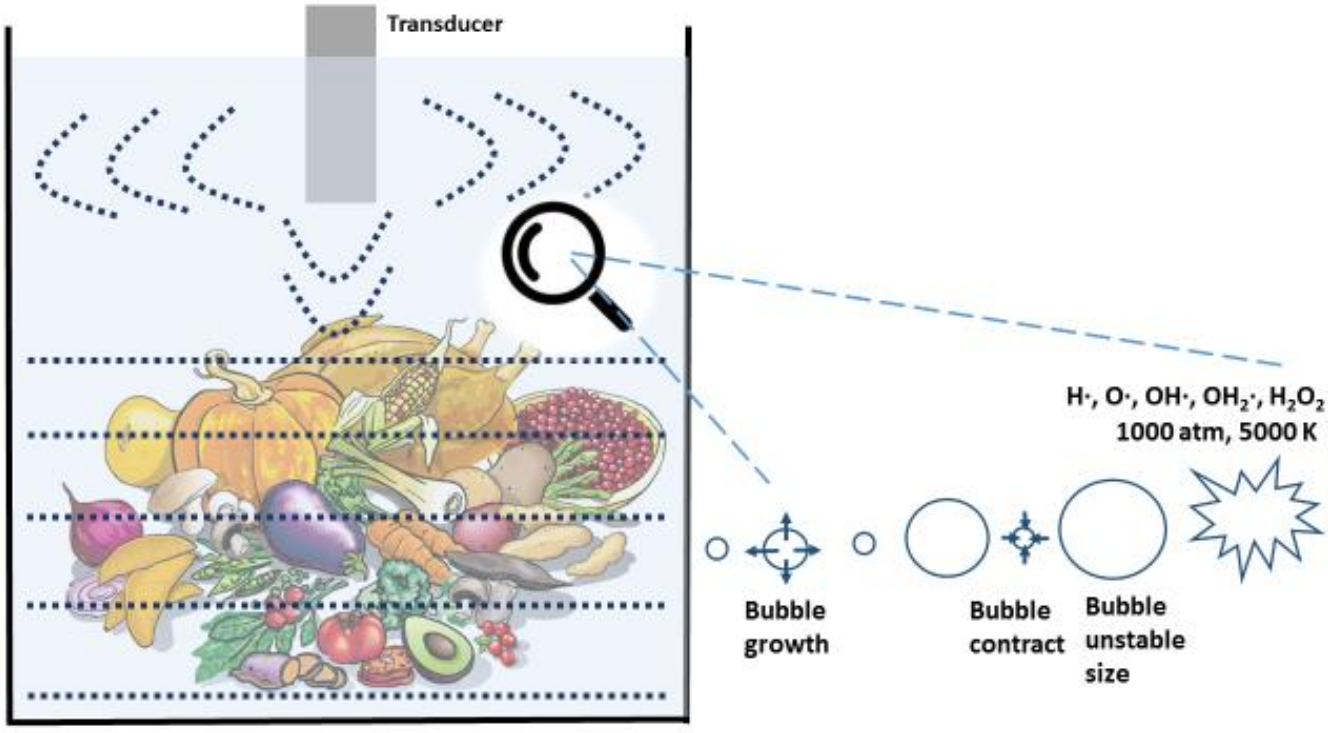

Figure 7: Graphical depiction of US processing of foods

The US frequency refers to the number of sound waves passing a stationary point per second. The wavelength is the distance of two identical points in the adjacent cycles of a waveform signal, while the amplitude is the maximum distance moved by a point on a vibrating medium from its equilibrium position (Williams 2012). Low-power US is applied at high frequencies (>100 kHz) as a non-destructive method for altering food properties and quality control. High-power US is applied at lower frequencies (<100 kHz) for cell disruption, tenderization, enzyme inactivation and emulsion formation (M. Sango, Abela et al. 2014, Ojha, Tiwari et al. 2018). The efficiency of US processing is evaluated by estimation of power, ultrasonic intensity and acoustic power density (Equations 12-14), with the US efficiency increasing when amplitude, treatment time and temperature increases (Abela, Sango et al. 2014):

Power (W) = $m \cdot c_p \cdot [\frac{dT}{dt}]_{t=0}$ (Equation 12)

where m (g) is the mass, c_p (J/g·K) is the specific heat capacity and $\frac{dT}{dt}$ (K/s) is the heating rate during sonication

Ultrasonic intensity (W/cm^2) = $\frac{4 \cdot P}{\pi \cdot d^2}$ (Equation 13)

where P (W) is the power and d (cm) is the diameter of the ultrasonic probe

Acoustic power density (W/ml) = $\frac{P}{V}$ (Equation 14)

where P (W) is the power and V (ml) is the medium volume.

US processing finds a wide variety of applications on the food sector (Awad, Moharram et al. 2012). The US-induced cell disruption by generated cavities inside tissues can lead to different applications, including microbial inactivation (Abela, Sango et al. 2014) and improved marination, pickling and extraction of nutritional compounds from a wide variety of food materials (Tiwari 2015, Ojha, Tiwari et al. 2018). Furthermore, US can improve heat transfer, thus having a positive effect on cooking, filtration and drying. US-induced cavitation is, also, able to positively influence mixing and homogenization of liquids, degassing and defoaming of foods, meat tenderization, freezing and crystallization (Ojha, Tiwari et al. 2018). Other applications of US processing include emulsification (Zhou, Zhang et al. 2021), altering of protein functionality (Su and Cavaco-Paulo 2021) and affecting enzymatic activity (Mawson, Gamage et al. 2011).

As mentioned, one of the major applications of US processing is extraction of nutrients from food materials, including plant, animal or marine matrices. For this purpose, US processing takes place either with a probe (Figure 7) or with a sonication bath. It can be applied as a pre-treatment, prior to the extraction, or as US-assisted extraction or combined with other food processing technologies, such as microwaves or enzymatic treatment (Ojha, Aznar et al. 2020).

Regarding edible insects, US processing has been used to facilitate protein extraction from different species, such as *Patanga succincta*, *Bombyx mori*, *Tenebrio molitor*, *Gryllus bimaculatus*, *Apis mellifera* larvae and pupae and adult *Schistocerca gregaria* (Choi, Wong et al. 2017, Mishyna, Martinez et al. 2019, Kingwascharapong, Chaijan et al. 2021). It has, also, been applied to enhance the lipid extraction from insects, such as *Alphitobius diaperinus L*., *Acheta domesticus* and *Tenebrio molitor* (Otero, Gutierrez-Docio et al. 2020, Gharibzahedi and Altintas 2022). US has, also been applied for obtaining bioactive extracts from *Acheta domesticus* and *Tenebrio molitor* (Del Hierro, Gutiérrez-Docio et al. 2020).

When applied to *Hermetia illucens*, US altered the characteristics of proteins, enhanced enzymolysis kinetics and increased the antioxidant activity of protein hydrolysates (Mintah, He et al. 2019, Mintah, He et al. 2019, Mintah, He et al. 2020). US can improve functional properties, as solubility and emulsifying capacity and antioxidant properties of proteins of *Patanga succincta* (Kingwascharapong, Chaijan et al. 2021), solubility, foaming and antioxidant capacity of protein hydrolysates from *Hermetia illucens* (Mintah, He et al. 2019), gelling properties and water holding capacity of proteins from *Hermetia illucens* (Kumar, Queiroz et al. 2022) and emulsifying capacity of proteins from *Tenebrio molitor* (Huang, Li et al. 2023). Finally, US has been successfully combined with enzymatic treatment to obtain antidiabetic peptides from *Tenebrio molitor* (Rivero-Pino, Espejo-Carpio et al. 2020).

1.4.5 Deep eutectic solvents (DES)

Deep eutectic solvents (DES) belong to the class of green solvents, among supercritical fluids and ionic liquids, sharing similar properties with ionic liquids (Smith, Abbott et al. 2014, Mišan, Nađpal et al. 2020). DES are composed from the eutectic mixture of hydrogen bond donors (HBDs) with hydrogen bond acceptors (HBAs), which have higher respective melting points (Figure 8) (Smith, Abbott et al. 2014, Hansen, Spittle et al. 2020). HBDs are usually ammonium salts or amino acids and HBAs are usually organic acids or carbohydrates (Mišan, Nađpal et al. 2020). The reduction of the mixture's melting point, in respect to the individual melting point of the HBA and the HBD, has been attributed to the shift of the charging due to the occurring hydrogen bond (Smith, Abbott et al. 2014).

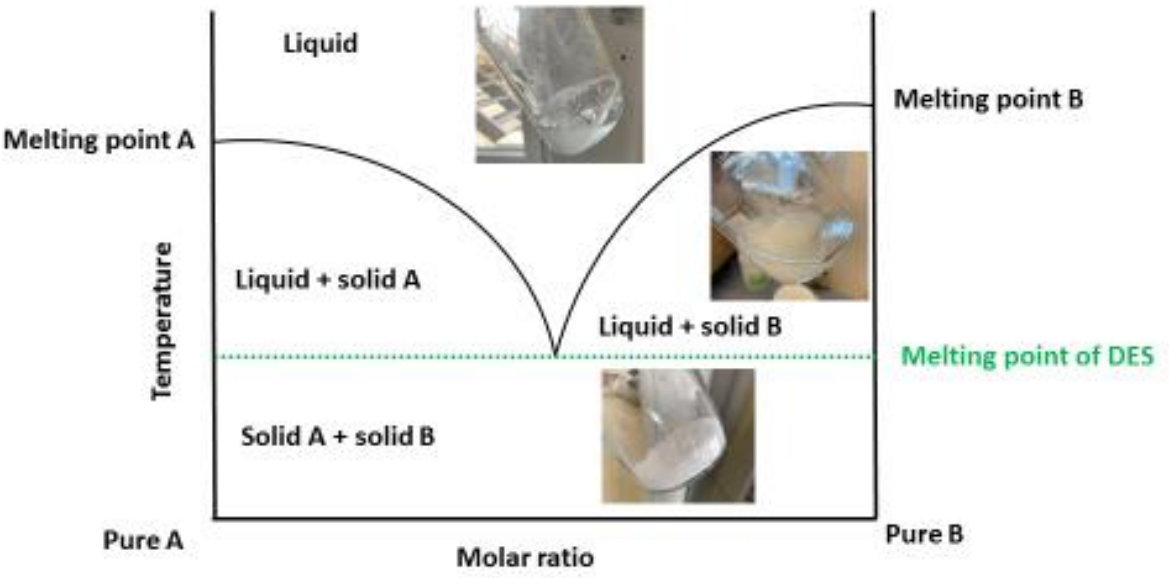

Figure 8: Phase diagram of DES based on the temperature and molar ratio of the components. Photos were taken during experiments with a mixture of Betaine/Urea.

Although DES have been described for the first time in 2003 (Abbott, Capper et al. 2003), they have obtained a growing interest in the latest years (Chen, Li et al. 2019). Their application in the agri-food sector is related to their advantages as non-toxic (Radošević, Bubalo et al. 2015, Halder and Cordeiro 2019), biodegradable (Radošević, Bubalo et al. 2015, Khandelwal, Tailor et al. 2016), cheap (Xu, Zheng et al. 2017) and easy to prepare (Hansen, Spittle et al. 2020).

DES are used as extraction medium of nutritional compounds from a wide variety of food matrices, food waste and biomasses. They have been thoroughly used for extraction of phenolic compounds, flavonoids, proteins, lignin, chitin, collagen, vitamins, pigments and metals (Chen, Li et al. 2019, Saini, Kumar et al. 2021). The processing pathway of extraction with DES starts with homogenizing a material with the DES to obtain a DES-based extract. This extract can be utilized directly or used to recover targeted compounds. This recovery

takes place with different methods such as liquid-liquid extraction, solid phase extraction, addition of water, extraction with supercritical fluid and crystallization. DES can remove, also, toxic compounds, like heavy metals, toxins and pesticides. In this case, the DES-based extract is eliminated. Finally, DES can be applied in bio-refineries, which are the conversion of a biomass feedstock into multiple valuable ingredients via a combination of processes (Mišan, Nađpal et al. 2020). DES are a promising method to isolate chitin, as well (Özel and Elibol 2021, Wang, Zhou et al. 2021, Li, Liu et al. 2022).

Regarding edible insects, they have been applied for chitin extraction from *Hermetia illucens* (Zhou, Li et al. 2019) and extraction of mycotoxins from *Acheta domesticus* flour (Pradanas-González, Álvarez-Rivera et al. 2021). However, their application to edible insects and house crickets specifically, is very scarce.

2. Thesis overview: motivation and objectives

Main hypothesis: House crickets are major candidates for introduction to future agri-food systems with sustainable practices. Rearing, inactivation and conventional food processing are the major steps in the processing pathways that are required for generation of cricket-based products. Their rearing, at a pre-harvest stage, and their processing pathways, at a post-harvest stage, were selected as the major parts of the production chain of cricket-based food ingredients. The potential compatibility of house crickets with innovative farming methods and the application of innovative and emerging food processes to house crickets were investigated to facilitate their integration in the future agri-food systems.

Objective 1: The crickets' easy cultivation process and low resource requirements give them potential for inclusion in vertical farming systems and co-cultivation with other organisms. However, the integration of house crickets in indoor co-cultivation systems has not been investigated. The first objective of the thesis was to evaluate the potential of house crickets for introduction in vertical farming and integrated farming by exploring their potential that are related to key aspects of these farming systems.

The artificial light sources, which are implemented in vertical farming, have a major importance due to their positive effect on the organisms that are grown with vertical farming. Additionally, a possible mineral-rich composition of the frass of the crickets would underline its potential as fertilizer. In order to introduce crickets in innovative cultivation systems, such as vertical farming and integrated farming, the interaction of the crickets with the artificial light sources such as PAR and UV-B, as well as the composition of their frass, were identified as key parameters that required investigation. The development of the crickets that were exposed to LEDs and UV-B irradiation was investigated. Since the existing research on this topic was

limited, the light treatment intensity was kept low to avoid possible harming effects on the crickets. Following the positive response of the crickets to the exposure to the LED/narrowband UV-B irradiation, the objective of further advancing the knowledge on the effect of this irradiation on the crickets arose. Therefore, the same irradiation system was tested at an elevated intensity, with monitoring their development and nutritional composition, aiming to reproduce irradiation conditions that are appropriate for other organisms. Additionally, the mineral composition of their frass was evaluated in order to confirm its suitability as a fertilizer for plant-organisms that can be cultivated together with the crickets.

Objective 2: The rich nutritional profile of the house crickets and their acceptance from the EU as novel food allows their utilization in a variety of food products. Cricket flour was selected as the focus of investigations, due to its invisibility in the final product, which would make it more acceptable to critical consumers and would facilitate the generation of tailored products. Emerging food processing technologies are environmentally friendly alternatives to conventional food processing. They can facilitate or replace conventional processes and positively affect food quality. However, their application on house crickets was limited and their effect on cricket-based products was poorly understood. The second objective of the thesis was to explore the potential of non-thermal processing for the production of cricket flour with reduced energy consumption and improved quality.

Pulsed electric field processing (PEF) was tested as a means to reduce conventional drying time and energy consumption, while electrohydrodynamic drying (EHD) was tested as a means of reducing energy consumption of the overall drying process. Both novel processing methods were evaluated for their effect on key quality aspects of the dried crickets. Both processes led to a reduction of the energy consumption of the overall drying process, while PEF had a significant positive effect on the quality of the produced flour. This finding led to the further exploration of the effect of PEF on the functional properties of the cricket flour, which showed an enhancement after the PEF treatment, as well.

Objective 3: One of the conventional processes that PEF treatment can improve is the extraction of intracellular compounds of food materials. Due to its high content in valuable nutrients, cricket flour is a considerable basis for extraction and isolation of nutrients. For this purpose several pretreatments can be applied in food materials, such as high pressure and ultrasound treatment, as well as green solvents and biological processes that are considered more environmentally friendly. The third objective of the thesis was to evaluate the potential of emerging technologies for the extraction of valuable compounds and the production of fractions from the cricket flour.

After observing the positive effect of PEF processing on protein solubility and considering its application for improving the extraction yield of intracellular compounds from food materials,

PEF processing was successfully implemented for improving conventional extraction of the main components of the house crickets, e.g. fat and proteins. b. The possibility of utilizing nutrients of house crickets could further advance by refining the cricket flour into its major compounds. Following the promising potential of PEF processing on fractionation of cricket flour, the possibility of applying high pressure (HP) and ultrasound (US) processing was explored. In order to reduce the environmental impact of this biorefinery, the possibility of replacing hazardous solvents with sustainable alternatives, e.g deep eutectic solvents (DES) was explored. One of the fractions that was generated from the bio-refinery was chitin. Chitin can be used to produce chitosan, which has a variety of applications due to its bioactivity and wastewater treatment capacity. Following the successful application of DES for isolating chitin, the applicability of environmentally friendly methods, including biological processes, to extract chitin from crickets was investigated and the produced chitosan was evaluated for its potential for application.

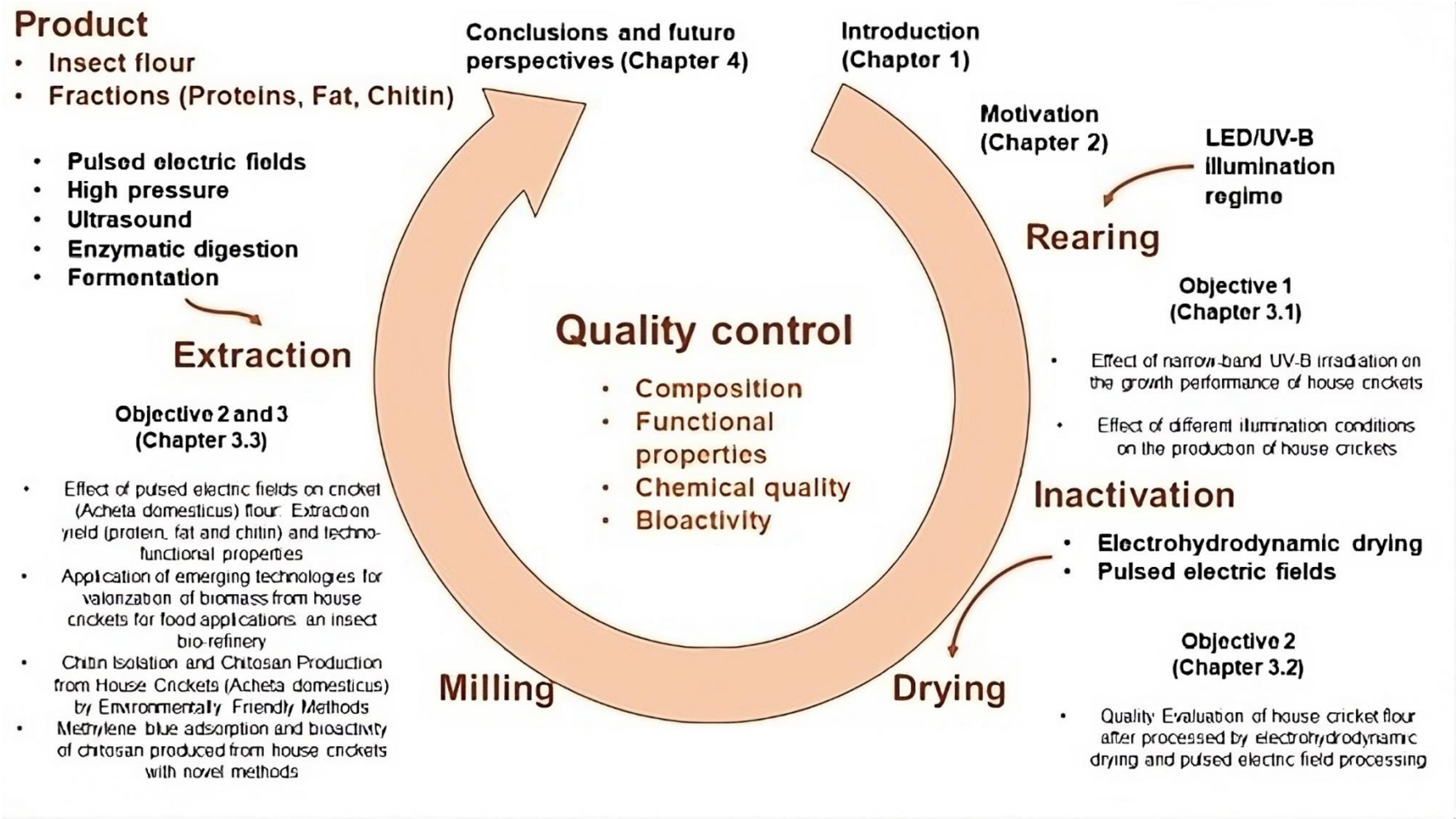

Figure 9: Graphical outline of the present thesis

3. Discussion

3.1 Implementation of a LED/narrowband UV-B irradiation regime in the house cricket rearing

There have been many innovations that have been proposed regarding the future agri-food systems. Some of them are related to farming, e.g. to reduce resource requirements due to indoor vertical farming or perform a better resource management and utilization with integrated farming. Another direction is to implement innovative protein and nutrient sources, e.g. edible insects, in the biodiversity of the future agri-food systems. Due to their low environmental impact and tradition of consumption as food worldwide, house crickets were considered for introduction in innovative farming systems.

The main parameter that would allow crickets to be included in such a system was the applicability of artificial light. The application of LEDs and UV-B irradiation on the cultivation of organisms, e.g. plants or algae, can have benefitial effects on them by triggering the production of certain metabolites. It can have, consequently, an effect on their growth and nutritional content. A PAR at a low intensity of 50 μmoles/ $m^{-2}s^{-1}$ of 6500K LEDs was applied on the rearing process of house crickets, after the 21rst day of rearing, with and without a narrowband UV-B radiation dose of 1.15 $KJm^{-2}d^{-1}$ of 285 nm. The effect of the UV-B exposure on the growth performance, e.g. weight and survival, as well as the composition of the crickets was evaluated. The crickets were unaffected from the narrowband UV-B radiation in terms of weight and composition. However, UV-B exposure did show a positive effect on their survival (%). Consequently, the same experiment was repeated but with an elevated PAR dose at 150 μmoles/ $m^{-2}s^{-1}$, which would better fit other organisms, e.g. plants. The positive effect of narrowband UV-B radiation at 285 nm was confirmed, with the maximum observed survival (%) being 78.18±2.29%. However, in this case, UV-B exposure had, also, a positive effect on the protein and chitin content of the crickets, while decreasing the fat content. The FCR was evaluated as well and was found unaffected by the light treatment and comparable or lower to conventional livestock. Finally, the frass of the crickets grown under different light treatments was evaluated for its potential as fertilizer and was found rich in minerals, e.g. Calcium, Potassium, Magnesium, Sodium, but also Sulfur. Therefore, it was concluded that house crickets have potential for inclusion in an indoor integrated farming system with other innovative edible organisms, e.g. plants and algae.

3.2 Application of non-thermal processing on the drying procedure for the generation of house cricket flour

The potential of applying non-thermal processing in the production of cricket-based products was explored. In that perspective, cricket flour was chosen in compare to whole crickets. The reason for that was that the invisibility of insects in food products would make their utilization easier, considering the disapproval of insects by some consumers for aesthetical reasons. Non-thermal processing was chosen because the reduction of temperature can positively affect the product quality. With that concern, PEF processing was considered as pre-treatment, because it has shown to accelerate the drying process of foods due to cell disintegration, including insects, and enhance the quality of food products. Additionally. EHDD was considered for replacement of the conventional oven drying of crickets, due to its potential of lower energy consumption, lower costs and low temperature during drying. After applying EHDD on house crickets, a moisture equilibrium was reached at a high moisture content of the crickets, making an additional step of over drying at 60 ^{o}C for 1 h necessary. However, the application of EHDD reduced the duration of oven drying and so the overall energy consumption more than 50%, while PEF did reduce the drying duration of over drying, as well, since equilibrium was reached faster. Therefore, PEF was, also, able to reduce energy consumption by approximately 14%. Additionally, PEF treatment was found to improve the protein solubility and antioxidant activity of cricket flour and reduce the content of histamine, which is an anti-nutrient, when combined with EHHD.

Following these findings, the potential of PEF processing on house cricket flour was further explored. Different PEF treatments at a range of SEC of 4.9-49.1 kJ/kg were applied. The effect of PEF on the flour's functional properties was initially explored. PEF was found to improve emulsifying and oil binding capacity, while leaving unaffected water binding and foaming capacity. The improvement of antioxidant activity was confirmed.

PEF processing was found to positively affect various product quality indexes, including bioactivity, e.g. antioxidant activity, functionality, e.g. emulsifying capacity and chemical properties, e.g. histamine content. Additionally, PEF can be successfully combined with conventional processes, e.g. drying and extraction, with a positive effect on the yield, duration and energy consumption. Based on these observations, PEF processing was considered appropriate for application in the production of cricket flour.

3.3 Application of novel food processing methods for the extraction of high value compounds from house crickets

Since PEF can be applied to improve the extractability of intracellular compounds of foods due to cell disruption, its effect on the extraction of fat, proteins and chitin from cricket flour was explored. PEF was found to increase the fat extraction yield and the protein extraction yield, even by applying the least intense treatment with a SEC of 4.9 kJ/kg. PEF did not affect chitin yield, however, because the process of isolating chitin is very exhaustive for the material and consequently PEF did not have an effect.

Considering the positive effect of PEF on the extraction of intracellular compounds of cricket flour, more emerging food processes were tested for enhancing extraction. In specific, HP processing and US processing were considered. US has been shown to positively affect the extraction of high-added value compounds from a variety of food materials, due to its effect on cell structure. Additionally, HP is a process that causes cell disintegration and has been shown to have potential for improving the extraction process of nutritional compounds from foods. These two processes were applied as pre-treatments to cricket flour at a range of conditions: HP at 200-500 MPa for 10 min and US at different amplitudes (25-50%) and processing times (5-10 min). A biorefinery process followed these pre-treatments. A biorefinery is the conversion of a biomass into valuable and functional products. In the case of house crickets, a biorefinery would be applied to recover their main nutritional components (fat, proteins and chitin), as performed for other insect species. Even though house crickets are not known to be a source of phenolics, they have been identified as one. Therefore, phenolic compounds, which are known for their health benefits, were considered in the biorefinery. Therefore, the need of designing tailored products and applying the beneficial components of the crickets directly would be addressed. An additional aspect that was considered, was the reduction of use of hazardous chemicals. The biorefinery consisted of two major processing steps. One was performed with a 3-phase system using hexane, water and methanol, where one phase would include the crude fat, one would be a functional phenolic extract with high antioxidant activity and the last one would be a protein and chitin rich fraction that would be used for further processing. The chemical solvent consisting of hexane and methanol was used in a range of volumes of 25-50 ml. The fat was recovered from the organic phase with hexane, whereas the hexane was recycled. The pretreatments did not affect the fat yield, but at the pathway where the higher volume of the chemical solvent mixture was used, US pretreatment did improve the extraction of phenolics with a consequential positive effect on the antioxidant activity.

Therefore, regarding the separation and recovery of proteins and chitin from the remaining fraction, only the two samples with the highest potential were considered. One of them was an

untreated sample that was homogenized with the lower volume of the chemical mixture (25 ml) and the other was an US treated sample at 50% amplitude for 5 min that was homogenized with the highest volume of the chemical mixture (50 ml). The remaining fraction was a pellet that was mixed with a DES, since DES are considered green solvents with potential for a successful chitin extraction from insects and crustaceans. The DES was a mixture of Betaine/Urea that was homogenized with the pellet of the two samples and two fractions were obtained. One was a protein-rich fraction with a protein content that reached a maximum of 90% and the other one was a chitin-rich fraction with a chitin content that reached a maximum of 77%, with differences between the two samples. Consequently, house crickets were considered an appropriate material for biorefining. All main components were successfully recovered at a yield that was considered acceptable. The applied process was easy and fast with a total duration of a few hours. Finally, the process was flexible among the two suggested pathways. One of them had the advantage of improving the quality of one of the fractions (the phenolic yield and antioxidant activity of the aqueous extract) and the other one had the advantage of lower energy consumption, due to the fewer processing steps, and lower amount of chemicals.

During the biorefinery process, one of the fractions that were recovered was chitin. Chitin is a valuable compound that is found in the exoskeleton of the insects, as well as in crustaceans and fungi. The importance of chitin is due to the product of its deacetylation; chitosan. Chitosan is biomolecule with a wide variety of applications in different sectors. Some of its main benefits are related to its antioxidant, antimicrobial and antitumor activity, while it finds application in medicine, dentistry and agriculture among many sectors. This process, even though it is successful for various insects, requires the utilization of hazardous chemicals (e.g. concentrated acidic and alkaline solutions), generating side streams that are not easily utilized and affecting the quality of the recovered chitosan. However, several alternatives have been suggested for chitin extraction from crustaceans, e.g. DES, fermentation, enzymatic digestion, MW treatment and green solvents. These alternatives were not explored much for edible insects or house crickets particularly. Therefore, all possible alternatives were applied to house crickets, at conditions that were considered optimum for other materials, and compared to the conventional method. Most of these methods were not as successful for removing proteins, as the conventional method for chitin extraction. However, all of them were comparable to the conventional method for removing minerals. An alternative processing pathway was constructed for chitin extraction using fermentation with *Lactococcus lactis* and digestion with bromelain as demineralization and deproteinization alternatives, respectively. This method was compared to the conventional one using a NaOH and an HCl solution for deproteinization and demineralization, respectively. The alternative method that implemented biological extraction steps resulted in a chitinous material with a chitin content of approx. 56%, in

compare to the conventional one that resulted in a 73% chitin content of the recovered material. However, the chitosan that was produced from both materials showed a higher purity (>80%) for both materials. The chitosan produced through the biological process pathway showed, also, better properties with respect to its applications, e.g. a low molecular weight of 86840 g/mole.

Due to the potential of this material, the possibility for its application was explored. Chitosan was produced from materials that were generated from cricket flour with the conventional chitin extraction method, the biological method and the chemical method enhanced by MW treatment in order to shorten the duration of the steps. These chitosans were compared to commercially bought chitosan. All chitosans showed a good antioxidant activity and antimicrobial activity against some commonly found pathogenic bacteria like *Escherichia coli, Staphylococcus aureus* and *Salmonella enterica ssp. Enterica Serovar Typhimurium*. The cricket-derived chitosan that was produced from all methods showed comparable results to the commercial one. All of them had low effective concentrations for both antioxidant activity (≤5.31 mg/ml) and antimicrobial activity (≤0.56 mg/ml). Chitosan and chitosan-based blends have been highlighted for their potential for wastewater treatment, as well. In particular, these materials have the capacity to adsorb methylene blue, among other ionic dyes that are considered water pollutants. Regarding the cricket-derived chitosan, all materials showed a methylene blue adsorption capacity at a dye concentration of 10 ppm. However, the chitosan that was produced from a chitinous material that was obtained with the biological processing pathway showed an exceptionally efficient methylene blue adsorption capacity. It was successful in removing the dye even at higher concentrations (up to 320 ppm) and had a maximum adsorption capacity of 643.899 mg/g, which is the highest one ever reported for a chitosan-based material.

4. Conclusions and future perspectives

Modern agriculture and agri-food systems are facing a variety of challenges and they are called upon to address them. One the one hand, there is climate change that leads to global warming, land scarcity and degradation of water quality. Climate change is coming to complete in the already existing problem of world hunger and expected rise in future global population. One the other hand, there are system shocks, e.g. the recent Covid-19 pandemic, which can disrupt food chains.

These challenges have to be considered in the conceptualization of the future agri-food systems, which need to be resilient, biodiverse and environmentally sustainable. Resilient agri-food systems are the ones that can withstand and recover from the shocks. Sustainable agri-food systems are the ones that have a positive or neutral effect on the environment.

The present work aimed to explore these possibilities. The production chain of food products based on house crickets was considered both in pre- and post-harvest steps. Several agri-food chains were suggested including house crickets that were reared with innovative farming methods and processe with emerging food processing technologies.

At a pre-harvest stage, house crickets were shown to be adaptive to vertical farming supported by LEDs and UV-B irradiation. The LED-UV-B irradiation was appropriate for application in the cricket rearing. The UV-B irradiation positively affected the survival rate of crickets at a range of PAR intensities, whereas at higher PAR intensities the protein and chitin content can be increased due to UV-B exposure. The frass that was collected throughout the rearing was evaluated and confirmed as a mineral-rich material that can potentially be utilized as fertilizer.

At a post-harvest stage, EHDD was successfully implemented to produce cricket flour with a major reduction in the energy consumption. PEF was implemented as a pretreatment and was efficient in reducing the drying time and energy consumption of the production of cricket flour. Additionally, the application of PEF on crickets had a positive effect on the key quality parameters and functional properties of the cricket flour.

Cricket flour was identified as a valuable source for extraction of valuable nutrients and for biorefining to high value fractions. PEF and US were efficient in improving the extractability of fat and proteins (PEF) and phenolic compounds (US), respectively. Following environmentally friendly methods, such as green solvents and biological processing, e.g. fermentation and enzymatic treatment, it was possible to isolate a chitin rich fraction from the cricket flour and produce chitosan. This chitosan was shown to have a important bioactive properties and be particularly useful in wastewater treatment to reduce the amount of methylene blue.

Future perspectives

House crickets showed adaptability to rearing conditions that would allow them to be included in an indoor integrated farming system, with UV-B radiation positively affecting their survival and composition. These findings allow the rearing of crickets with different organisms simultaneously in order to observe their possible interactions and affected growth performance and composition. This system should be evaluated in terms of energy consumption and profitability, while the included organisms should be further evaluated for their consumer acceptability. Additionally, the possibility of using food waste as feed for the crickets should be investigated.

Regarding the processing of harvested insects, PEF treatment can have a positive effect on the oxidative stability and microbial load of foods and therefore its potential on the self-life of the crickets should be evaluated. Considering the application of PEF on reducing contaminants

and the promising preliminary result of reducing histamine, PEF can be also applied on house crickets to assess their chemical safety.

Regarding US and HP, both of them show a variety of applications for food quality and safety control, which has not been thoroughly explored for house crickets. For instance, US and HP can be applied to reduce the microbial load of cricket-derived products. Additionally, these processes can modify the material's properties, leading to a variety of applications for generation of emulsions, reduction of the undesired activity of enzymes and generation of products with tailored properties.

Each non-thermal process that was applied in the present work can be applied in different steps of the overall production process and its effect on each processing step, as well as the final product should be further evaluated. Additionally, the effect of processing methods on the quality of the isolated nutrients, e.g. oxidation of lipids and proteins, should be evaluated.

The processing pathways that were evaluated in the present thesis were applied on lab scale. Apart from their proposed advantages, these processing pathways should be evaluated for their applicability at industrial scale. This evaluation can be performed from a technical perspective, but also via a cost analysis that will facilitate their application. Furthermore, the applicability of novel food processing methods should be explored for more products, e.g. whole fresh or dried insects.

Finally, since the main concern of the present thesis was to propose environmentally friendlier processes and method in the cricket production process, the question of the environmental sustainability of this production process should be assessed. The processing pathways for producing cricket-based products should be optimized in terms or waste reduction or utilization of possible side streams, reduction of energy consumption and possible gas emissions. In this perspective, a life cycle assessment should be applied to confirm the sustainability of inclusion of house crickets in the agri-food systems.

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6. Published works

6.1 Effect of narrowband UV-B irradiation on the growth performance of house crickets

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Article

Effect of Narrowband UV-B Irradiation on the Growth Performance of House Crickets

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Abstract: Indoor co-cultivation systems can answer to the need for sustainable and resilient food production systems. Rearing organisms under light-emitting diodes (LEDs) irradiation provides the possibility to control and shape the emitted light spectra. UV-B-irradiation (280–315 nm) can positively affect the nutritional composition of different plants and other organisms, whereas information on edible insects is scarce. To evaluate the potential effect of the photosynthetically active radiation (PAR) and LED-emitting LEDs on the rearing and nutritional quality of edible insects, house crickets (*Acheta domesticus*) were reared from the age of 21 days under controlled LED spectra, with an additional UV-B (0.08 W/m^2) dose of 1.15 KJm^2 d^{-1} (illuminated over a period for 4 h per day) for 34 days. UV-B exposure showed no harm to the weight of the crickets and significantly increased their survival by ca. 10% under narrowband UV-B treatment. The nutritional composition including proteins, fat and chitin contents of the insects was not affected by the UV-B light and reached values of 60.03 ± 10.41, 22.38 ± 2.12 and 9.33 ± 1.21%, respectively, under the LED irradiation. Therefore, house crickets can grow under LED irradiation with a positive effect of narrowband UV-B application on their survival.

Keywords: resilient food systems; alternative food source; urban rearing; edible insects; LED/narrowband UV-B

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1. Introduction

It is estimated that by 2050, the world population will reach 10 billion people [1]. With many people facing hunger already, global food production needs to increase in the future [2]. However, agriculture is a major contributor to the greenhouse gas emissions [3] and water consumption [4], while its impact on soil degradation can lead to scarcity of arable land [5]. There is a growing interest in resilient food systems since food security can be disrupted and strongly affected by many unpredictable factors [6].

A possible solution lies in the further development of urban, peri-urban, or rural agricultural systems with sustainable practices [7,8]. Environmental controlled systems, allowing the co-cultivation of different organisms, including plants and animals, provide a potential response to the need for a sustainable and qualitative production system [9].

Driven by technical developments such as narrowband light emitting diodes (LEDs), indoor cultivation of plants and crops is advancing [10,11]. Artificial narrowband emitting UV/LEDs have been tested for the cultivation of several organisms, affecting the presence

of secondary plant metabolites including phenolics, carotenoids and glucosinolates [12] and modulating the phenolic content of algae [13].

Diversifying crops and animal products by including alternative food sources such as insects can help build resilient food systems with increased yields and more stable national and regional value chains [14,15]. Due to their low environmental impacts [16] and high nutritional value [17], edible insects are considered a suitable alternative food sources in the future [18]. Given their comparatively easy rearing [19], high reproduction rate and short oviposition period [20], and more importantly their highly valuable nutritional profile, with a high lipid content and a protein content that can reach up to 70% [17], house crickets (*Acheta domesticus*) are attractive for introduction into future food systems. This species of edible insect is already being used as feed and food [21] and were recently accepted as a novel food under Regulation (EU) 2015/2283 [22]. House crickets offer several advantages due to their low feed conversion ratio (<2) [23–25], high percent that is available for food utilization with respect to conventional livestock and low water requirements [21]. Furthermore, house crickets are also reported to contain bioactive compounds, e.g., phenolic compounds [26] and vitamins [27].

Even though irradiation can play a crucial part in maintaining circadian rhythms [28] and edible insects, including house crickets, have been shown to synthesize vitamin D due to exposure to UV-B irradiation [29], the effect of artificial irradiation on their rearing circle has not been thoroughly explored yet. The present study aims to explore the possibility of introducing house crickets to an indoor co-cultivation system, by testing their response to an artificial LED-emitted illumination system, implemented in the rearing of house crickets with and without simultaneous UV-B exposure. The potential impact of this LED/narrowband UV-B irradiation regime on the nutritional composition and growth parameters of the insects was evaluated.

2. Materials and Methods

2.1. Experimental Organisms and Rearing Conditions

Adult crickets were purchased from TropicShop (Nordhorn, Germany) and reproduced at the lab-scale rearing facility at the Institute for Agricultural Engineering and Bioeconomy (ATB) so that the crickets could be raised from birth and their complete life circle could be monitored and controlled. The egg-laying substrate consisted of a mixture of sand and coconut fiber (20:1) and was kept wet at all times. After hatching, crickets were placed inside a 22 L transparent polypropylene box (39 × 28 × 28 cm) and reared for 20 days at 32 °C, 70% humidity according to Fernandez-Cassi et al. [30] inside a climatic chamber (WK-600/40 Weiss, reQutec, Borken, Germany), while exposed only to natural sunlight, transmitted through the window of the chamber. Before the LED rearing experiment and narrowband UV-B exposure, the insects were raised together until they reach the age of 20 days, in order to reach a handling size of about 0.034 ± 0.004 g/cricket and separate into different boxes. At the age of 21 days, crickets were transferred to the facilities of the Leibniz Institute of Vegetable and Ornamental Crops (IGZ) for further investigations.

2.2. Rearing Box Light Transparency

To test for light transparency in experimental rearing boxes, light attenuation for PAR (400–700 nm) and narrowband UV-B (280–315 nm) were determined prior experiment, using a handheld spectrometer and the corresponding software Ocean View 2.0 (Ocean Insight, Orlando, FL, USA). For this purpose, an experimental box was placed in the climatic chamber (Polyklima, Freising, Germany) exposed to intensities of PAR = 50 µmol m^{-2} s^{-1} of a 6500 K LED and narrowband UV-B = 0.04 W/m^2 of 285 nm LED. Five different points (four corners, one center) were measured within the box, open and with closed lid, resulting in absorbance of 26% PAR and 39% narrowband UV-B, respectively. Therefore, the boxes were considered narrowband UV-B transparent, and the obtained values were used to calculate the experimental irradiation dosage.

2.3. LED Rearing Experiment and Narrowband UV-B Exposure

Considering the introduction of crickets to indoor co-cultivation systems, their response to LED/narrowband UV-B irradiation was explored. Ten experimental rearing boxes were placed in a climate chamber (Polyklima, Freising, Germany) in the facilities of the Leibniz Institute of Vegetable and Ornamental Crops (IGZ). The climate chamber was set to selected conditions and a photoperiod of 8h at an irradiation strength of 50 μmol m^{-2} s^{-1} of 6500 K LEDs. To avoid overpopulation that could potentially affect the rearing procedure [31], 60 crickets were placed inside each box, resulting in a total of 600 individuals. To test the potential effect of narrowband UV-B on the cricket physiology, half of the boxes (n = 5) were exposed to an additional narrowband UV-B dose of 1.15 KJm^{-2} d^{-1} of a 285 nm LED for a period of 34 days. As UV-B light can be used for insect pest control of plants [32] and in order not to damage the development of the insects, the light intensity was kept lower (0.08 W/m^2) compared to the one used for plants (0.34 mW/cm^2 = 3.4 W/m^2) [33] or algae (470–650 μW/cm^2 = 4.7–6.5 W/m^2) [13]. Facing the issue of crickets hiding in the UV-B non-transparent shelter material on the 4th experimental day the narrowband UV-B irradiation time was changed. Without changing the dose, the narrowband UV-B intensity to the irradiation time was shortened from 8 to 4 h to provide higher UV-B intensities of 0.08 W/m^2, keeping the same photoperiod of 8 h/day, at the time when fresh food was available and feeding activity was the highest. Therefore, the crickets were lured out from their hiding places and exposed as intended at least for a certain time to the experimental narrowband UV-B irradiation

During the experiment, crickets were fed three times per week with a dried commercial pellet (TropicShop, Nordhorn, Germany) and hydrogel mixed with water. To provide shelter, four pieces of egg carton were placed inside each box and changed weekly, while each box was cleaned daily to ensure hygiene.

At the experimental end at an age of 53 days, crickets were inactivated by shock freezing at −195.8 °C and stored at −80 °C until lyophilization and further processing.

2.4. Growth and Survival Parameters

During the experiment, two different growth parameters were estimated three times per week: (i) average weight (Equation (1)) and (ii) survival percent (SP; Equation (2)). These parameters were estimated using the following Equations of Mole and Zera [34]:

$$\boldsymbol{m_{aver.}}(\text{g/cricket}) = \frac{\boldsymbol{m_{total}}(\boldsymbol{g})}{\boldsymbol{total\ number\ of\ crickets}} \quad (1)$$

$$\text{SP } (\%) = \frac{\boldsymbol{N_i}}{\boldsymbol{N_0}} \cdot \mathbf{100}\ \% \quad (2)$$

where $\boldsymbol{N_i}$ is the number of crickets on day $\boldsymbol{i}$ and $\boldsymbol{N_0}$ is the number of crickets on the first day of the measurements. The growth of the crickets was monitored from their age of 21 days, so at that point the SP was considered equal to 100%.

2.5. Mathematical Modeling of Cricket Growth and Survival

The values of the individual weight (g/cricket) and the SP (%) of the population were expressed as a function of the rearing time (days), with the individual weight w (g/cricket) with the time (days) being correlated with a sigmoidal equation (Equation (3)):

$$\text{w } (\text{g/cricket}) = \frac{\boldsymbol{w_f}}{\mathbf{1} + \boldsymbol{e}^{-\boldsymbol{k}\cdot(\boldsymbol{t}-\boldsymbol{t_0})}} \quad (3)$$

where $\boldsymbol{w_f}$ (g/cricket) is the weight of the crickets at the end of the rearing circle when crickets are harvested, $\boldsymbol{t_0}$ (s), which is the sigmoid midpoint of the curve, represents the time that the first crickets reached adulthood and $\boldsymbol{k}$ (1/s) represents the growth rate.

The mathematical model used to correlate the SP (%) with the time (days) was an exponential decay equation (Equation (4)):

$$\text{SP } (\%) = \text{a} \cdot \boldsymbol{e}^{-\boldsymbol{k} \cdot \boldsymbol{t}} + \boldsymbol{SP_f} \quad (4)$$

where $\boldsymbol{SP_f}$ (%) is the SP (%) at the end of the rearing circle, $\boldsymbol{k}$ (1/s) is the rate of reduction of the SP (%) during the rearing and a is a constant variable. Apart from Equations (3) and (4), the experimental data were expressed as functions of time using several equations, including linear, quadratic, inverse, power, exponential and logarithmic equations. Additionally, the logarithm of the values of the survival percent (%) was modeled as a function of time with a linear equation. However, none of these equations had the high value of regression coefficient and repeatability of Equations (3) and (4). The software used for constructing the models was IBM SPSS Statistics 23 (IBM Corp., Armonk, NY, USA).

2.6. L Composition Analysis

Freeze-dried crickets were milled into a fine powder using a Retsch GM 200 Mill (Retsch GmbH, Haan, Germany). The Ash content (n = 5) of the sample was estimated gravimetrically, after placing the samples at 550 °C and measuring the difference in weight. Moisture content (n = 5) was determined by placing the samples at 105 °C for 48 h and measuring the difference in weight. The protein content (n = 15) of the samples was estimated after hydrolyzing the samples with 6 N, HCl for 24 h at 98 °C and estimating the free amino nitrogen on the hydrolysates [35]. Fat content (n = 5) was estimated gravimetrically using the Folch method by mixing the material with a chloroform/methanol (2:1) solvent for 1 h, centrifuging and adding water to the supernatant at a volume of 0.2-times of the supernatant volume and mixing for another 30 min [36]. Chitin content (n = 15) was calculated by measuring glucosamine and N-acetyl-glucosamine, after hydrolysis with dilute sulfuric acid [37]. The results were expressed on a dry matter basis. For determination of the total phenolic content (TPC) (n = 15), 0.5 g of sample was mixed with 5 mL in an 80% methanol solution and the mixture was homogenized for 4 min. After centrifugation, the supernatant was collected, and the pellet was mixed with a 70% acetone solution. The mixture was homogenized for 4 min and after centrifuging the supernatant was collected. The two supernatants were mixed, and the liquid was removed with a rotary evaporator R-100 (Büchi, Flawil, Switzerland). The remaining extract was solubilized in 5 mL ethanol, filtered with a 0.45 μm filter and the TPC of the liquid was determined with the Folin–Ciocalteau method [38]. The TPC was expressed as mg GAE/100 g dry matter. All chemicals were of analytical grade and were purchased from Carl Roth GmbH (Karlsruhe, Germany).

2.7. Statistical Analysis

The individual weight (g/cricket) and SP (%) were analyzed with a Linear Mixed Model Analysis (LMM), which considered both the irradiation regime and the time (days) as fixed terms and the (irradiation*time) as a random term. The SP (%) did not follow a normal distribution and therefore was normalized with IBM SPSS Statistics 23 (IBM Corp., Armonk, NY, USA) prior to the analysis. The constant parameters of the models, as well as the values of the composition analysis were compared with an Analysis of Variance (ANOVA) with a level of significance of 0.05. Levene's test was used to test homogeneity. The software used for all analyses was IBM SPSS Statistics 23 (IBM Corp., Armonk, NY, USA). The tables of the output of the statistical analysis are given as supplementary material.

3. Results and Discussion

3.1. Rearing of Crickets under Different Light Regimes

Both irradiation regimes led to the growth of crickets with almost equal individual weight in all replicates of all rearing systems (Figure 1). Both irradiation regimes led to the growth of crickets with almost equal individual weight in all replicates of all rearing systems. According to the Linear Mixed Model Analysis (LMM) analysis (Tables S1 and S2), the UV irradiation had no significant effect on the individual weight

of the crickets (F = 3.317, df = 1, p = 0.076). At the end of the rearing, crickets reared under LED/narrowband UV-B irradiation regime at 285 nm had an individual weight of 0.402 ± 0.035 and 0.369 ± 0.035 g/cricket, respectively.

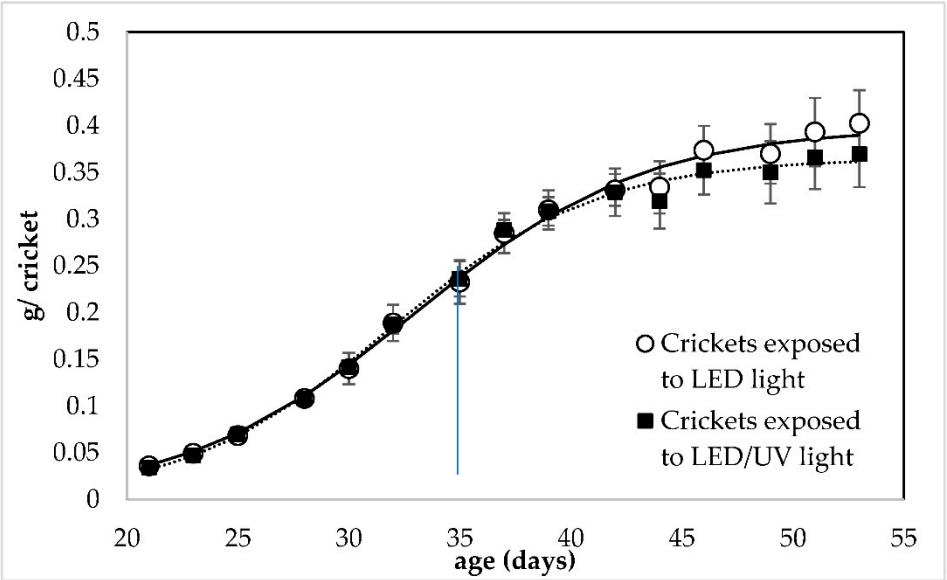

Figure 1. Growth of *Acheta domestica* (individual weight) reared under LED irradiation without (○) and with narrowband UV-B at 285 nm (▪). Error bars represent the standard deviation of multiple replications of the measurements (n = 5). Dashed lines represent the fitting of experimental data to Equation (3). The small line that is vertical to the x-axis indicates the time when crickets reached adulthood (35 days of age).

A significant effect of the irradiation type on the survival of the crickets was observed (F = 4.82, df = 14, p = 0.031, Tables S3 and S4). At the crickets' age of 35 days, when the first adults were observed, the crickets exposed to LED irradiation showed a SP of 74.67 ± 7.83%, while the crickets exposed to the LED/narrowband UV-B irradiation showed a SP (%) of 78.63 ± 4.63%. At the end of the rearing, crickets reared under LED and LED/narrowband UVB irradiation regime at 285 nm showed a SP of 61.53 ± 4.61 and 70.51 ± 8.53%, respectively. UV-B irradiation at 285 nm did not affect (p > 0.05) the number of adult crickets that were harvested. In the two rearing systems with LED and LED/narrowband UV-B irradiation at 285 nm, the number of crickets that reached adulthood was 91.24 ± 4.65% and 94.30 ± 4.82%, respectively.

The present study investigated the response and growth performance of house crickets reared under a LED/narrowband UV-B regime that could be implemented in a co-cultivation system. Crickets were successfully reared under the LED/narrowband UV-B irradiation regime at 285 nm. The narrowband UV-B at 285 nm did not affect the weight, growth and the nutritional value of the crickets; however, it showed a positive effect on their survival (16.67% higher survival). This result indicates that the LED/narrowband UV-B irradiation at 285 nm did not cause any damage to the crickets. On the contrary, it can be implemented in their rearing system. The values of the individual weight and survival of the crickets reported by the present study (Figures 1 and 2) are within the range reported by other studies focusing on the growth performance of house crickets. Specifically, it is reported that house crickets can weigh approximately 0.4 g/cricket after 50 days of rearing [39,40] and have a 55% of survival when reared at standard conditions and harvested at adulthood [41]. This indicated that crickets reared in both treatments could grow normally using the LED regime with and without the narrowband UV-B exposure.

UV light in the UVA dominating range of 300–400 nm has been known to be used for pest control due to its lethal effect on insects, such as moths [32]. Since the aims of the present study were to focus on the effect of narrowband UV-B (285 nm) on house

crickets and ensure their survival, the light intensity was kept at the minimum level possible. Furthermore, crickets had been expected to hide under the egg carton during the photoperiod [20]. Despite our efforts to coordinate feeding time and UV-B exposure, active avoidance could be a further reason for no effect on the growth of the insects. These reasons could explain the low effect of UV-B irradiation on cricket physiology.

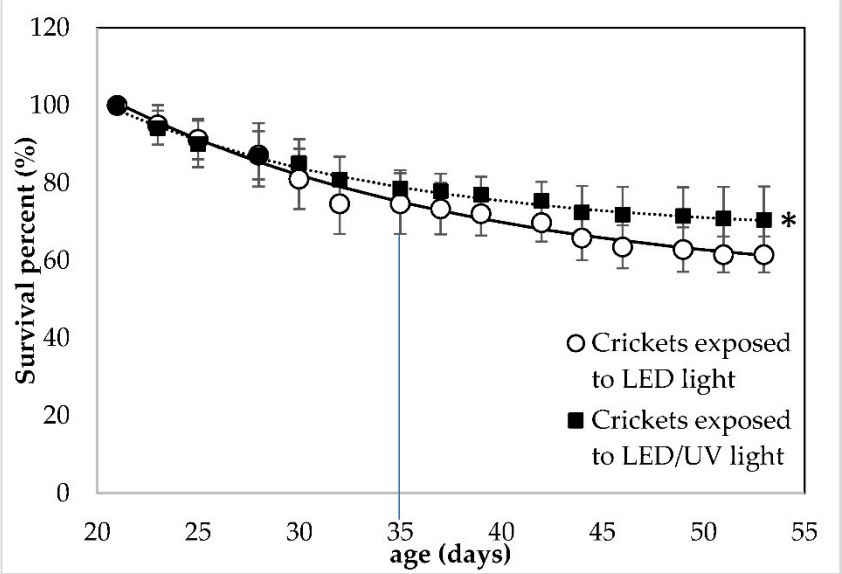

Figure 2. Survival percent (%) of *Acheta domestica* reared Under LED without (○) and with narrowband UV-B irradiation at 285 nm (▪). Error bars represent the standard deviation of multiple replications of the measurements (n = 5). Dashed lines represent the fitting of experimental data to Equation (4). The small line that is vertical to the x-axis indicates the time when crickets reached adulthood (35 days of age) and (*) indicates significant differences between data obtained from different treatments.

3.2. Mathematical Modeling

The parameters of Equations (3) and (4) that were applied to the experimental data obtained from the two rearing systems are presented in Tables 1 and 2, respectively. It was observed that all parameters of Equation (3) show no significant differences between the two rearing systems ($p > 0.05$). However, regarding Equation (4), it was observed that the parameter SP_f, which re the SP at the end of the rearing was significantly higher ($p < 0.05$) when Equation (4) was to the data obtained from the crickets that were exposed to the UV-B light. Both equations had a good fitting on the experimental data, since in all cases the standard errors are low and the regression coefficient (R^2) is high (>0.980).

Table 1. Estimates of the parameters of Equation (3) (individual weight as a function of rearing time) that were applied to the average data obtained from the cricket *Acheta domestica* rearing with the two irradiation regimes.

	w_f (g/Cricket)	k (1/s)	t_0 (s)	R^2
Crickets exposed to LED light	0.398 ± 0.007 [a]	0.192 ± 0.011 [a]	32.960 ± 0.342 [a]	0.995
Crickets exposed to LED/narrowband UV-B light at 285 nm	0.365 ± 0.005 [a]	0.217 ± 0.011 [a]	31.854 ± 0.273 [a]	0.996

Parameters are expressed as mean ± SD. Superscript letters indicate significant differences in the same model parameter between the two rearing systems (n = 5).

Table 2. Estimates of the parameters of Equation (4) (survival percent as a function of rearing time) that were applied to the average data obtained from the cricket *Acheta domestica* rearing with the two irradiation regimes.

	a	***k* (1/s)**	***SP*$_f$ (%)**	**R^2**
Crickets exposed to LED light	150.603 ± 19.754 [a]	0.055 ± 0.009 [a]	53.111 ± 3.371 [b]	0.986
Crickets exposed to LED/narrowband UV-B light at 285 nm	138.525 ± 16.189 [a]	0.070 ± 0.007 [a]	66.895 ± 1.203 [a]	0.993

Parameters are expressed as mean ± SD. Superscript letters indicate significant differences in the same model parameter between the two rearing systems (n = 5).

The data on weight and survival obtained from each replication of rearing under each irradiation regime (n = 5) were fitted to Equations (3) and (4), respectively. Afterwards, the average value of the model parameters obtained for each replicate of each treatment was used as initial parameters for the model that was applied to the average values of the experimental data. In all replicates of both irradiation regimes, both Equations (3) and (4) were found to have a good fitting on the experimental data, with a high regression coefficient ($R^2 \geq 0.985$ for Equation (3) for all replicates and $R^2 \geq 0.880$ for Equation (4) for all replicates). It was, therefore, considered that both Equations (3) and (4), apart from the good fitting on the experimental data, show repeatability when applied to express the individual weight (g/cricket) and survival percent (%) of the crickets as a function of their age (d), respectively.

The parameters of Equations (3) and (4) follow the trend of the experimental data. Both w_f, and k, the rate of the weight increase, showed no significant differences. Furthermore, t_0 was not significantly affected by the UV-B exposure at 285 nm. This was confirmed by the experimental observation that on the 35th day of the rearing the first adult crickets were observed. Regarding Equation (4), parameter SP_f was significantly ($p < 0.05$) higher in the model of the SP of the crickets exposed to UV-B, which confirmed the positive effect of the narrowband UV-B exposure at 285 nm. This indicated that the LED/narrowband UV-B regime at 285 nm could facilitate the inclusion of house crickets in an indoor co-cultivation system.

3.3. Composition Analysis

The composition of the crickets that were harvested from the two rearing systems is presented in Table 3. The UV light had no significant effect ($p > 0.05$) on the composition of the insects. Residual moisture in the samples, after freeze-drying, was approximately 5%. All insects showed a high amount of fat that was higher than 20% on a dry basis, as well as a high amount of chitin that was estimated to be approximately 10% on a dry basis, for all samples. Finally, the protein content of the crickets exposed to LED light and LED/narrowband UV-B light at 285 nm containing 60% and 65% of proteins on a dry basis, respectively, without significant differences ($p > 0.05$).

Table 3. Composition analysis of the *Acheta domestica* that were exposed to the two irradiation regimes. Results are expressed as a percentage of dry matter.

	Crickets Exposed to LED Light	**Crickets Exposed to LED/Narrowband UV-B Light at 285 nm**
Dry matter (%)	95.25 ± 0.39 [a]	95.69 ± 0.13 [a]
Ash (%)	4.74 ± 0.08 [a]	4.69 ± 0.25 [a]
Fat (%)	22.38 ± 2.12 [a]	25.08 ± 2.23 [a]
Proteins (%)	60.03 ± 10.41 [a]	65.59 ± 5.38 [a]
Chitin (%)	9.33 ± 1.21 [a]	10.19 ± 2.05 [a]
TPC (mg GAE/100 g)	161.22 ± 10.26 [a]	175.88 ± 37.84 [a]

Parameters are expressed as mean ± SD. Superscript letters indicate significant differences between the two rearing systems on the content of dry matter (n = 5), ash (n = 5), fat (n = 5), proteins (n = 15), chitin (n = 15) and TPC (n = 15).

Similarly, their nutritional composition was not affected by the crickets' weight. UV-B light has been shown to affect the total phenolic content (TPC) of other organisms, such as the microalgae *Chlamydomonas nivalis* [13]. However, this was not confirmed for the crickets. Nevertheless, the composition of the adult house crickets estimated in the present study agrees with the one reported in the literature [17].

4. Conclusions

The co-cultivation of different organisms can be a response to the demand for more sustainable and resilient agricultural systems. UV-B irradiation is relevant in the indoor cultivation of plants and other organisms. Crickets could be successfully reared under artificial LED irradiation system (400–700 nm). An LED/narrowband UV-B irradiation regime operating for 8 h daily, with a 4 h narrowband UV-B exposure, did not have a significant effect on the weight or the composition of the crickets. Nevertheless, narrowband UV-B exposure resulted in enhanced survival of the crickets, offering the possibility of increasing the number of crickets at the harvesting stage. The obtained experimental data on individual weight and survival were fitted into mathematical models, which confirmed experimental findings. However, further studies are needed to understand the effect of the light quality and intensity on the crickets and try to implement the same irradiation regime for several organisms, in order to further advance the design of co-cultivation systems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/foods11213487/s1, Table S1: Statistical parameters of fixed effects of LMM analysis that was performed for the individual weight of the crickets as presented by IBM SPSS Statistics 23 (IBM Corp., Armonk, NY, USA); Table S2: Statistical parameters of random effects of LMM analysis that was performed for the individual weight of the crickets as presented by IBM SPSS Statistics 23 (IBM Corp., Armonk, NY, USA); Table S3: Statistical parameters of fixed effects of LMM analysis that was performed for the survival percent of the crickets as presented by IBM SPSS Statistics 23 (IBM Corp., Armonk, NY, USA); Table S4: Statistical parameters of random effects of LMM analysis that was performed for the survival percent of the crickets as presented by IBM SPSS Statistics 23 (IBM Corp., Armonk, NY, USA).

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Data Availability Statement: All the data can be found in this study. Any inquiries or additional data can be requested from the corresponding author.

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6.2 Effect of different illumination conditions on the production of house crickets

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Potential of house crickets *Acheta domestica* L. (Orthoptera: Gryllidae) as a novel food source for integration in a co-cultivation system

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Abstract

Environmental controlled indoor cultivation combined with a co-cultivation of different organisms can address the challenges of agri-food systems and lead towards resilience. Due to their numerous food and feed applications and low environmental impact, house crickets (*Achaeta domestica*) are a promising candidate for indoor cultivation systems. They have been shown, also, to positively respond to UV-B radiation. The present study explored the potential of house crickets for application in indoor farming and co-cultivation systems, by applying PAR at elevated intensity combined with narrowband UV-B irradiation at 285 nm and observing its effect on the development of the house crickets, their nutritional composition and the mineral content of their frass, which has potential as fertilizer. Narrowband UV-B irradiation increased the survival rate of the house crickets by >20%, while not affecting their individual weight (g/cricket), in compared to house crickets grown in the same conditions without the narrowband UV-B irradiation (elevated intensity PAR). Furthermore, narrowband UV-B irradiation increased by 16.18% and 67.95% the protein and chitin content, respectively, compared to the house crickets reared in the same conditions without UV-B irradiation. Finally, all the frass samples showed a rich mineral profile containing Ca, K, Mg, Na and S, with narrowband UV-B irradiation affecting its composition throughout the rearing process. It was indicated that house crickets can adapt physiological for integration to indoor farming and co-cultivation systems. Thus, it is suggested that house crickets, as edible insects, have significant potential to be cultivated with other organisms for the circular economy.

Keywords *Acheta domesticus*, house cricket, LED/narrowband UV-B, frass, feed conversion ratio, composition

Introduction

Modern agri-food systems are presently confronted with a multitude of challenges, primarily stemming from the escalating global population. World population is growing and expected to reach 10 billion people by 2050 (FAO, 2017). Driven by anthropogenic caused environmental and climate changes, soil degradation leads to land scarcity, and declining crop production (Gomiero, 2016; Webb et al., 2017). Consequently, world hunger is increasing, estimated to affect up to 828 million people in 2021 (about 46 million people more than in 2020) (Unicef, 2022). Moreover, Agriculture is also reported to be a major contributor to greenhouse gas emissions (Schmidt & Merciai, 2014) and water footprint (D'Ambrosio, De Girolamo, & Rulli, 2018). Consequently, resilient food production systems are of global interest (Constas, d'Errico, Hoddinott, & Pietrelli, 2021).

As a possible solution, sustainable urban and rural agricultural systems becoming in the scientific focus (Moreau, Adams, Mullinix, Fallick, & Condon, 2012; Weindl et al., 2020). Considering of this, fostering regional and local agri-food systems and supporting the combined (co-)cultivation of different organism groups like plants, invertebrate and vertebrate animals has gained importance in recent years (Reddy, 2016). Invertebrates, like insects, play an important role in the development of stable regional agri-food systems (Queiroz et al., 2021). Edible insects are expected to play a major role in the future agri-food systems due to their low environmental impact (Van Huis, 2013), rich nutritional composition (Rumpold & Schlüter, 2013) and environmentally friendly breeding (Smetana, Palanisamy, Mathys, & Heinz, 2016). Among edible insects, house crickets (*Acheta domestica*) are already traditionally used as food and feed in wide parts of the world (Van Huis, 2013). In Europe, they are suggested as food ingredient (Rossi et al., 2021) and recently approved by EFSA as novel food (EFSA Panel on Nutrition et al., 2021). They offer the advantage of an easy rearing process (Caparros Megido, Haubruge, & Francis, 2017), even at household scale, as e.g. in Kenya (Ayieko, Ogola, & Ayieko, 2016) and they are rich in nutrients with a high protein content (Rumpold & Schlüter, 2013). Moreover, even their frass can be applied in the agricultural sector, e.g. use as fertilizer (Food & Feed, 2019; Ojha, Bußler, & Schlüter, 2020). Hereby, cricket species are among the insects that are considered for frass utilization (Bulak et al., 2020).

House crickets have also indicated potential for urban indoor cultivation systems and co-cultivation with other organisms. The suitability of house crickets for integrated indoor cultivation is being explored already in order to assess various cultivation conditions, e.g. innovative composite materials that are appropriate for co-cultivating them with other

organisms, such as macroalgae (Fricke et al., 2022). The potential of utilization of house cricket frass has been explored, as well (Wantulla, van Zadelhoff, van Loon, & Dicke, 2023). Moreover, able to trigger the nutritional composition and disease resistance in different organisms such as plants, research on UV-B controlled artificial illumination is progressing (Fitzner, Fricke, Schreiner, & Baldermann, 2021; Kühnhold, Schreiner, Kunzmann, & Springer, 2023; Schreiner et al., 2012). In a recent study on house crickets, a positive UV-B effect on house cricket has been found by additionally applying an artificial narrow band LEDs of 285nm under LED illumination (50 µmol $m^{-2}s^{-1}$ of a 6500K LED) (Psarianos, Fricke, et al., 2022). (Oonincx et al., 2018) also reported that low irradiance UV-B exposure increased vitamin D3 levels in house crickets. Based on this, it is hypothesized that farming house crickets can be possible in a co-cultivation system with other organisms under joint conditions. Because of this, elevated PAR intensity (150 µmol $m^{-2}s^{-1}$), used for laying in the range for breeding of common crops such as bok choi (Harun, Mohamed, Ahmad, & Ani, 2019), tomato (N. Lu et al., 2012), and also more innovative food sources such as halophytes (Fitzner et al., 2021) or jellyfish (Kühnhold et al., 2023), was also applied to house crickets in the current study. However, there is no detailed information on the effects of different visible light intensities and narrowband UV-B applications on the physiological adaptation and metabolism of house crickets. Therefore, the current study aimed to further investigate the effects of LED/narrowband UV-B irradiation on the growth performance, nutritional values, and frass components of house crickets.

Materials and methods

Experimental insects and rearing conditions

Adult house crickets were purchased from Tropic Shop (Nordhorn, Germany). They were placed in 22 L-transparent polypropylene boxes (39x28x28 cm) inside climatic chambers (Polyklima, Freising, Germany) that were operating to the rearing conditions defined by (Fernandez-Cassi et al., 2019) at 32ºC, a LD 8:16 h photoperiodic condition and 70% humidity. For nutrition a commercial cricket feed (Tropic Shop, Nordhorn, Germany) in pellet form were provided and a water/hydrogel mixture served as water source. A sand/ coconut fiber mixture (20:1) served as egg-laying substrate and was frequently sprayed with water to be always kept humid. The adult insects were exposed for reproduction for 1 week and afterwards inactivated by freezing at -20ºC.

The eggs were incubated at the same photoperiodical regimes until hatching, which was observed after approximately 10 days. Then the small house crickets (nymphs) were reared in

the same conditions until they reached an age of 20 days. Afterwards they were separated into three experimental treatments with different light regimes. At the beginning of the experiment, the light transparency of the experimental boxes was tested with a hand held spectrometer and the corresponding software Ocean View 2.0 (Ocean Insight, USA), as described in the study of (Psarianos, Fricke, et al., 2022). The first (Control) treatment operated at irradiation strength of 50 μmol $m^{-2}s^{-1}$ of a 6500K LED with a photoperiod of 8 h. This treatment was already applied for house cricket rearing during a former study (Psarianos et al. 2022). The second treatment (Elevated intensity PAR) was similar to the control, but with an irradiation strength of 150 μmol $m^{-2}s^{-1}$. The third treatment (LED/narrowband UV-B) was the application of 150 μmol $m^{-2}s^{-1}$ of a 6500K LED at a photoperiod of 8 h/day and an additional narrowband UV-B dose of 1.15 $KJm^{-2}d^{-1}$ of a narrowband (285 nm) LED at an intensity of 0.08Wm^{-2} for 4 h/day. Each light treatment was replicated 5 times and each replicate consisted of 50 house crickets inside the 22-L polypropylene transparent box.

The insects were exposed the different light treatments for 4 weeks and then they were harvested and inactivated by freezing at -20°C. The whole rearing experiment was performed in the facilities of the Leibniz Institute of Vegetable and Ornamental Crops (IGZ) in Großbeeren, Germany.

Growth performance

The rearing was monitored three times per week during the 4 weeks that the house crickets were exposed to the different treatments. The growth parameters that were estimated were individual weight (Equation 1) and the survival percent (Equation 2) (Mole & Zera, 1993):

$$m_{aver.}\text{(g/cricket)} = \frac{m_{total}(g)}{total\ number\ of\ crickets} \quad \text{(Equation 1)}$$

$$\text{SP (\%)} = \frac{N_i}{N_0} \cdot 100\ \% \quad \text{(Equation 2)}$$

where N_i and N_0 are the house cricket population on each day and on the first day of the monitoring, respectively.

Considering the first day of the monitoring of the rearing and the day of harvesting, the total feed conversion ratio (FCR) for these 4 weeks was estimated using Equation 3:

$$\text{FCR} = \frac{feed\ ingested\ per\ cricket\ (g)}{weight\ increase\ per\ cricket\ (g)} \quad \text{(Equation 3)}$$

where lower values of FCR indicate a higher efficiency (Fry, Mailloux, Love, Milli, & Cao, 2018).

Nutritional composition of house crickets

After the house crickets were inactivated, they were lyophilized for 7 days () and grinded into fine powder using a laboratory Retsch GM 200 Mill (Retsch GmbH, Haan, Germany). Afterwards, their nutritional composition was estimated. Ash content was estimated by measuring the difference in weight after placing them inside an ash furnace at 550^{o}C for 48 h, and fat content was estimated gravimetrically after a Soxhlet extraction (Psarianos, Dimopoulos, et al., 2022). Protein content was estimated with a free amino nitrogen analysis following a hydrolysis with HCl, 6 N for 24 h at 98^{o}C, as described by (Dimopoulos, Tsantes, & Taoukis, 2020). Chitin content was estimated by measuring glucosamine content following a hydrolysis with dilute sulfuric acid (Zamani, Jeihanipour, Edebo, Niklasson, & Taherzadeh, 2008). Results were expressed as a percentage of dry matter (%).

Frass evaluation

The frass of the insects from each replicate of each treatment was collected once per week and evaluated for its mineral composition as follows.

Frass samples were initially dried at 60^{o}C for 48 h, weighed, and milled with a ball mill. Then, an approximate amount of 0.2 g of each sample was placed in a digestion tube, mixed with 6 ml oh HNO_3, and left in a fume hood for 5 min. Then the tubes were closed and analyzed with an inductively coupled plasma - optical emission spectrometry (ICP-OES) system (iCAP6300 Duo, Thermo Scientific) with an Autosampler (ASX-520, CETAC Technologies), a pump rate of 75 U/min, and a wavelength range of UV/Vis. The auxiliary gas flow was 1.5 l/min, and the atomizer gas flow was 0.5 l/min. The wavelengths that correspond to each mineral are presented in Table 1 of Supplementary Material. The analysis was performed in the facilities of the Department of Microbiome Biotechnology of the Leibniz Institute of Agricultural Engineering and Bioeconomy (ATB). Custom multi-standards at 1000 mg/l were used that contained Mo, S, Ti (multi-standard 1451) and Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Se, Tl, V, Zn (multi-standard 1450), and that were obtained from VHG Labs, Inc. (New Hampshire, USA). Results were expressed as mg/kg of fresh matter of frass.

Statistical analysis

Individual weight and SP (%) were analyzed with a Linear Mixed Model Analysis (LMM) that considered the irradiation regime conditions and the experimental time as fixed terms and the

(irradiation regime conditions) *(experimental time) as a random term. Data of SP (%) were normalized prior to the analysis. The FCR, estimated minerals of the frass and nutrients of the house crickets were analyzed with an Analysis of Variance (ANOVA) with a confidence level of 0.05, and Tukey's-B Test was applied Post Hoc to separate means obtained from different replicates of the same measurement. All analyses were performed using IBM SPSS Statistics 23 (IBM Corp., Armonk, N.Y., USA).

Results

Growth parameters of house crickets under different irradiation regimes

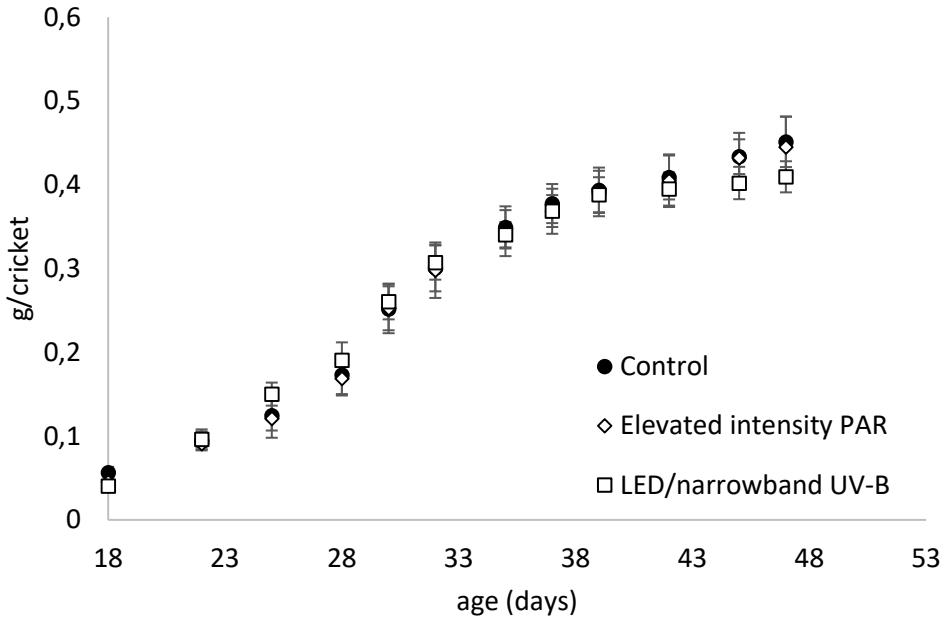

Figure 1: Growth of *Acheta domestica* (individual weight) reared under a control light treatment at irradiation strength of 50 µmol $m^{-2}s^{-1}$ (●), an elevated intensity PAR at irradiation strength of 150 µmol $m^{-2}s^{-1}$ (◊) and a combined LED/ narrowband UV-B treatment at 150 µmol $m^{-2}s^{-1}$ PAR and a UV-B dose of 1.15 $KJm^{-2}d^{-1}$ (□). Error bars represent the standard deviation of multiple replications of the measurements (n=5).

The different irradiation treatments did not significantly affect the individual weight of the house crickets (p= 0.252) (Figure 1). House crickets reared under the control conditions had a weight of 0.45±0.04 g/cricket at the end of the study. House crickets reared under the elevated intensity PAR conditions showed an individual weight of 0.44±0.04 g/cricket at the end of the experiment, while the ones exposed to the LED/ narrowband UV-B showed a weight of 0.40±0.02 g/cricket at harvesting day.

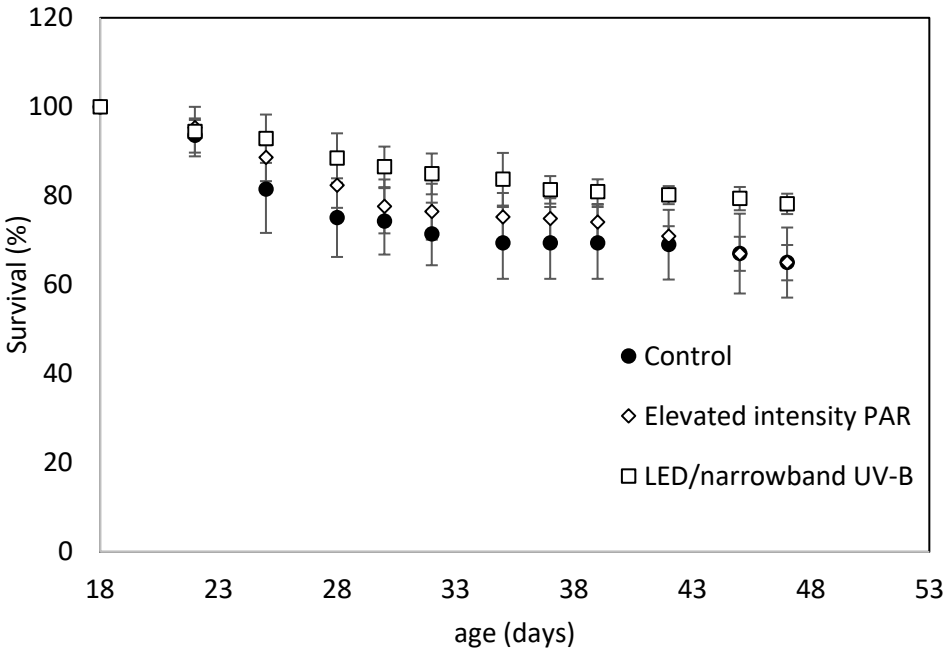

Figure 2: Survival (%) of house crickets reared under a control light treatment at irradiation strength of 50 µmol $m^{-2}s^{-1}$ (●), an elevated intensity PAR at irradiation strength of 150 µmol $m^{-2}s^{-1}$ (◊) and a LED/narrowband UV-B irradiation regime at 285 nm at irradiation strength of 150 µmol $m^{-2}s^{-1}$ and a UV-B dose of 1.15 $KJm^{-2}d^{-1}$ (□). Error bars represent the standard deviation of multiple replications of the measurements (n=5).

Figure 2 presents the survival (%) of the house crickets reared under the different light treatments. It was observed that the different irradiation conditions had a significant effect ($p<0.001$) on the survival of the reared house crickets. At the end of the rearing, insects reared under the control conditions had a survival of 65.00±7.87%, insects reared under elevated intensity PAR showed a survival of 64.98±3.97%, while insects exposed to the LED/ narrowband UV-B had a survival of 78.18±2.29%. Accordingly, it was observed that at the end of the experiment, the narrowband UV-B increased relatively the house cricket survival by 20.32% to the house crickets reared at the same elevated intensity PAR conditions.

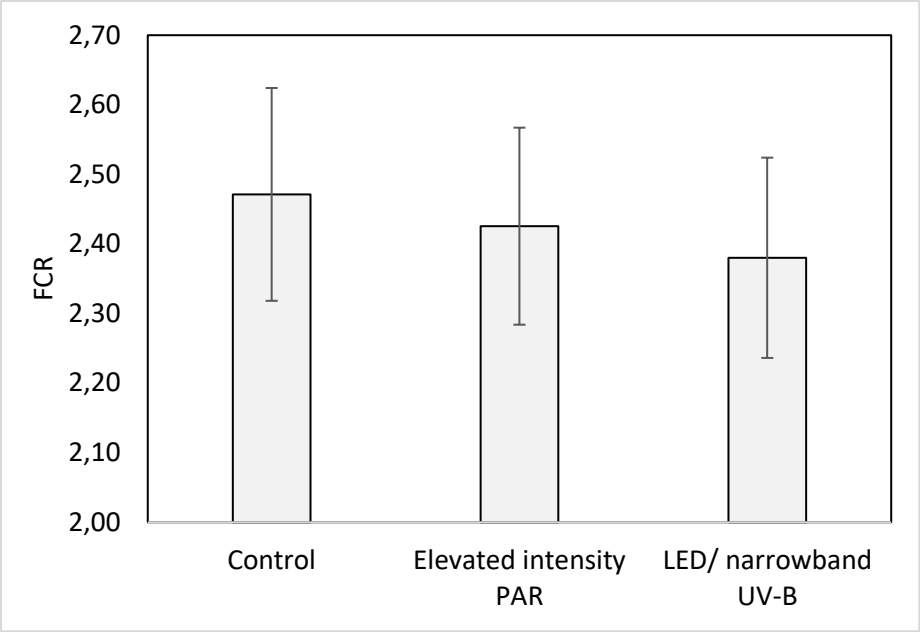

Figure 3: Feed conversion ratio (FCR) of house crickets reared under a control light treatment at irradiation strength of 50 µmol $m^{-2}s^{-1}$, an elevated intensity PAR at irradiation strength of 150 µmol $m^{-2}s^{-1}$, and a LED/narrowband UV-B irradiation regime at 285 nm at irradiation strength of 150 µmol $m^{-2}s^{-1}$ and a UV-B dose of 1.15 $KJm^{-2}d^{-1}$. Error bars represent the standard deviation of multiple replications of the measurements (n=5).

The three different irradiation treatments did not significantly affect the FCR of the house crickets (p=0.627) (Figure 3). The FCR of the house crickets reared under the control conditions, the elevated intensity PAR and the LED/ narrowband UV-B was 2.47±0.15, 2.43±0.14 and 2.38±0.14, respectively.

Effect of light treatment on the nutritional composition

Table 1: Nutritional composition of *Acheta domestica* after exposition to the three irradiation regimes for a period of 35 days.

*	Control	Elevated intensity PAR	LED/ narrowband UV-B
Ash (%)	3.97 ± 1.23^{a}	3.93 ± 0.99^{a}	3.22 ± 0.45^{a}
Fat (%)	17.57 ± 0.78^{a}	15.66 ± 1.09^{b}	14.75 ± 1.19^{b}
Proteins (%)	54.33 ± 6.29^{a}	53.56 ± 4.42^{a}	62.22 ± 6.71^{b}
Chitin (%)	5.02 ± 0.84^{a}	2.67 ± 0.90^{b}	4.48 ± 0.62^{a}

*Results were expressed as percentage of dry matter. Parameters are expressed as mean ±SE. Superscript letters (a,b…) within a line indicate significant higher values of the applied light treatments on the content of ash (n=5), fat (n=5), proteins (n=15) and chitin (n=15).

The nutritional composition of the insects was significantly affected by the light treatments (Table 1). The total fat content of the house crickets was significantly decreased by the elevated intensity PAR (p=0.003), independent from additional UV-B exposure. The protein content was not affected by the elevated intensity PAR, but significantly increased under additional UV-B (p=0.007), reaching a value of 62.22 ± 6.71%. Finally, chitin was significantly decreased by the application of elevated intensity PAR but showed no decline by applying LED/narrowband UV-B.

Evaluation of frass

Table 2: Mineral composition of frass obtained from *Acheta domestica* that were exposed to the three irradiation regimes in the last week of the rearing

*	Control	Elevated intensity PAR	LED/ narrowband UV-B
Ca (g/kg FM)	36.96 ± 4.13^{a}	38.37 ± 3.14^{a}	39.73 ± 1.03^{a}
K (g/kg FM)	22.19 ± 2.43^{a}	22.74 ± 1.54^{a}	23.86 ± 0.54^{a}
Mg (g/kg FM)	5.84 ± 0.58^{a}	6.01 ± 0.54^{a}	5.06 ± 0.46^{a}
Na (g/kg FM)	7.54 ± 0.69^{a}	9.47 ± 2.35^{a}	16.28 ± 2.23^{b}
Pb (mg/kg FM)	< LOD	0.92 ± 0.00^{a}	1.19 ± 0.13^{b}
S (g/kg FM)	5.28 ± 0.46^{a}	5.49 ± 0.38^{a}	5.85 ± 0.07^{a}

*Results were expressed as g/kg of fresh matter (FM) for all minerals except Pb content that was expressed as mg/kg FM. Parameters are expressed as mean ±SE. Superscript letters (a,b…)within a line indicate significant higher values of the applied light treatments (n=15).

Table 2 presents the mineral composition of the frass that was obtained in the last week of rearing. The mineral composition of the frass that was collected during the rest days of the rearing is provided as supplementary material. The frass collected from the different rearing conditions in the first week (Supplementary Table 2) did not show any significant differences between different treatments (p>0.05 for all minerals). In the second week (Supplementary Table 3) apart from Ca, Pb and S that did not vary significantly (p>0.05), the frass collected from insects from the Control treatment was significantly poorer in K (p=0.006) and Mg (p=0.007) and significantly richer in Na (p=0.001). In the third week of rearing the frass (Supplementary Table 4), collected from house crickets reared under all irradiation regimes,

did not vary significantly ($p>0.05$), apart from the Na content of the frass collected from house crickets exposed to LED/ narrowband UV-B, which was significantly lower ($p=0.001$). In the last week of rearing, the Na content of the frass collected from insects exposed to LED/ narrowband UV-B was significantly higher ($p<0.001$), but the Pb content was also significantly higher ($p=0.006$).

Discussion

The present study showed that the elevated intensity PAR combined with the narrowband UV-B irradiation at 285 nm, had a significant effect on the survival, metabolism and nutritional composition of house crickets, nocturnal insects.

The exposure of house crickets to narrowband UV-B irradiation had a positive effect on their survival rate and did not affect the individual weight (g/cricket). This finding confirms the outcome of a former study, where the applied narrowband UV-B irradiation at 285 nm combined with a lower PAR intensity of 50 μmol $m^{-2}s^{-1}$ increased the survival rate of house crickets by 16.67% and did not affect their weight (g/cricket) (Psarianos, Fricke, et al., 2022). These findings are probably linked to the induced physiological strengthening in aspects of immunity and metabolism in house crickets exposed to narrowband UV-B irradiation since it enhanced the synthesis of Vitamin D, an immunomodulator agent, and antimicrobial peptides (Oonincx et al., 2018; Sabockytė, McAllister, Coates, & Lim, 2023). On the other hand, prolonged UV-B (320–290 nm) exposure decreased the survival and immune response of the model insect *Galleria mellonella* (Sabockytė et al., 2023). For this reason, the intensity and duration of UV-B irradiation combined with LED used in the current study provide useful data for insect farming facilities to enhance the survival ability of house crickets. It is also worth noting that previous studies suggest that increased access to water increases the survival rate of house crickets due to the maintenance of internal homeostasis during the physiological mechanisms of cells (Bawa, Songsermpong, Kaewtapee, & Chanput, 2020; McCluney & Date, 2008). In light of these confirmations, if it is possible to figure out whether the water needs of house crickets change in response to different light intensities and narrowband UV-B irradiation in future research, the optimum rearing conditions required for further increased survival rate in the co-cultivation system will also be detailed.

Furthermore, the elevated intensity of PAR and the narrowband UV-B irradiation did not affect the FCR of the house crickets that was for all cases approximately 2.4. The estimated FCR values are comparable to the FCR of chicken, which is reported as 1.85±0.21, but lower than

the FCR of pigs and cattle that are reported as 3.85±1.63 and 8±2.83, respectively (Fry et al., 2018). The lower FCR of house crickets, in compared to the one reported for conventional livestock is a further indication that they are an appropriate alternative to traditional livestock (Bawa et al., 2020; Finke, 2002; Nakagaki & Defoliart, 1991; Wilkinson, 2011). Our findings are broadly consistent with those of other studies showing that house crickets are high-potential feed converters due to their ectothermic metabolism and use of less energy for hemimetaboly development. However, the FCR of house crickets can be manipulated by the rearing conditions, e.g. diet, and reach even lower values (<2) (Bawa et al., 2020). Thereby, the abiotic and biotic factors used in the current co-cultivation system enhanced the feed conversion potential of house crickets regardless of the lighting compared to previous studies.

The protein, fat, ash, and chitin content of house crickets that is determined in the present study is within the range reported in the literature. In particular, the protein content was expected to be 55-70%, the lipid content between 10 and 24%, the ash content 2-4% and the chitin content up to 9% (Psarianos, Dimopoulos, et al., 2022; Rumpold & Schlüter, 2013). Therefore, the house crickets reared under the three different irradiation regimes are a valuable food source. Along with that, the tested illuminations caused significant changes in the nutritional value of house crickets (Table 1).

The applied UV-B showed further beneficial effects on the nutritional profile, by increasing the protein content by 16.17% compared to the house crickets reared under the same PAR without the narrowband UV-B exposure. Prior studies have demonstrated that protein content of house crickets can be influenced by a variety of factors, as for instance the diet (Bawa et al., 2020; Gutiérrez, Fresch, Ott, Brockmeyer, & Scherber, 2020; Riekkinen, Väkeväinen, & Korhonen, 2022), life stage (Finke, 2002), environmental conditions and gender (Gutiérrez et al., 2020). In the case of the present study, all house crickets reared under the different light treatments grew under the same diet and temperature conditions. Therefore, it is concluded that the differences in the protein composition are a result of the narrowband UV-B irradiation. The data obtained here indicate that narrowband UV-B exposure positively affects the protein metabolism of house crickets through biochemical changes. As mentioned in the interpretation of data on survival rates, UV-B may have promoted the synthesis of special proteins that play a role in the immune and endocrine systems to improve insect growth and development. Insect antioxidant defense and melanization-based innate immunity can be modulated depending on the intensity and duration of UV-B exposure (Debecker, Sommaruga, Maes, & Stoks, 2015; Oonincx et al., 2018; Sabockytė et al., 2023; Sang et al., 2016). In some instances, while lower doses of UV-B can induce melanin production and thus activate the humoral and hemocyte-

mediated immune responses to increase physiological resistance, excessive UV-B exposure can suppress immune functions, making insects more susceptible to pathogens and physiological stressors (Ben-Yakir & Fereres, 2016; Debecker et al., 2015; Güven, Pandir, & Hatice, 2015; Sabockytė et al., 2023; Schlein, 1975). Because of these reasons, narrowband UV-B exposure might modulate positively the biosynthesis pathway of several biomolecules (e.g., vitamin D, antimicrobial proteins, biogenic amines) and antioxidant enzyme activities, resulting in an increase in the protein content of house crickets as well as survival. To clarify this assumption, more detailed studies are needed on whether different light intensities and narrowband UV-B induce specific metabolites such as phenolic and other antioxidant compounds and immune-related proteins to improve disease resistance, as in plants (Meyer, Van de Poel, & De Coninck, 2021).

Moreover, the applied narrowband UV-B neutralized the relative reduction of the chitin content (46.81%) that was observed on the house crickets grown under the lower and elevated PAR intensity. Chitin, the primary component of insect exoskeletons, plays a significant role in the physiological adaptation of insects to environmental stressors (Merzendorfer & Zimoch, 2003). Because of this, changes in chitin biosynthesis and degradation derived by enzymatic pathways can be associated with protein metabolism of house crickets treated with narrowband UV-B irradiation. Additionally, disrupting or reducing normal chitin metabolism, which may play a crucial role in their defense against abiotic stresses, is likely to have detrimental effects on survival. For this reason, the chitin content of house crickets indicates that the elevated-intensity PAR treatment had a negative effect on the chitin metabolism because of the re-allocation of energy metabolism to survival (Table 1). Therefore, elevated-intensity PAR can induce light-dependent physiological stress due to their nocturnal metabolism.

Changes in the protein and chitin content of house crickets might also be linked to narrowband UV-B and elevated light intensity-induced energy allocation from the lipid storage of house crickets. It is known that environmental changes can alter the endocrinal state of insects in order to mediate their energy metabolism (Hori, Shibuya, Sato, & Saito, 2014; Perić-Mataruga, Nenadović, & Ivanović, 2006; Sang et al., 2016; Woodring, Meier, & Rose, 1988). This can impact the utilization of metabolites such as lipids and glycogen storage to support energy allocation for physiological and developmental processes. House crickets have been reported, also, to show synthesis of octopamine as a response to a 14-h photoperiod (Woodring et al., 1988). Octopamine, the insect stress hormone, is a biogenous amine that regulates adipokinetic hormone (AKH) secretion from intrinsic neurosecretory cells, and thus it has been related to lipolysis (Farooqui, 2012; Stohs, 2015). Moreover, (J. Lu et al., 2022) reported that exposure

to UV induced the insulin metabolism in black soldier fly larvae (*Hermetia illucens*) to facilitate their sugar and starch digestion, influenced their lipid metabolism and fat accumulation, and improved their metabolism of cysteine and methionine (J. Lu et al., 2022). A recent study also showed that when house crickets were exposed to similar light conditions at a lower light intensity with and without the same dose of narrowband UV-B irradiation as in the present study, no differences were observed in their composition (Psarianos, Fricke, et al., 2022). Therefore, it is hypothesized that the differences observed in Table 1 are a result of changes in the protein and lipid metabolism of the house crickets, as they have been similarly reported for the black soldier fly larvae. Therefore, exposure to elevated PAR intensity and narrowband UV-B doses may induce AKH levels to stimulate the lipid mobilization from the fat body of house crickets to provide energy for overall metabolism and survival. However, more studies are needed to confirm this hypothesis and understand the mechanism of this finding. As a result, the positive effect of UV-B irradiation at 285 nm on the protein and chitin content of the house crickets makes it appropriate for application.

The effect of the irradiation regime on the house crickets was observed in their isolated frass, as well. The house crickets that grew under the elevated intensity PAR and the LED/ narrowband UV-B generated frass with elevated levels of Na, Pb, K, and Mg. These changes support the hypothesis that endocrinal and metabolic changes may occur in insects exposed to different light irradiation regimes. It has been reported that some insects, including house crickets, can increase vitamin D synthesis due to UV-B exposure (Oonincx et al., 2018). (Antwis & Browne, 2009) also reported that UV-B exposure can lead to calcium accumulation in vertebrates due to the synthesis of vitamin D (Antwis & Browne, 2009). Even though invertebrates need lower levels of calcium than vertebrates, it is still important to their development (Cohen, 2003; Ortiz et al., 2016). So, it is indicated that the improved survival rate that was observed due to the narrowband UV-B irradiation was a result of the synthesis of vitamin D by the house crickets. The influence of the irradiation regime on the mineral composition of the frass was expected since the irradiation regime affected the nutritional composition and survival rate of the house crickets. However, further studies are required to confirm this physiological phenomenon. Also, the minerals found in the frass of the house crickets *A. domestica* have been identified in the frass of various insects, including different cricket species such as *Gryllus bimaculatus* (Beesigamukama, Subramanian, & Tanga, 2022; Ferruzca-Campos et al., 2023). This mineral composition supports the possible applications of frass as a biofertilizer (Food & Feed, 2019). The frass of house crickets in particular has been identified as a source of phosphorus, potassium, calcium, magnesium, sulfur, sodium, iron,

copper, manganese, and zinc and has been underlined for its potential as a biostimulant for tomato plants (Ferruzca-Campos et al., 2023). So, the application potential of the house cricket frass indicates house crickets as a potential species that is suitable for contributing in the circular bioeconomy (Ojha et al., 2020) and for co-cultivation.

Conclusions

In the present study, house crickets as edible insects were evaluated for their potential suitability with innovative agrifood systems that are expected to have broad applications in the coming future. Their possible integration in production systems based on indoor farming and co-cultivation of different organisms was investigated by applying a LED/narrowband UV-B regime that has been successfully applied for cultivation of other organisms. Initially, they to LED/narrowband UV-B irradiation at 285 nm, which has been shown to trigger positive metabolic responses from various organisms, which increased house crickets' survival rates, as well as protein and chitin contents. Furthermore, the frass of the house crickets was evaluated for its mineral composition, due to its known potential for utilization as fertilizer. The frass was confirmed to have a mineral profile that would enable its further use, making insects suitable for concepts of circular economy and co-cultivation with other organisms that are cultivated for food applications. Consequently, given that narrowband UV-B treatment together with lower PAR intensity improved the nutritional quality of house cricket biomass, our data suggest that house crickets were evaluated as compatible with cultivation in indoor farming for food applications.

Conflict of interests

The authors declare no conflict of interests.

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Supplementary material

Table 1: Wavelength of detection of minerals that were analyzed

Element	Wavelength (nm)	Element	Wavelength (nm)
Al	394,401; 396,152	Ni	216,556; 221,647; 231,604
B	208,959; 249,773	PO_4^{3-}	177,495; 185,942;
Ba	233,527; 455,403	Pb	216,9; 220,353
Ca	315,887; 317,933; 318,128; 422,6	S	182,034;
Cd	214,438; 226,502; 228,802	Sb	206,833; 217,581
Co	228,616; 230,7; 237,8	Se	196,090
Cr	205,552; 267,716; 284,325	Sn	189,989; 283,999
Cu	224,700	Sr	407,771; 421,552
Fe	238,204; 240,488; 259,940	Ti	190,8; 351,0
K	404,7; 766,490; 769,896	Tl	351,9; 352,9
Mg	279,553; 285,213	V	290,8; 311,0
Mn	257,610; 260,596	W	207,911; 209,8
Mo	202,030; 203,844; 204,598	Zn	202,548; 206,200; 213,856
Na	330,2; 589,592; 818,326		

Table 2: Mineral composition analysis of the frass obtained from *Acheta domestica* that were exposed to the three irradiation regimes in the first week of the rearing. Results were expressed as g/kg of fresh matter (FM) for all minerals except Pb content that was expressed as mg/kg FM.

	Control	Elevated intensity PAR	LED/ narrowband UV-B
Ca (g/kg FM)	29.61 ± 1.44[a]	28.73 ± 2.49[a]	29.54 ± 0.94[a]

K (g/kg FM)	21.36 ± 1.11[a]	21.37 ± 1.97[a]	22.06 ± 0.85[a]
Mg (g/kg FM)	4.76 ± 0.24[a]	4.59 ± 0.48[a]	4.75 ± 0.13[a]
Na (g/kg FM)	8.48 ± 0.75[a]	8.15 ± 1.24[a]	7.06± 0.56[a]
Pb (mg/kg FM)	1.34 ± 0.67[a]	1.45 ± 0.24[a]	1.36 ± 0.53[a]
S (g/kg FM)	5.02 ± 0.15[a]	5.04 ± 0.29[a]	5.19 ± 0.25[a]

589 Parameters are expressed as mean ±SE. Superscript letters (a,b,…) indicate significant differences between the
590 two rearing systems on the content of minerals (n=15).

591 Table 3: Mineral composition analysis of the frass obtained from *Acheta domestica* that were
592 exposed to the three irradiation regimes in the second week of the rearing. Results were
593 expressed as g/kg of fresh matter (FM) for all minerals except Pb content that was expressed as
594 mg/kg FM.

	Control	Elevated intensity PAR	LED/ narrowband UV-B
Ca (g/kg FM)	30.88 ± 0.71[a]	32.62 ± 1.53[a]	31.70 ± 1.06[a]
K (g/kg FM)	22.21 ± 0.51[a]	23.16 ± 0.74[b]	23.74 ± 0.55[b]
Mg (g/kg FM)	4.68 ± 0.12[a]	4.94 ± 0.19[b]	5.03 ± 0.12[b]
Na (g/kg FM)	13.24 ± 0.76[a]	10.79 ± 0.70[b]	11.23 ± 0.79[b]
Pb (mg/kg FM)	3.00 ± 0.36[a]	3.39 ± 0.44[a]	2.78 ± 0.83[a]
S (g/kg FM)	4.72 ± 0.17[a]	4.88 ± 0.18[a]	4.94 ± 0.05[a]

595 Parameters are expressed as mean ±SE. Superscript letters (a,b,…) indicate significant differences between the
596 two rearing systems on the content of minerals (n=15).

597 Table 4: Mineral composition analysis of the frass obtained from *Acheta domestica* that were
598 exposed to the three irradiation regimes in the third week of the rearing. Results were expressed
599 as g/kg of fresh matter (FM) for all minerals except Pb content that was expressed as mg/kg
600 FM.

	Control	Elevated intensity PAR	LED/ narrowband UV-B

Ca (g/kg FM)	33.99 ± 3.85[a]	35.58 ± 1.84[a]	35.77 ± 3.24[a]
K (g/kg FM)	21.74 ± 1.16[a]	20.92 ± 1.25[a]	22.01 ± 1.21[a]
Mg (g/kg FM)	4.45 ± 0.37[a]	4.55 ± 0.29[a]	4.67 ± 0.37[a]
Na (g/kg FM)	30.72 ± 4.02[a]	26.13 ± 3.43[a]	19.94 ± 2.67[b]
Pb (mg/kg FM)	1.15 ± 0.52[a]	1.36 ± 1.16[a]	1.44 ± 0.31[a]
S (g/kg FM)	5.33 ± 0.34[a]	5.45 ± 0.17[a]	5.40 ± 0.29[a]

601 Parameters are expressed as mean ±SE. Superscript letters (a,b,…) indicate significant differences between the
602 two rearing systems on the content of minerals (n=15).

603

6.3 Quality Evaluation of house cricket flour after processed by electrohydrodynamic drying and pulsed electric field processing

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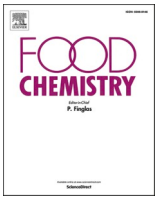

Quality evaluation of house cricket flour processed by electrohydrodynamic drying and pulsed electric fields treatment

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ABSTRACT

House crickets are expected to play a significant role in the future food sector. Electrohydrodynamic (EHD) drying offers an environmentally friendly alternative to conventional drying methods. Pulsed electric fields (PEF) is a non-thermal process that facilitates conventional processes. EHD was applied to house crickets with and without PEF pretreatment, and the effect of PEF and EHD on the quality of the insects was evaluated. PEF pretreatment positively affected the oven drying at 60 °C by reducing its duration and thus decreasing the energy consumption by 14.22%. Moisture removal of EHD was not sufficient to replace oven drying, but when combined with oven drying, the overall energy consumption was reduced by >50%. PEF processing also increased the protein solubility (53.07% higher than the respective control) and antioxidant activity (24.05% higher than the respective control) of the oven-dried samples and reduced the histamine content of the EHD-dried samples (25.87% lower than the respective control).

1. Introduction

The world population is rising and is expected to be almost 10 billion people by 2050, with a major increase in global food demand (Faostat, 2019). This abrupt change threatens our food security which requires appropriate actions such as replacing alternative food resources. Edible insects are one of the alternatives to address these issues as part of the future food systems. They provide a high nutritional composition (Rumpold and Schlüter, 2013) with a low environmental impact (Van Huis and Oonincx, 2017). House crickets, in particular, have been proposed, already, as food ingredients (Rossi et al., 2022), and are accepted in the EU as novel food (EFSA Panel on Nutrition et al., 2015).

Processing insects is essential for extending their self life and controlling their safety and quality. Drying is one of the major processes to extend their shelf life. Different drying methods are reported for processing edible insects, including oven drying, solar drying, freeze drying, vacuum drying, fluidized bed drying and microwave drying. Oven drying at temperatures between 50 and 80 °C is the most frequently applied method in the industry (Parniakov et al., 2021). The drying method is reported to affect product characteristics, like color, due to browning reactions and shrinkage, as well as the overall product quality (Parniakov et al., 2021). Additionally, drying has been reported to affect the histamine levels of some foods (Lin et al., 2014). Histamine is a chemical hazard, that can be found in insects (Chomchai and Chomchai, 2018), and its content has to be maintained below a certain limit.

Electrohydrodynamic (EHD) drying is a novel non-thermal drying method by which moisture is removed from the product mainly due to convection and partially electroporation. It consists of a high voltage power supply and repeated arrays of two electrodes (emitter and collector). Applying a high voltage difference between the electrodes ionizes the air around the emitter. The movement of these ions from the emitter towards the collector induces the so-called ionic wind. This ionic wind provokes convective dehydration on drying materials. EHD can be a promising alternative to conventional drying due to its scalability and significantly low energy consumption (Iranshahi et al., 2020).

Another novel food process that has promising potential for integration in the edible insect production is pulsed electric fields (PEF). PEF refers to the application of high voltage and short duration pulses to a

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food product that is placed between two electrodes. PEF processing causes cell disruption due to electroporation, which enhances the transport phenomena between the intracellular and extracellular environment of the material, accelerating conventional processes like drying (Raso et al., 2016). Furthermore, PEF has been reported to enhance the quality of foods, while being appropriate for processing insects, enhancing conventional drying, and extracting nutrients (Shorstkii, 2022; Psarianos et al., 2022).

The present study explores the applicability of EHD drying and PEF pretreatment on house crickets to reduce the drying time and positively affect the quality of the final product.

2. Materials and methods

Living house crickets (*Acheta domestica*) were purchased from Tropic Shop (Nordhorn, Germany) and were inactivated by freezing at −20 °C and then thawed at 25 °C for 2 h and used for further experiments. All chemicals were purchased from Carl Roth Gmbh & Co. Kg (Karlsruhe, Germany), unless stated otherwise. The moisture content of the samples was estimated by measuring the weight difference after placing them at 105 °C for 48 h and was equal to 72.05 ± 0.98 g/100 g of crickets.

2.1. Pulsed electric field (PEF) treatment

Whole fresh insects (25 g) were mixed with 100 ml of water, placed inside a batch chamber with a 40-mm electrode distance and processed with an HVP-5 (DIL, Quackenbrück, Germany) PEF system. The system was connected to an oscilloscope (Voltcraft, DSO-1062D, Conrad electronics, Hirschau Germany) that was used to monitor the pulses. The picture of the pulse is presented in the supplementary material (Fig. S1). The oscilloscope had two channels, one that corresponded to the voltage and one that corresponded to the current. The insects were treated with 500 almost rectangular pulses with pulse width of 25 μs at the following conditions: 4.4 kV/cm, 41.6 A, 20 Hz and a temperature that did not exceed 25 °C after the treatment. The specific energy input was calculated using Eq. (1) (Raso et al., 2016)

$$w_{spec.}(kJ/kg) = n/m\hat{A}\cdot\int_0^{\infty} V(t)\bullet I(t)dt \quad (1)$$

where n is the number of pulses, m (kg) is the mass of the sample, V(t) and I(t) are the voltage and the current as a function of the treatment time, and t (s) is the time. Afterwards, the samples were sieved and blotted with a paper towel to remove excess water. Untreated samples were placed inside water at the same ratio for the whole treatment to make samples comparable.

2.2. Drying process

2.2.1. Oven drying

The whole fresh insects were placed inside a conventional oven dryer and were spread in a monolayer in drying trays of 30 cm × 30 cm. The crickets were dried at a constant temperature of 60 °C and an average air velocity of 1 m/s. The weight of each tray was recorded at different time intervals until equilibrium was reached. The total energy consumption was measured using the MegaPower™ Plug Power Meter (Digiparts, Canada).

2.2.2. Electrohydrodynamic (EHD) drying

The set-up of the EHD drying was based on the work of (Iranshahi et al., 2020) and took place inside a lab-scale chamber of 40 × 40 × 70 cm. The set-up consisted of an emitter electrode connected to a positive high voltage power supply and a collector electrode connected to a negative high voltage power supply. The insects were placed on the plate collector (30 cm × 30 cm). A multimeter (Keysight U1253B, Santa Rosa, CA, USA) and a 1000:1 high voltage probe (Testec HVP-40, Testec Elektronik GmbH, Germany) were used to measure the discharge energy consumption, while a MegaPower™ Plug Power Meter (Digiparts, Canada) was used to measure the total energy consumption. The drying set-up was connected to a digital scale (PG5001-S, Mettler-Toledo, Greifensee, Switzerland) to measure the weight difference directly during the drying process. The insects were dried for 580 min to ensure equilibrium was reached. The energy consumption E (J) was calculated by multiplying discharge voltage V (V), current I (A) and drying time t (s):

$$E = V\hat{A}\cdot I\hat{A}\cdot t \quad (2)$$

2.2.3. Drying curves

For both oven and EHD drying methods, the moisture load M_t was calculated for every time interval t as $M_t = (m_t\text{-}m_s)/m_s$, where m_t (g) and m_s (g) are the mass of the sample at time t and after being completely dried, respectively. Afterwards, the moisture ratio (MR) was calculated as $MR = (M_t\text{-}M_e)/(M_0\text{-}M_e)$, where M_t, M_e and M_0 are the moisture load of the samples at each time interval t, at equilibrium and at the beginning of the process (t = 0 min), respectively. To minimize errors caused by fluctuation, it was considered that $M_e = 0$ (Ostermeier et al., 2018). The moisture ratio was expressed as a function of drying time t (min) with the models that are presented on Table 1.

The models were evaluated based on the correlation coefficient R^2, the reduced χ^2 (Eq. (2)) and the root mean square error RMSE (Eq. (3)):

$$\chi^2 = \frac{\sum_{i=0}^{N}(MR_{i,p} - MR_{i,e})^2}{N - n} \quad (2)$$

$$\text{RMSE} = \sqrt{\frac{\sum_{i=0}^{N}(MR_{i,p} - MR_{i,e})^2}{N}} \quad (3)$$

where $MR_{i,p}$ and $MR_{i,e}$ are the calculated and experimental values of the MR, N is the number of observations and n is the number of parameters of the model. Higher values of the R^2 (close to 1) and lower values of χ^2 and RMSE indicate a better fitting of the model to the experimental data. The regression analysis was performed with IBM SPSS Statistics 23 (IBM Corp., Armonk, N.Y., USA).

2.3. Product quality

Prior to each analysis, the insects were dried with a laboratory Retsch mill (Retsch Grindomix, Retsch GmbH, Germany) to become a homogenous material. Afterwards the milled samples were stored at room temperature in order to be further evaluated. All quality parameters were measured within two weeks.

2.3.1. Bulk density

Bulk density was estimated gravimetrically by placing the samples in a volumetric cylinder of a known volume and measuring the weight. The results are expressed as (g/cm^3).

Table 1
Models used to express the MR as a function of time. k corresponds to the drying rate (1/s), a and b are coefficients of the equations, n is an exponent and t is the drying time (min).

Model name	Equation	Reference
Newton	$MR = e^{-k\bullet t}$	(Demir et al., 2004)
Page	$MR = e^{-k\bullet t^n}$	(Sarimeseli, 2011)
Henderson and Pabis	$MR = a\hat{A}\cdot e^{-k\bullet t}$	(Rahman et al., 1997)
Logarithmic	$MR = a\hat{A}\cdot e^{-k\bullet t} + b$	(Sarimeseli, 2011)
Medilli	$MR = e^{-k\bullet t^n} + b\hat{A}\cdot t$	(Midilli et al., 2002)
Wand and Singh	$MR = 1 + a\cdot t + b\cdot t^2$	(Wang and Singh, 1978)

2.3.2. Color

Color was measured with a Minolta chroma meter (CM-2600D, Konica Minolta Inc., Japan) with CIELab system, illuminant D_{65} (daylight), SCE (specular component excluded) mode, and 10° observer angle. The colorimeter was calibrated with standard white plate prior to the analysis.

The overall color change, ΔE was estimated with Eq. (4):

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (4)$$

where L^*, a^*, and b^* correspond to the color coordinate system parameters of the CIELAB system. L^* corresponds to the light–dark spectrum (0 (black) to 100 (white)),a^* corresponds to the red-green spectrum (−60 (green) to 60 (red)) and b^* corresponds to the yellow–blue spectrum ranging from (−60 (blue) to 60 (yellow)) of the samples. Color was measured for insects samples before (L_0^*, a_0^*, and b_0^*) and after drying (L^*, a^*, and b^*).

2.3.3. Total phenolic content (TPC)

An amount of 0.5 g of samples was homogenized with 12 ml of a ethanol/acetic acid/water solvent (50:8:42) for 1 h and then samples were centrifuged at 3900 χg for 10 min and the supernatant was collected (Bolat et al., 2021). The TPC of the extract was measured with the Folin-Ciocalteau method by mixing 0.1 ml of extract with 7.9 ml of water, 0.5 ml of the Folin reagent (1 N) and 1.5 ml of a saturated Na_2CO_3 solution and incubating the mixture for 30 min at 40 °C in the dark. Then the absorbance was measured at 765 nm. Gallic acid was used for the calibration curve (100–1000 mg/L) and the results were expressed as g gallic acid equivalent (GAE)/ 100 g.

2.3.4. Antioxidant activity

The obtained extract from the crickets that was described in Section 2.3.3 was used for estimating the antioxidant activity.

2.3.4.1. Free radical scavenging activity (DPPH). The free radical scavenging activity was estimated by mixing 0.1 ml of extract with 3.9 ml of a freshly prepared DPPH solution at $6{\cdot}10^{-5}$ M and incubating in the dark at room temperature for 15 min. Then, the absorbance of the mixture was measured at 515 nm against a blank that contained methanol instead of sample extract. Trolox was used for the calibration curve (0.1–1 mM) and results were expressed as g trolox equivalent (TE)/100 g (Brand-Williams et al., 1995).

2.3.4.2. Ferric reducing iron power (FRAP). FRAP was measured by mixing 0.5 ml of extract with 0.5 ml solium phosphate buffer (0.2 M, pH = 6.6) and 0.5 ml of potassium ferricyanide 1%.and incubating at 50 °C for 20 min. Afterwards, 0.5 ml of TCA 10% were added, followed by the addition of 2 ml water and 0.4 ml of ferric chloride 0.1%. The absorbance of the mixture was measured at 700 nm after vortexing. Trolox was used for the calibration curve (50–500 μM) and the results were expressed as g TE/100 g (Jakovljevic et al., 2014).

2.3.4.3. Cheletating ability. Chelating ability was estimated by mixing 0.1 ml of extract with 3.7 ml methanol and 0.1 ml of 2 mM solution of ferrous chloride, incubating the mixture at room temperature for 3 min and then adding 0.2 ml of a 5 mM ferrozine solution. The mixture was incubated at room temperature for 10 min and then the absorbance was measured at 562 nm. EDTA was used for the calibration curve (0.25–2 mg/ml) and the results were expressed as g EDTA equivalent/100 g (Dinis et al., 1994).

2.3.5. Protein solubility

To estimate protein solubility, 0.5 g of samples were homogenized with 10 ml of water at room temperature for 1 h and then the mixture was centrifuged at 3900g for 10 min. Protein content was measured in the supernatant with the Lowry method (Bolat et al., 2021). Bovine serum albumin was used for the calibration curve (40–300 μg/ml) and the results were expressed as g protein/100 sample.

2.3.6. Maillard reaction

The progress of Maillard reaction was measured by measuring the concentration of hydroxylmethylfulfural (HMF). Briefly, 2 ml of the supernatant that was obtained from the process described in section 2.3.3 was mixed with 2 ml of a 12% TCA solution and 2 ml of a 25 mM TBA solution. The mixture was incubated at 40 °C for 50 min and then the absorbance was measured at 443 nm (Cohen et al., 1998). Standard HMF was used for the calibration curve (0.4–25 mg/ml) and results were expressed as g HMF/100 g.

2.3.7. Polyphenoloxidase (PPO) activity

For measuring the PPO activity, 0.2 ml of sample was homogenized with 5 ml of 0.1 M sodium phosphate buffer (pH of 6.5) for 60 min at room temperature. Then, they were centrifuged at 3900g for 20 min at 8 °C. Afterwards, the PPO activity was measured by mixing 2.25 ml of reaction buffer (0.5 mM of SDS in 0.1 M sodium phosphate buffer) with 0.15 ml of the supernatant 0.3 ml of a 0.5 M proline solution and 0.3 ml of a catechol solution (2.2 mg/ml in reaction buffer). The absorption of the mixture was measured every 30 s, for 10 min and the PPO activity was calculated from the slope of the curve of the absorption and time (Reinkensmeier et al., 2016).

2.3.8. Histamine concentration

Histamine was extracted from the samples by mixing 2 g with 20 ml of a 0.85% NaCl solution for 2 min. Then the mixture was centrifuged at 3900g for 10 min at 4 °C and the supernatant was collected, diluted 1:2 with the saline solution and then mixed with 0.5 g of a salt mixture (6.25 g sodium sulfate and 1 g trisodium phosphate monohydrate). Then 2 ml of butanol were added, the mixtures were mixed for 1 min and then centrifuged at 3900g for 10 min. The upper layer is collected, evaporated and solubilized with 1 ml of water. Then 5 ml of a 1.1% sodium carbonate solution and 2 ml of the reaction reagent were added. Mixtures were incubated at room temperature for 5 min and the absorption was measured at 496 nm. The reaction reagent was prepared by mixing 1.5 ml of a 0.9% sulfanilic acid solution in 4% HCl with 1.5 ml of a 5% $NaNO_2$ solution inside an ice bath. After 5 min, another 6 ml of the $NaNO_2$ solution was added and after 5 min 41 ml of water was added (Patange et al., 2005). Standard histamine (Merck, Darmstadt, Germany) was used for the calibration curve (5–50 μg/ml) and results were expressed as mg histamine/100 g.

2.3.9. Volatile profile

The volatile profile was measured using a GC–MS coupled with a solid phase microextraction (GC–MS-SPME) technique, according to (Rossi et al., 2021). Briefly, samples were placed inside a vial and preheated at 45 °C for 10 min.Then, a fiber (SPME Carboxen/ PDMS, 85 μm, Stalleflex Supelco, Bellefonte, PA, USA) was positioned inside the vial to absorbe the volatile molecules for 40 min. Volatiles were desorbed in the injector of a gas-chromatograph for 10 min and separated through a column Chrompack CP-Wax 52 CB (Chrompack, Middelburg, Olanda) with a length of 50 m and internal diameter of 0.32 mm. The analysis was performed using an Agilent Technology 7890A gas chromatograph, Network GC System combined with a Network Mass Selective detector HP 5975C mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). The volatile peaks were identified via comparison of mass spectral data of molecules from the NIST library (NIST/EPA/NIH Mass spectral Library, Version 1.6, United States of America) of 2011 and WILEY (sixth edition, United States of America) of 1995. 4-methyl-2-pentanol at 10.000 mg/kg was used as standard at a concentration of 20 mg/kg for each sample.

2.4. Statistical analysis

All processing pathways were repeated twice and each analytical method was repeated in triplicate (n = 6). Statistical differences among means of data obtained for samples that were subjected to different processing pathways are explored with an one-way analysis of variance (ANOVA) at a significance level of 0.05, with Duncan's test applied post hoc to separate means. Data that did not follow a normal distribution were normalized prior to the analysis. Principal component analysis (PCA) was performed using Statistica software (version 8.0; StatSoft., Tulsa, OK) on raw data to highlight the statistical variance among the volatile profiles.

3. Results and discussion

3.1. Drying curves

According to the drying curves (Fig. 1), PEF has a significant effect on the drying of the crickets. The oven-dried insects that were subjected to PEF treatment showed a lower moisture ratio throughout the whole drying process. Additionally, the drying time was reduced with PEF. The oven-dried untreated samples reached a drying equilibrium at 180 min of drying, while the PEF treated ones at 150 min of drying. Regarding the application of PEF alone as a pretreatment for the drying of crickets to produce cricket flour, it is hypothesized that due to PEF-induced electroporation, the moisture removal from the crickets was enhanced, leading to a lower moisture content and moisture ratio in the final product.

However, EHD drying was not sufficient, for both untreated and PEF-treated samples. For both samples, EHD drying reached equilibrium at 210 min, when the water content of the insects was not reducing further. The insufficiency of EHD drying for insects could be attributed to the low temperature of the drying (EHD operates at room temperature ≈25 °C). Preliminary tests were performed with oven drying of insects at 30 °C, which similarly showed that low-temperature convective drying could not dry the insects to a moisture of <5% (data not shown), which is shown to be sufficient for avoiding spoilage (Kamau et al., 2018). Furthermore, the positive effect of PEF on the drying kinetic was not observed for the EHD-dried samples.

The drying curves of house crickets at 60–80 °C have been reported to show a rapid reduction in the first 60 min of the oven drying and reach equilibrium after 360 min (Bawa et al., 2020). In the present study the rapid reduction of the drying curve in the first 60 min of drying was observed as well for the oven dried samples. However, the equilibrium was reached faster in the case of the present study, which could be attributed to the low initial amount of sample that was 25 g in compare to the study of Bawa et al. (2020), who reported the longer drying time for 200 g of crickets until constant weight (Bawa et al., 2020). The loading density of the tray during the oven drying can reduce the drying rate, as shown for okra (Emmanuel and Fakayode, 2011). The oven drying of house crickets in a single-layer position of crickets has been reported to be faster than the multi-layer setting at the same temperature (Fröhling et al., 2020). However, the oven drying settings of house crickets can differ, as for instance in the case of Khatun et al. (2021), who reported 20 h of drying at 65 °C of a thick layer of crickets (Khatun et al., 2021).

The application of PEF treatment has been widely reported to improve the drying rate of foods and accelerate the drying process due to an increased vapor transfer between the intracellular and extracellular environment of foods. This could lead to a reduction of the energy consumption of the drying process (Punthi et al., 2022). This effect has been reported for various food materials, e.g. potatoes (Lebovka et al., 2007), apples (Wiktor, 2013) and onions (Ostermeier et al., 2018). The positive effect of PEF on the drying kinetics has been reported for edible insects, as well. PEF pretreatment has been shown to increase the drying rate of oven drying of black soldier fly larvae (Shorstkii, 2022; Alles et al., 2020), as well as the drying rate of freeze drying and infrared drying of black soldier fly and yellow mealworm larvae (Bogusz et al., 2023,2022; El Hajj et al., 2023). Therefore, the positive effect of PEF pretreatment on the drying kinetic of house crickets was somewhat expected.

Regarding EHD drying, the present study is the first one to report its application on edible insects. It has been applied successfully on plant materials, such as slices of apples (Hashinaga et al., 1999) and carrots (Ding et al., 2015). Additionally, EHD drying has been combined with a PEF pretreatment in the case of apple slices, with the PEF treatment leading to a 39% reduction of the drying time (Iranshahi et al., 2023). However, in the case of house crickets EHD drying alone was not efficient in drying the crickets, while no effect of the PEF pretreatment was observed for the samples subjected to EHD drying.

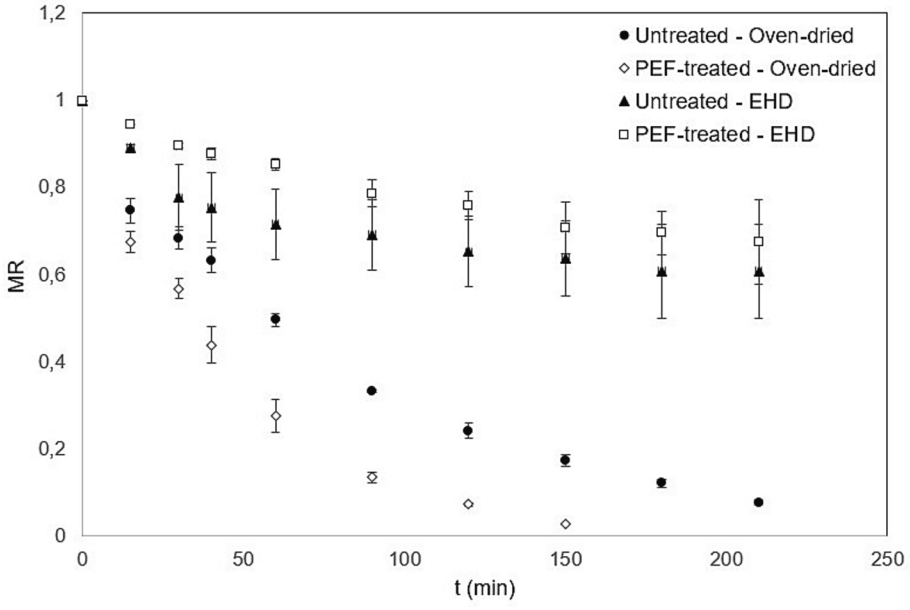

Fig. 1. Moisture ratio (MR) of samples during the drying process. Error bars represent the standard error of means of the MR that was obtained from different replications of the same process.

Drying of insects is facilitated as temperature increases (Shorstkii, 2022). Milder temperatures would cause a partial protein denaturation, induced by heating or oxidation, even at 40 °C, which would affect surface hydrophobicity and thus protein complexes (Shorstkii, 2022; Chelh et al., 2006). This increase of protein hydrophobicity could lead to alignment of the proteins at the water/oil interface and, depending on the amino acid sequence and degree of denaturation, cause a steric hindrance due to the generation of protein parts that extend from the lipid to the water fraction (Damodaran, 2005; Timilsena, 2016). Therefore, the driving force of moisture removal from insects has been reported to be stronger and the effect of PEF treatment more evident at higher temperatures (Shorstkii, 2022).

When the EHD drying reached equilibrium and the moisture removal would not progress further, the samples were not considered dried. Therefore, after the EHD drying, they were placed in a drying oven at 60 °C for 1 h, at the same conditions described in Section 2.2.1, so that they were properly dried. The final moisture content that was targeted for the samples was <5%, which according to the moisture absorption isotherm for house crickets at 25 °C would correspond to a water activity $a_w < 0.4$ (Kamau et al., 2018). The EHD drying was considered sufficient for the insects when combined with a short oven drying treatment, similarly shown for mushrooms (Taghian Dinani, 2014). The combination of the two drying methods stemmed from the fact that EHD drying is recognized for its significantly lower energy consumption compared to other drying techniques. Consequently, by merging these methods, it was anticipated that a dry end product could be achieved with even lower energy consumption. Moreover, EHD is a low-temperature drying method which can lead to higher product quality by preserving heat-sensitive compounds (Iranshahi et al., 2023).

These samples were considered for further characterization and compared to the samples that were subjected to only oven-drying, with and without a PEF treatment. The drying processes that were evaluated for their effect on the insect matrices were oven-drying and EHD-drying followed by a shorter oven-drying step. The effect of PEF treatment on the insects when combined with both drying pathways, was considered as well.

Table 2
Evaluation of models applied to the drying curves of samples subjected to different processing pathways.

Model		Untreated Oven-dried	PEF treated Oven-dried	Untreated EHD-dried	PEF treated EHD-dried
Newton	R^2	0.990	0.994	0.564	0.927
	χ^2	0.1807	0.1266	0.2903	0.3107
	RMSE	0.4033	0.3355	0.5112	0.5288
Page	R^2	0.993	0.994	0.975	0.994
	χ^2	0.0008	0.0007	0.0005	0.0001
	RMSE	0.0252	0.0240	0.0193	0.0089
Henderson and Pabis	R^2	0.993	0.995	0.809	0.965
	χ^2	0.0009	0.0007	0.2682	0.3585
	RMSE	0.0267	0.0239	0.4632	0.5355
Logarithmic	R^2	0.993	0.996	0.984	0.995
	χ^2	0.0011	0.0007	0.0003	0.0001
	RMSE	0.0275	0.0218	0.0154	0.0088
Midilli	R^2	0.994	0.873	0.827	0.959
	χ^2	0.1753	0.2120	0.1697	0.1700
	RMSE	0.3243	0.3432	0.3191	0.3194
Wand and Singh	R^2	0.997	0.966	0.880	0.990
	χ^2	0.0029	0.0055	0.0034	0.0001
	RMSE	0.0482	0.0619	0.0519	0.0108

Table 2 shows the applicability of the models to the drying curves of the samples. The application of these models is essential to increase the predictability of the processes (Wiktor, 2013). Even though EHD drying was insufficient to dry the insects completely, the models that showed a good fitting to the experimental data were applicable to both drying methods and for PEF-treated and untreated samples. The ones that had a good fitting for all samples were the Page model and the Logarithmic model with $R^2 > 0.97$ for all samples and χ^2 and RMSE being <0.001 and <0.1, respectively. The other models were considered inappropriate due to low values of R^2 or high values of χ^2.

3.2. Product quality

Table 3 shows the evaluated key indicators of the product quality of the insects. The bulk densities of the EHD-dried samples were significantly ($p < 0.05$) higher than the one of the oven-dried samples due to a higher amount of remaining water in the material. The overall color difference of the EHD-dried samples was significantly ($p < 0.05$) higher than the oven-dried ones. Similarly, the L^* values of the oven-dried samples were higher. One reason for the color difference could be the Maillard reaction, but the EHD-dried samples showed a significantly lower HMF content ($p < 0.05$), while PEF not affecting the HMF levels. Another reason could be the browning enzymatic activity. However, no PPO activity was observed in the samples. Finally, the color differences could be explained due to oxidative reactions in the sample, which were also confirmed by the volatile profile.

EHD drying did not affect the TPC or the antioxidant activity ($p > 0.05$). However, PEF treatment did increase the free radical scavenging activity and the ferric iron reducing power of the oven-dried samples by 22.90% and 24.05%, respectively. However, this positive effect was not transferred to the EHD-dried insects, which showed no significant differences due to the PEF treatment ($p > 0.05$). Since there are no differences in the TPC ($p > 0.05$), the differences in antioxidant activity were attributed to antioxidant peptides, which have been reported to increase their antioxidant activity and show changes in their properties due to PEF processing (Lin et al., 2017).

The effect of PEF on proteins was observed due to the significant (p

Table 3
Key indicators of the quality of the insects obtained from each processing pathway. Values are presented as mean ± standard deviation (SD). Results are expressed g/100 g wet matter. Superscript letters (a,b,c, and, d) indicate significant differences ($p < 0.05$) among the means of values obtained from replicates of the same measurement ($n = 6$).

	Untreated Oven-dried	PEF treated Oven-dried	Untreated EHD-dried	PEF treated EHD-dried
Bulk density (g/cm^3)	245.56 ± 17.60^a	257.78 ± 15.59^a	303.33 ± 9.19^b	322.22 ± 13.77^c
△E	12.86 ± 1.83^a	10.27 ± 1.22^b	20.56 ± 1.35^c	15.31 ± 1.60^d
TPC (g GAE/100 g)	0.91 ± 0.09^a	0.98 ± 0.18^a	1.00 ± 0.06^a	0.98 ± 0.06^a
Free radical scavenging activity (g TE/100 g)	0.83 ± 0.08^a	1.02 ± 0.15^b	0.95 ± 0.10^{ab}	0.91 ± 0.16^{ab}
FRAP (g TE/100 g)	0.79 ± 0.04^a	0.98 ± 0.10^b	1.16 ± 0.25^c	0.99 ± 0.06^{bc}
Chelating ability (g EDTA equivalent/100 g)	2.75 ± 0.24^a	2.79 ± 0.23^a	2.63 ± 0.18^a	2.29 ± 0.26^b
Protein solubility (g protein/100 g)	5.71 ± 0.47^a	8.74 ± 0.44^b	4.20 ± 0.26^c	3.41 ± 0.78^d
HMF (g HMF/100 g)	3.93 ± 0.66^a	3.97 ± 0.49^a	1.95 ± 0.37^b	2.46 ± 0.46^b
Histamine (mg/100 g)	89.09 ± 18.58^a	90.39 ± 10.52^a	127.27 ± 17.49^b	94.35 ± 5.83^a

< 0.05) increase in the protein solubility from the oven-dried samples (53.07% increase in compare to the control). This increase in solubility could also be attributed to an enhancement of extractability because PEF can enhance the extraction of insect proteins due to electroporation (Psarianos et al., 2022). However, protein solubility was significantly ($p < 0.05$) lower for EHD-dried samples than for oven-dried samples. The EHD-dried samples were subjected to both drying processes and for a longer time, which would cause a higher surface hydrophobicity due to denaturation (Chelh et al., 2006), thus reducing solubility.

Regarding the histamine content, there were no significant differences between the oven-dried samples and the PEF-treated EHD-dried one ($p > 0.05$), but the untreated EHD-dried sample had a significantly higher histamine content ($p < 0.05$). Histamine is produced from histidine due to a reaction with the enzyme histidine decarboxylase that can be produced by bacteria (Epps, 1945). PEF processing and thermal treatment have been reported to reduce enzymatic activity (Huang et al., 2012). However, the lack of PEF treatment and the mild temperature levels of the EHD-dried sample could be responsible for a higher enzymatic activity of histidine decarboxylase. Even though this sample was oven-dried for 1 h, after the EHD drying, it is possible that the histamine was already formed before the sample was subjected to a higher temperature.

From the analysis of the volatile profile of the samples, there were 56 identified molecules belonging to different classes of compounds, including aldehydes, ketones, alcohols, acids, esters, alkanes and pyrazines. Compounds are presented as supplementary material (Table S1).

According to the obtained results, EHD-dried samples, with or without PEF pretreatment, had a higher concentration of volatile compounds than the oven-dried ones. In particular, EHD samples were characterized by a higher amount of acids and pyrazines while oven-dried samples showed the same trend but in lower amounts. Acids reached concentrations of 172.22 ± 10.55 mg/kg and 124.78 ± 28.57 mg/kg in the untreated and PEF-treated EHD-dried samples, respectively, while lower level were measured in oven-dried samples without and with PEF (54.74 ± 1.80 mg/kg and 42.42 ± 3.73 mg/kg, respectively). Acetic acid was the most abundant acid followed by short chain fatty acids.

Pyrazines were the second most abundant class of volatiles reaching amounts of 49.45 ± 7.83 mg/kg and 87.41 ± 46.04 mg/kg in the non-treated and PEF-treated EHD-dried samples, respectively, and 16.21 ± 3.77 mg/kg and 18.26 ± 0.89 mg/kg in the non-treated and PEF-treated oven-dried samples, respectively. The presence of pyrazines in crickets has been reported (Rossi et al., 2021) and was expected since animals use them as attractive or deterrent compounds, depending on the situation (Müller and Rappert, 2010). Although heating and cooking are the main cause of pyrazine formation, their amount in oven-dried samples was lower. This could be explained by pyrazine low vapour pressure and evaporation process that could have reduced their content.

To highlight differences among samples, PCA analysis was carried out on raw data obtained from the analysis of the volatile molecule. Fig. 2 a and b represent the projections of the samples and variables in the spaces contained by the two main components PC1 and PC2, which account for 46.12 % and 32.35 %, respectively, of the total variance among the different samples. Independently from the treatment, oven-dried samples clustered together in the right side of the factorial space and were separated from the EHD-dried samples along the PC1. Instead, EHD-dried samples were separated based on the PEF treatment along PC2. The projection of variables on factor planes showed that different volatile molecules affected the grouping of samples along PC1 and PC2. In fact, most of the pyrazines were found in the PEF-treated EHD-dried samples, while short-chain fatty acids (including Propanoic acid, Butanoic acid, Hexanoic acid, Heptanoic acid and Butanoic acid, 3-methyl-) were found in both EHD-dried samples. The major volatile molecules in oven-dried samples were aldehydes and ketones, such as Pentanal, Hexanal, Propanal, 2-methyl-, 1-Octen-3-one, and 3-Penten-2-one, 4-methyl-. These molecules may be the result of lipid oxidation and fatty acid degradation (Xia and Budge, 2017).

3.3. Energy consumption

The energy requirements of all processing pathways are a summary of the energy requirements of each process separately. The specific energy consumption (*SEC*) of the PEF treatment was 40.21 kJ/kg and the energy that was required for the sample that was subjected to the treatment was 5.03 kJ. For the oven drying and the EHD drying, the power usage was 604 and 12.7 W, respectively. Even though the EHD drying reached equilibrium at approximately 210 min (experimental data), the EHD drying was performed for a maximum of 580 min for all samples. Therefore, the 580 min were considered for the calculation of the energy and not the 210 min (Fig. 3). PEF treatment was able to reduce the energy consumption by 14.22% due to a reduction in the drying time (from 305,928 to 262,425 kJ/kg). However, the processing pathway that includes EHD drying, with and without implementing PEF processing (105,086 and 105,287 kJ/kg, respectively), required less than half of the energy that was consumed for conventional oven drying. Therefore, even though EHD drying by itself was not sufficient to replace oven drying, it can be implemented as an intermediate step to reduce the oven drying time and therefore significantly reduce energy requirements.

Apart from the low energy requirement, EHD drying has been shown to be much more cost-effective than conventional oven drying, when investment cost, cost of drying per kg dried material, payback period and net present value are considered (Iranshahi et al., 2023). PEF has been characterized, also, by cost-efficiency due to its low energy costs and high efficiency as a pretreatment. It poses, however, the drawback of the high setup cost (Ghoshal, 2023; Toepfl and Knorr, 2006).

4. Conclusions

The applicability of EHD drying was tested on house crickets and PEF was used as a pretreatment to facilitate the drying process and enhance the product quality. PEF treatment, when combined with conventional oven drying, could reduce the energy consumption of the process and improve product quality, e.g. antioxidant activity and protein solubility. EHD drying be implemented as an intermediate drying step that would reduce the oven drying to 1 h, with a major decrease in energy consumption and a retention of higher amount of volatile molecules. When the EHD/oven drying processing pathway was combined with PEF processing, there was a further positive effect on product quality due to the reduction of the histamine content. The present study underlines the potential of EHD drying and PEF processing in edible insect production with possible industrial applications due to the higher scale applicability of both processes.

CRediT authorship contribution statement

Marios Psarianos: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Kamran Iranshahi:** Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **Samantha Rossi:** Investigation, Methodology, Validation, Visualization, Writing – review & editing. **Davide Gottardi:** Methodology, Writing – review & editing. **Oliver Schlüter:** Conceptualization, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

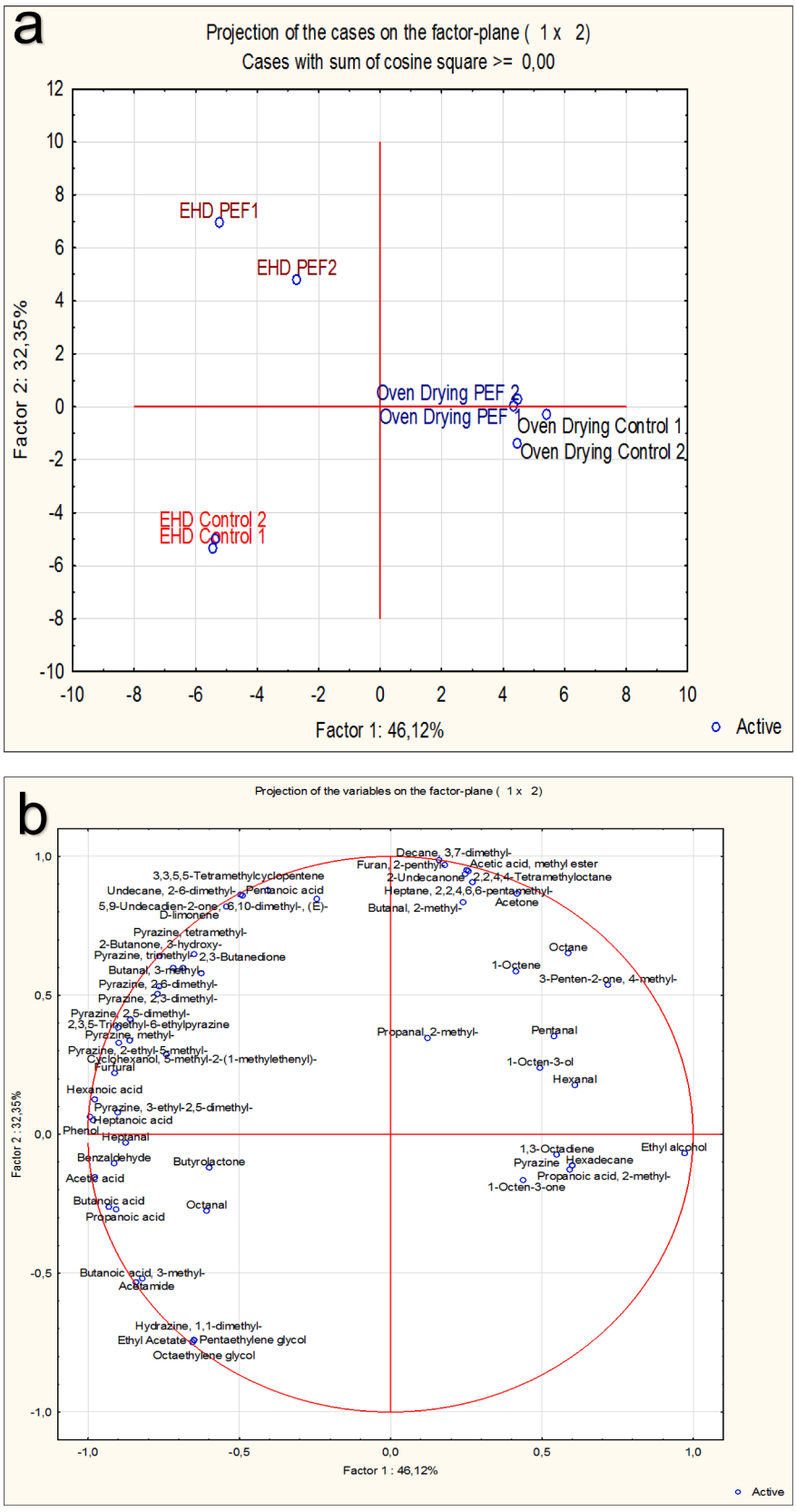

Fig. 2. Projection of cases (a) and variables (b) obtained by PCA elaboration of volatile molecules of the samples that were generated from each processing pathway, samples treated with PEF or untreated (control) and dried with oven drying or EHD drying. The data are shown in duplicate (1, 2).

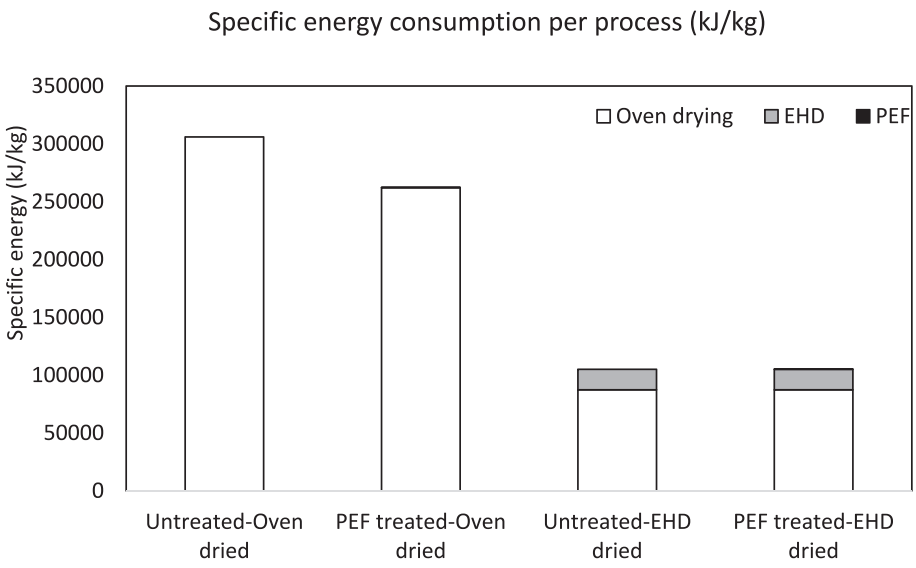

Fig. 3. Energy consumption of the processing pathways that were used for the cricket samples.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.138276.

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6.4 Effect of pulsed electric fields on cricket (Acheta domesticus) flour: Extraction yield (protein, fat and chitin) and techno-functional properties

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Effect of pulsed electric fields on cricket (*Acheta domesticus*) flour: Extraction yield (protein, fat and chitin) and techno-functional properties

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ABSTRACT

Edible insects are an important source of proteins, fat, and chitin, which need to be extracted to develop tailored products with a controlled composition. Pulsed electric fields (PEF) is a non-thermal technology that can enhance the extraction. This study explores the effect of PEF on the extraction of protein, fat and chitin from cricket flour, as well as the material's functional properties. House crickets (*Acheta domesticus*) were treated with PEF at several conditions (4.9–49.1 kJ/kg). PEF treatment with 4.90 kJ/kg increased the extraction yields of protein (>18%) and fat >40%), while the treatment at 24.53 kJ/kg increased the oil binding and emulsifying capacity and antioxidant activity of the cricket flour by 28.10, 64.88 and 58.20%, respectively. Water binding capacity and foaming capacity were not affected by the PEF treatment. These results outline PEF as a suitable pretreatment for the valorization of house cricket biomass with possible industrial application.

1. Introduction

Edible insects have been identified as a source of a variety of nutritional compounds suitable for human consumption including proteins, unsaturated fatty acids and chitin (Rumpold & Schlüter, 2013a). Furthermore, considering their suitability for mass production and the fact that their rearing can be environmentally friendly, edible insects are emerging as an important resource to feed the world population in the future (Rumpold & Schlüter, 2013b). There is a need to develop tailored insect-based products with a controlled composition, high nutritional value and stability (Purschke et al., 2018). Furthermore, in western countries, consumers culturaly resist to eating whole insects due to their appearance and image as pests (Chen, Feng, & Chen, 2009). Insects are more accepted by consumers as food ingredient, in a non-directly visible form (Schösler, De Boer, & Boersema, 2012). Therefore, extracting their nutritional compounds or using in form of flour might make it easier to utilize them in the food sector.

A strong interest has been reported regarding the house cricket (*Acheta domesticus*), because it is relatively easy to raise (Caparros Megido, Haubruge, & Francis, 2017), has a good nutritional profile (Rumpold & Schlüter, 2013a) and has already been used as food for animals and humans in some European countries (Van Huis, 2013). The processing of insects is paramount for safe and high quality insect-based foods. In this regards, effects of conventional food processing techniques on house crickets have been investigated. Several drying methods and other heat treatments such as boiling and steaming have been reported to increase the microbial safety of house crickets (Nyangena et al., 2020) (Fröhling, Bußler, Durek, & Schlüter, 2020; Klunder, Wolkers-Rooijackers, Korpela, & Nout, 2012). Furthermore, pulverization and storage temperature have been underlined as an important factor affecting the shelf life of the crickets (Kamau et al., 2018), while the nutritional quality of the crickets has been shown to be affected by different cooking methods (Manditsera, Luning, Fogliano, & Lakemond, 2019; Porusia, Rauf, & Haryani, 2020).

House crickets have also been studied as a resource for protein extraction and fractionation (Laroche et al., 2019; Ndiritu, Kinyuru,

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Kenji, & Gichuhi, 2017; Udomsil, Imsoonthornruksa, Gosalawit, & Ketudat-Cairns, 2019; Yi et al., 2013), with a reported 20–40% protein yield in a liquid fraction and an approximately 60–75% purity (Laroche et al., 2019; Ndiritu et al., 2017). Furthermore, they have been used as a starting material for fat extraction and isolation with several methods (Laroche et al., 2019; Tzompa-Sosa, Yi, van Valenberg, van Boekel, & Lakemond, 2014) and solvents (Ramos-Bueno, González-Fernández, Sánchez-Muros-Lozano, García-Barroso, & Guil-Guerrero, 2016). The fat yield of the house crickets has been reported to reach 25% (Laroche et al., 2019).

During the last decades, novel food processing technologies have emerged, which have been shown to improve the extraction yield of the intracellular compounds of a material. One of these technologies is pulsed electric fields (PEF), which involves the application of electric field at high intensity in the form pulses for a very short period (μs) on a sample that is placed between two electrodes. PEF processing leads to an increase of the transmembrane potential of the sample's cells, creating pores on the cell membrane, a phenomenon known as electroporation. For electroporation to occur, the applied field intensity should surpass a critical value. Electroporation can be temporary or permanent, depending on the level of the applied field intensity in comparison to the critical value (Raso-Pueyo & Heinz, 2010). PEF has been applied as a means to improve protein extraction from olive pomace, microalgae and mushrooms (Varvara Andreou, Psarianos, Dimopoulos, Tsimogiannis, & Taoukis, 2020; Buchmann, Brändle, Haberkorn, Hiestand, & Mathys, 2019; Xue & Farid, 2015) and fat extraction from olives, microalgae and sunflower seeds (V Andreou et al., 2017; Lai, Parameswaran, Li, Baez, & Rittmann, 2014; Shorstkii, Mirshekarloo, & Koshevoi, 2017) . Regarding the application of PEF on insects, a bio-refinery study included the application of PEF and reported the enhancement of the drying process of *Hermetia illucens* larvae and the increase of amino acid content on the extracted fat from the larvae, even though the fat extraction yield was not affected (Alles et al., 2020). Optimization of PEF-assisted drying of *H. illucens* larvae has reported enhancement of the drying process and a higher effect of temperature than PEF on the energy consumption of the process (Shorstkii et al., 2020). In another study intense PEF treatment led to a higher cell disintegration of *Tenebrio molitor* and enhanced the pressing extraction of lipids (Smetana, Mhemdi, Mezdour, & Heinz, 2020).

Consequently, PEF can be considered a promising process for utilization of insects in the food sector. However, to the best of our knowledge, there is no study exploring the effect of PEF on house crickets. Therefore, the aim of this study was to implement PEF treatment in the production of house cricket flour, which can be used directly as a food ingredient or as a substrate for extraction of nutritional compounds. Consequently, the effect of PEF treatment was explored both on the functional properties of the flour and the extraction yield of proteins, fat and chitin.

2. Materials and methods

2.1. Sample preparation

Living house crickets (*A. domesticus*) were purchased at an adult age from Tropic-Shop (Nordhorn, Germany) and were stored for 2 h inside a cold room at 4 °C to reduce their movement activity. Afterwards they were freeze inactivated at -20 °C packed in plastic pouches. Before any treatment, the insects were washed with cold water to remove impurities and then ground fresh for 10 s with a Retsch Mill (Retsch Grindomix, Retsch GmbH, Germany).

2.2. Pulsed electric fields (PEF) pretreatment and extraction

Treatments were performed on the fresh, grinded insects without any addition of water, with the ELCRACK HVP-5 (DIL, Quackenbrück, Germany) PEF system inside a batch chamber with a 40-mm electrode gap width. During the treatments, the pulse was monitored with an oscilloscope (Tektronix TDS 1012, Beaverton, OR, USA) with two channels. One channel showed a positive pulse that corresponded to applied peak voltage and the other channel showed a negative pulse that corresponded to the current. The measured values were shown by the oscilloscope by the peak-to-peak readings. The pulse was bipolar and near rectangular. Treatments were carried out at 1.5 kV/cm. The nominal pulse width and the pulse frequency were kept constant at 15 μs and 20 Hz, respectively. The number of pulses applied ranged between 100 and 1000 (energy input between 4.9 and 49.1 kJ/kg). The temperature of the sample was measured before and after treatment with a digital thermometer (General Tools & Instruments, NJ). Before treatment, temperature was equal to 20 °C and after treatment never exceeded 32 °C.

The energy input was calculated via Eq. (1) (Raso et al., 2016):

$$W\ (kJ/kg) = n \cdot \frac{1}{m} \cdot \int_0^{\infty} V(t) \cdot I(t) dt \quad (1)$$

where, n is the number of pulses, m(kg) is the mass of the treated sample, and V(Volt) and I(Ampere) are the voltage and current at time t(s), respectively.

After the PEF treatment, the samples were frozen at −20 °C overnight and freeze-dried at -20 °C for 48 h, using a Christ Alpha 1–4 LD Plus (Osterode, Germany) freeze dryer. Since the increase of temperature can affect negatively the functional properties of the material (Lucas-González, Fernández-López, Pérez-Álvarez, & Viuda-Martos, 2019), a freeze-drying process was preferred. The water content of the crickets was 66.96 ± 2.20%.

All the chemicals were purchased from Carl Roth (Kalsruhe, Germany), unless stated otherwise. Fig. 1 shows the flow process diagram of the treatments applied in the present study.

2.3. Characterization of the material

2.3.1. Composition analysis of the non-PEF-treated insect flour

The approximate composition of the house crickets was determined with standard methods from literature. Moisture content was determined after placing the sample in an oven at 105 °C for 48 h and calculating the weight difference. Total ash was determined after burning the sample into an oven at 550 °C for 8 h. Total protein content was measured with a ninydrin-based assay (Starcher, 2001), after hydrolyzing the proteins with HCl 6 N for 24 h at 98 °C. Total carbohydrates were measured with the phenol-sulfuric acid protocol (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), after hydrolyzing the carbohydrates with sulfuric acid 12 M, for 2 h at 98 °C and diluting at 100 mL with sodium acetate buffer 0.2 M, pH = 5. Crude fat was determined with a Soxhlet apparatus operating for 6 h with n-hexane as solvent at 68° C. Chitin content was determined by measuring the content of glucosamine and N-Acetyl-Glucosamine (Zamani, Jeihanipour, Edebo, Niklasson, & Taherzadeh, 2008). Standard chitin was used for the calibration curve.

2.3.2. Functional properties

2.3.2.1. Water binding capacity (WBC). Half a gram of cricket powder was weighed into 15 mL centrifuge tubes and mixed with 2.5 mL of water, which was also weighed with the sample. Mixtures were vortexed for 60 s, and then centrifuged at 4000 ×*g* for 20 min. After discarding the supernatant, the pellet was weighed. Using the following equation, WBC is calculated and expressed as g water/g d.w. (Bußler, Steins, Ehlbeck, & Schlüter, 2015).

$$WBC\ (g\ water/g\ dw) = \left(m_f - m_0\right)/m_{0,dw} \quad (2)$$

where, m_0 is the initial weight of the sample, m_f is the final weight of the wet sample and $m_{0,dw}$ is the initial weight of the sample based on dry matter.

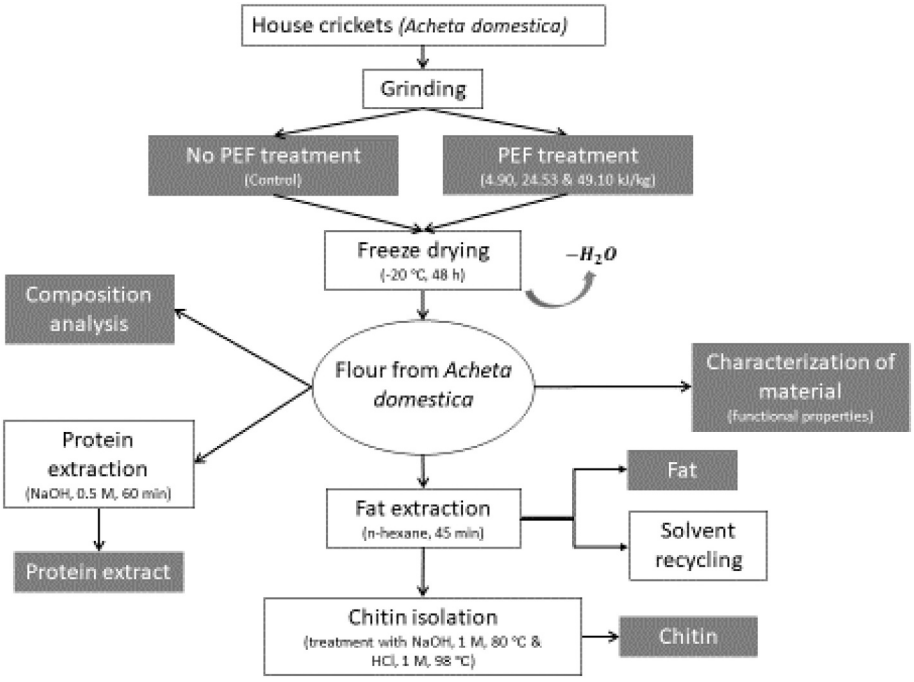

Fig. 1. Processing lines of house crickets combined with a PEF treatment.

2.3.2.2. Oil binding capacity (OBC). For determination of the OBC a similar procedure as for the WBC was followed. In summary, 0.5 g of cricket powder was weighed and mixed with 2.5 mL of commercial rapeseed oil, which was also weighed. Mixtures were vortexed for 60 s, and then centrifuged at 4000 ×*g* for 20 min. After removing the supernatant, the pellet was weighed and OBC was calculated using Eq. (2) and expressed as g oil/g d.w. (Schwenke et al., 1981).

2.3.2.3. Emulsifying capacity (EC). One gram of cricket powder was added to 50 mL H_2O and afterwards 50 mL of commercial rapeseed oil was added. Then the mixture was homogenized for 15 min at 9500 rpm (T-25 Ultra turrax, IKA, Staufen, Germany). The emulsion was centrifuged at 10000 ×*g* for 10 min in order to be broken. The height of the resulting emulsified layer (H_{EL}) and the total height of solution (H_S) were used to calculate the EC (%) based on the following equation (Yasumatsu et al., 1972):

$$\text{EC}\ (\%) = \frac{H_{EL}}{H_S} \times 100 \tag{3}$$

2.3.2.4. Antioxidant activity. 0.5 g of cricket powder was mixed with 5 mL of an 80% methanol solution and the mixture was vortexed thoroughly for 60 s. Afterwards, the mixture was centrifuged at 4 °C, 3200 ×*g* for 10 min and the supernatant was collected. The pellet was suspended in 5 mL of a 70% acetone solution and vortexed thoroughly for 1 min. The mixture was centrifuged at 4 °C, 3200 ×*g* for 10 min and the supernatant was collected and mixed with the one obtained from the previous centrifugation. The liquid was placed in a rotary evaporator (Buchi R-100, Flawil, Switzerland) connected with a vacuum pump (Buchi V-100) and an interface (Buchi I-100) set at 45 °C. After the solvents were completely removed, the solids were suspended in 5 mL of methanol. The mixture was centrifuged at 4 °C, 7000 ×*g* for 10 min and the supernatant was collected. Antioxidant activity was measured with the DPPH radical scavenging assay. (Lucas-González et al., 2019) Briefly, 0.1 mL of supernatant was mixed with 3.9 mL of a $6{\cdot}10^{-5}$M DPPH solution and incubated for 30 min in the dark at room temperature. Afterwards, the absorbance was measured at 515 nm. Methanol was used as a blank. Trolox was used for the calibration curve and the results were expressed as μg Troxol equivalents (μg TE/g d.w.).

2.3.2.5. Foaming capacity (FC). Cricket powder was added to water to obtain a mixture of 1% *w/v*. The mixture was homogenized for 1 h at room temperature. Afterwards the mixture was whipped for 2 min using a disperser at 12000 rpm. The height of the foam was measured 0.5 min and 30 min after the dispersion. The foaming capacity was calculated using Eq. (4):

$$\text{FC}\ (\%) = \frac{V_t}{V_0} \cdot 100 \tag{4}$$

Where, V_t is the volume (mL) is the volume of the foam at time t after the dispersion and V_0 is the volume (mL) of the initial liquid (Purschke et al., 2018).

2.4. Isolation of the valuable compounds

2.4.1. Fat extraction

Five grams of insect powder were added to 200 mL n-hexane (>95%). The mixture was stirred for 45 min within capped glass containers to avoid evaporation of the solvent. After the extraction, the mixture was centrifuged at 3200 ×*g*, 10 min, 15 °C and the supernatant was collected and moved to a rotary evaporator system, as described above, until the solvent was completely evaporated (Ravi et al., 2019). The extracted fat yield was expressed as g fat/100 g d w.

2.4.2. Protein extraction

The powder was mixed with NaOH, 0.5 M at a solid/liquid ratio of 1:50 and stirred for 60 min at room temperature (Rausch, 1981). During the extraction, 2 mL of solid-liquid mixture were withdrawn at fixed time intervals of 15 min and centrifuged at 10000 ×*g*, 10 min, 20 °C. The supernatant was isolated and stored at 4 °C for further analysis. The extracts remained for a maximum of 24 h stored at 4 °C before their protein content was determined.

2.4.3. Chitin isolation

The defatted samples were mixed with NaOH, 1 M (s/l ratio = 1:50) and incubated at 80 °C for 22 h, under agitation, in order to remove the proteins. The solvent was removed with centrifugation at 10000 ×*g*, 10 min, after the mixtures were cooled down to room temperature. The

pellets were placed on filter paper and washed with hot water at 60 °C (Kaya et al., 2015; Mohammed, Williams, & Tverezovskaya, 2013; Percot, Viton, & Domard, 2003). Afterwards they were added to HCl, 1 M (s/l ratio 1:30) and further incubated for 2 h at 98 °C under agitation, in order to remove the minerals (Mahmoud, Ghaly, & Arab, 2007). The solvent was removed with centrifugation at 10000 ×*g*, 10 min. Finally, the pellet was washed with hot water (60 °C) and the samples were dried in a vacuum oven at 55 °C. The obtained solid constitutes the extracted chitin. Results were expressed as g chitin/100 g d.w.

2.4.4. Determination of the protein content of the extracts

The soluble protein content of the extracts was determined using the Bradford micro-assay (Bradford, 1976) as modified by Carl Roth (Kalsruhe, Germany), using the commercial 5-X Bradford reagent (Carl Roth, Kalsruhe, Germany). In summary, 800 μL of the diluted (1:400 or 1:500) extracts were mixed with 200 μL of Bradford reagent and incubated for 15 min at 25 °C. The absorbance of the mixtures were measured at 595 nm using a UV/Vis spectrometer (Spectronic Unicam UV1, Thermo Fisher Scientific, Waltham, MA, USA). The results were translated to protein concentration via a standard curve prepared with bovine serum albumin. The protein content was expressed as g protein/100 g d.w.

2.4.5. Scanning electron microscopy (SEM) analysis of chitin

The morphology of chitin was studied with a scanning electron microscope (Quanta 200, FEI Oregon, USA/ voltage 12.5 kV, LFD detector, Spot size 4.5, magnification 12000X) with 11.34 nm gold layer coating applied prior to SEM (SC7620 Mini Sputter Coater, Quorum Technologies, West Sussex, UK/ 90 s, 18 mA, 1 KV), in order to make their surface reflect the electron beam.

2.5. Statistical analysis

All experiments were conducted at least in triplicate. Significant differences between data obtained from samples treated at different conditions and between the coefficients of the models were identified using a one-way analysis of variance (ANOVA). Duncan's multiple range test was applied post-hoc to separate means with significant differences at a significance level of 0.05. Data that did not follow a normal distribution were normalized before being analyzed. The software used was IBM SPSS Statistics 23 (IBM Corp., Armonk, N.Y., USA). The error bars on each graph indicate the standard error of measurement of several repetitions of the same process and measurement.

3. Results and discussion

3.1. Characterization of the material

The composition of the crickets (see Table 1) was in agreement with previous studies reported in the literature. House crickets have been reported to contain a high portion of proteins that ranges between 64 and 70% on dry basis (Lucas-González et al., 2019; Rumpold & Schlüter, 2013a), but also a significant amount of fat that ranges between 18 and 22% on dry basis (Rumpold & Schlüter, 2013a; Williams, Williams, Kirabo, Chester, & Peterson, 2016). The ash content of the crickets that is reported by the present study is within the range reported in the literature, which is between 3.5 and 5% on dry basis (Rumpold & Schlüter, 2013a; Williams et al., 2016), but is still considered relatively low. However, their composition shows a high variation (Rumpold & Schlüter, 2013a), which can be attributed to the composition of their feed (Nakagaki & Defoliart, 1991).

Table 1
Composition (g/100 g dry weight) of the adult house cricket flour.

Compounds	g/100 g dry weight
Moisture	10.97 ± 2.41
Proteins	72.45 ± 1.30
Crude fat	18.19 ± 0.63
Ash	3.97 ± 0.96
Carbohydrates	6.64 ± 0.15
Chitin	7.34 ± 0.73

Values are presented as mean ± SD.

As shown in Fig. 2a, PEF treatment had a significant effect ($p < 0.05$) on the oil binding capacity. The OBC of the flour that was subjected to the most intense PEF treatment conditions was increased from 2.27 to 3.21 g oil/g d.m. (41.3% increase). However, no significant differences ($p > 0.05$) were observed between the two samples treated at an energy input of 24.53 and 49.10 kJ/kg. The water binding capacity and foaming capacity of the flours were not affected by PEF treatment, since no significant differences ($p > 0.05$), between the samples treated with PEF and the untreated one, were observed. PEF induced cell permeabilization enhances transport phenomena between the intracellular and extracellular environment. However, since the cytoplasm of the eukaryotic cells consists mainly of water (Shepherd, 2006), it is assumed that after disrupting cell membranes, water can only enter the cell more easily, but cannot be bound by the sample. The values of the OBC reported in the present study are similar to the one reported in the literature, while the WBC is lower than the one reported by the literature. Specifically, WBC and OBC of freeze-dried house cricket flour have been reported to be 3.82 and 2.86 g of water or oil per g of sample, respectively. Both properties have also been reported to decrease, when oven-drying at 60 °C is used instead. The difference is attributed to alteration in protein structure and protein hydrophobicity (Lucas-González et al., 2019). However, there was no thermal effect during the PEF processing during the present study to suggest a similar alteration of protein structure. Therefore, the differences of OBC are greatly attributed to electroporation. A similar trend has been reported for freeze-dried cricket powder, after a high-pressure treatment at 500 MPa, where the WBC was not affected, but the OBC increased (Bolat, Ugur, Oztop, & Alpas, 2021).

PEF treatment also had a significant effect ($p < 0.05$) on the emulsifying capacity (Fig. 2c). Even the least intense PEF treatment condition led to an increase of the EC by 22.1%, while the most intense treatment led to a 74.7% increase of the EC. There is a strong correlation between protein content of a material and EC (Bußler, Rumpold, Jander, Rawel, & Schlüter, 2016). The sample was in contact with the water fraction, meaning that some proteins were exposed to it during that time. Therefore, the increase of emulsifying capacity could be explained due to the increased extractability of the proteins to the water fraction that could enhance hydrophobic interactions (Jung, Murphy, & Johnson, 2005), as well as by the enhanced oil binding capacity of the flours.

The EC reported in the present study is lower than the one reported by the literature, after using similar emulsifying parameters. Specifically, the EC of house cricket flour has been reported to range between 39 and 59% (Kim, Setyabrata, Lee, Jones, & Kim, 2017; Lucas-González et al., 2019) These differences are attributed to the different methods for preparing the emulsions. In specific, Lucas-González et al. (2019) homogenized the samples with water before they put the oil in the mixture, which could lead to a higher amount of proteins being extracted in the water fraction, leading to a higher EC. Furthermore, Kim et al. (2017) used a higher amount of sample. In specific, they used 7 g of samples and 200 mL of water and oil, while in the present study 1 g of powder is mixted with 100 mL of oil and water. The different ratio could lead to a different EC.

Further, PEF treatment led to a significant ($p < 0.05$) increase of the antioxidant activity of the samples (Fig. 2e). In specific, the sample treated at 24.53 kJ/kg showed a 58.20% higher antioxidant activity than the untreated sample. However, the sample treated with the most intense condition showed an increase of antioxidant activity of 29.57%, in comparison to the control, as shown in Fig. 2e. It is important to note, that the antioxidant activity of the flour is measured from an extract obtained by the flour. PEF has been shown to increase the extraction

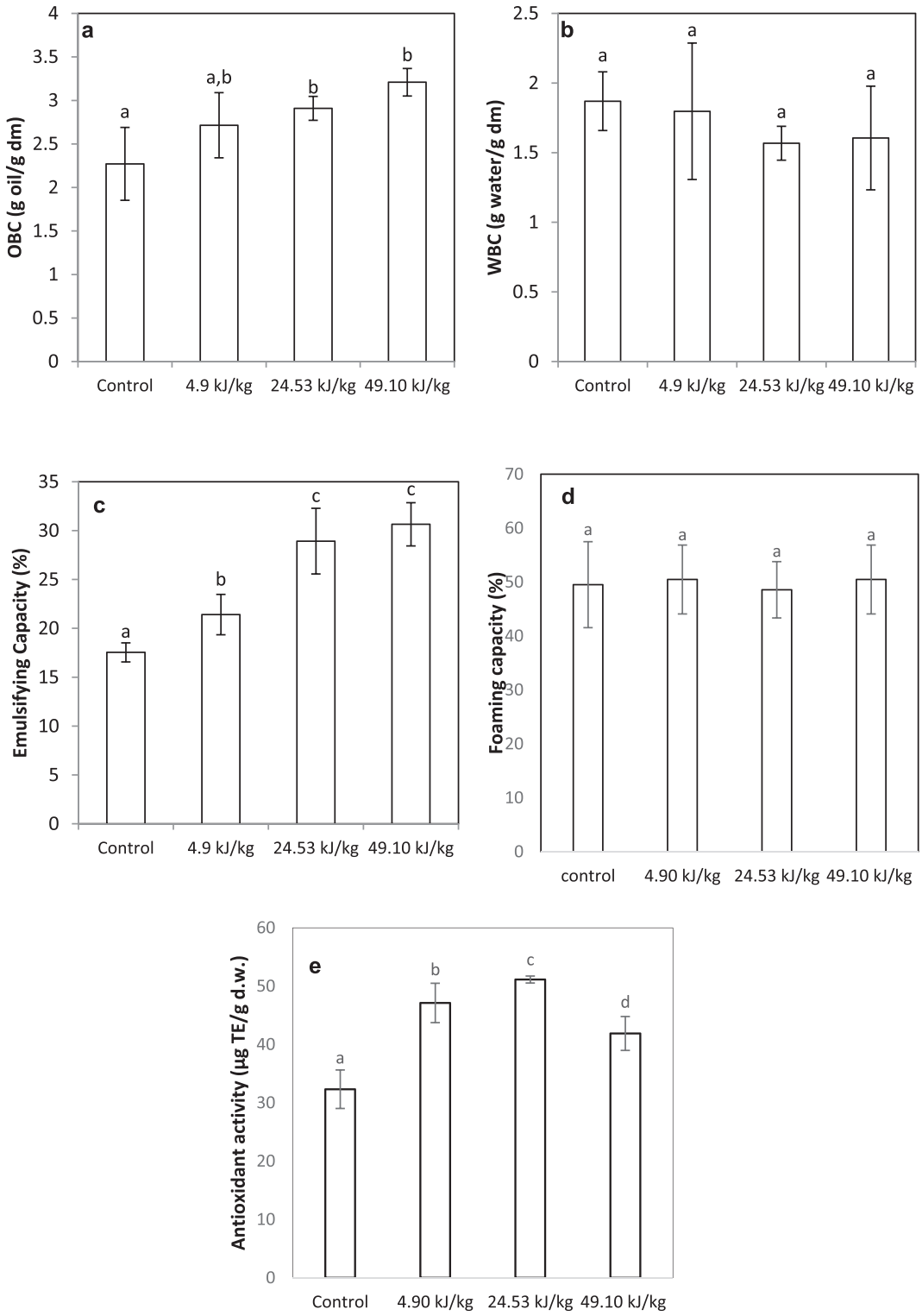

Fig. 2. Effect of PEF pretreatment on the functional properties of the cricket flour: (a) oil binding capacity (g oil/g d.m.), (b) water binding capacity (g water/ g d. m.), (c) emulsifying capacity (%), (d) foaming capacity (%), (e) antioxidant activity (µg TE/g d.w.). The error bars indicate the standard errors of measurements. Superscript letters (a,b,c…) indicate the significant differences ($p < 0.05$) between the means of the functional properties of PEF-treated samples and the untreated sample (control).

yield of antioxidant compounds from food materials, leading to an enhanced antioxidant activity of the extract itself (Varvara Andreou et al., 2020). However, the antioxidant activity of some peptides has been reported to decrease, after a PEF treatment due to possible changes to the functional groups of the peptides (Liang, Cheng, & Wang, 2018). This could explain the slight decrease of antioxidant activity of the sample that was subjected to the most intense treatment.

3.2. Effect of PEF pretreatment on the isolation of valuable compounds

3.2.1. Fat extraction

As shown in Fig. 3, every PEF pretreatment led to a significant ($p < 0.05$) increase of the isolated fat yield. The fat isolated from samples treated on different PEF conditions did not show any variation, making 4.90 kJ/kg the most appropriate treatment, since it is less energy consuming. Specifically, pretreating the fresh material with PEF at 4.90 kJ/kg led to a 41.75% increase of the fat yield.

The results obtained by the present study differed from the study of Alles et al. (2020), who did not observe an increase on the oil yield after PEF treatment of freeze-dried biomass of *H. illucens*. Specifically, they reported an approximately 30% oil yield from all samples, including the untreated one, after pressing the insect mass with a screw press. It is possible that their control extraction procedure was exhaustive enough to obtain the highest possible yield, since the screw press they used was preheated at 100 °C. Additionally, the age of the crickets affects their fat body mass, lipid and protein content (Anand & Lorenz, 2008). The crickets used in the present study are adults, which were expected to contain less fat than crickets at lower instars, while the lipid content of the fat body of the adult house crickets is approximately 65% (Woodring, Clifford, & Beckman, 1979). Furthermore, the crude fat of black soldier fly larvae was, also dependent on the age of the insects, while showing the highest value (approximately 30%) at the later larval and pre-pupal stages (Liu et al., 2017). Additionally, the amount of fat body and fat accumulation of insects differs among species (Arrese & Soulages, 2010). It was considered that these differences among the species and age of the insects played a significant role in the difference of yield. However, PEF treatment has been successful in enhancing the lipid extraction yield from *T. molitor,* while increasing the yield of the insect juice obtained by pressing from 41 to 55%. PEF has been also shown to cause cell disruption to *T. molitor* larvae samples (Smetana et al., 2020).

Apart from PEF, the fat extraction yield from house crickets has been reported to increase after an ultrasound treatment by direct sonication, reaching a yield of 24.85 g extract/100 g d.w . However, the yield was depended on the extraction medium as well (Otero, Gutierrez-Docio, Del Hierro, Reglero, & Martin, 2020). On the contrary, high pressure processing has been shown not to affect the fat extraction yield from house crickets (Ugur, Bolat, Oztop, & Alpas, 2020), but it has been observed to enhance the extraction of phenolic compounds from house cricket powder (Bolat et al., 2021).

The fat yield obtained from the control samples was similar to that obtained with extraction using hexane by Ramos-Bueno et al. (2016), although they obtained a higher yield using direct methylation. Additionally, the extracted fat from the control reported by the present study (14.52 g/100 g dry weight or 4.79 g/100 fresh weight) was lower than one obtained from *A. domesticus* by Tzompa-Sosa et al. (2014) with a Folch lipid extraction (8% in fresh weight basis) and Soxhlet extraction (6% in fresh weight basis). This result could be attributed to the different extraction procedures, even though the yield was increased after PEF processing of the material. The fat yield from the untreated material that is reported by the present study was lower than the range reported by the literature, but the PEF pre-treatment offered the possibility of a yield increase. Furthermore, considering that most of the lipid content of insects is stored as body fat (Canavoso, Jouni, Karnas, Pennington, & Wells, 2001), and therefore easily extracted, a high fat yield was expected after applying fat isolation processes that were exhaustive for the material.

3.2.2. Protein extraction

It was observed that during the whole duration of the extraction, the different treatments resulted in a significant ($p < 0.05$) increase of protein yield compared to the untreated one, but showed no significant differences ($p > 0.05$) in comparison to each (Fig. 4) other. In specific, after 15 min of extraction, the yield from the sample treated at 4.90 kJ/kg was 32.47% higher, while after 60 min of extraction the yield was 18.62% higher. Moreover, after 15 min of extraction, the yield increase between the control and the samples treated with 24.53 and 49.10 kJ/kg was 30.47 and 39.55%, respectively. The yield increase between the untreated sample and the samples treated with 24.53 and 49.10 kJ/kg, after 60 min of extraction, was 22.76 and 16.55%, respectively. Therefore, it was concluded that 4.9 kJ/kg is an appropriate treatment condition, leading to an 18.62% increase of the protein yield after 60 min.

Yi et al. (2013) performed aqueous protein extraction on N_2-frozen house crickets, among other insect species, followed by a centrifugation step to separate the extract and pellet and reported an approximately 20% of the total protein in the supernatant, while measured crude protein content to be 21.5% of the fresh weight (70.8% of moisture) of the house crickets. Therefore, the protein content of the supernatant (liquid fraction) based on dry weight was calculated and was equal to 14,73%. Ndiritu et al. (2017) used the method of Yi et al. (2013) for protein fractionation and reported a 32% yield of the liquid fraction with a 66% protein content on dry basis, meaning they isolated 21.12% of the proteins on the aqueous fraction. Laroche et al. (2019) used mild alkaline extraction conditions (0.25 M NaOH) and heating at 40 °C to obtain a 30% protein yield with an approximate of 75% purity on dry basis from defatted house cricket powder and Udomsil et al. (2019) performed a pH-dependent extraction with mild heating (up to 60 °C) and reported

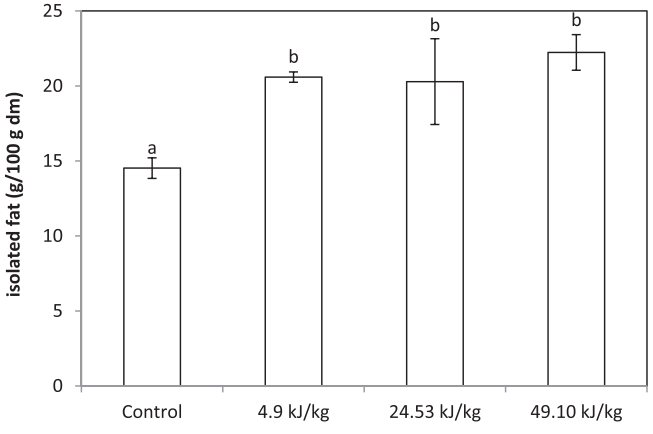

Fig. 3. Effect of PEF pretreatment on the fat extraction yield (g fat/100 g d.w.). The error bars indicate the standard errors of measurements. Superscript letters (a,b,c…) indicate the significant differences ($p < 0.05$) between the means of the functional properties of PEF-treated samples and the untreated sample (control).

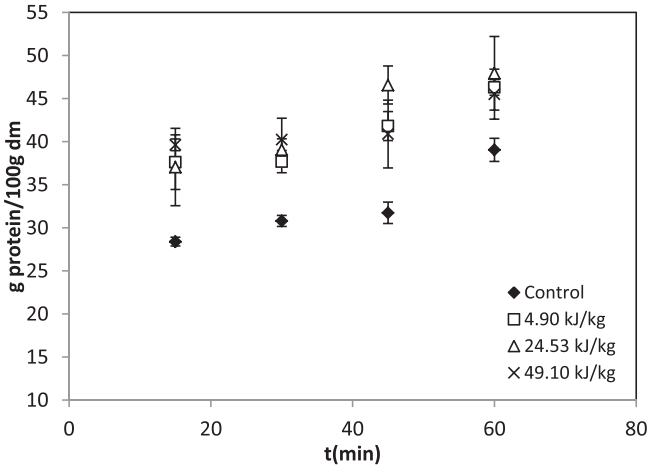

Fig. 4. Effect of PEF pre-treatment on the protein extraction (duration of 15, 30, 45 and 60 min) yield from insect flour. The error bars indicate the standard errors of measurements.

a maximum protein yield of 25 mg/100 g. However, the combination of a PEF treatment with an increased pH has been shown to further enhance the protein extraction yield (Parniakov et al., 2015).

Furthermore, Smetana et al. (2020) reported that PEF led to an increase of the oil yield in the pressed extract from *T. molitor*, when the protein content of the extract was low (~1 g/L) and not affected by PEF. Finally, according to Alles et al. (2020), PEF did not increase the oil yield after pressing, but did increase the amino acid content of the oils, indicating a slight effect of PEF on protein removal.

The protein yield observed in the present study after 15 min of extraction from the untreated freeze-dried material was slightly higher (28.4 g/100 g d.w.) than the one reported by previous studies. This difference is attributed to the alkaline conditions of the extraction. The lack of effect of PEF on the protein extraction, as well as the low yield, reported by Smetana et al. (2020) differed from the results of the present study. However, the efficiency of an extraction is related to the ability of the solvent to fully penetrate the cells of the material (Mercer & Armenta, 2011). It is considered that the low efficiency of the protein extraction presented by Smetana et al. (2020) was due to the exclusion of solvents from the extraction.

3.2.3. Chitin isolation

The chitin isolation yield obtained from all different samples did not vary significantly ($p > 0.05$) and it was equal to 10.10 ± 1.50 g chitin/100 g d.w. Even though PEF was shown to increase protein and oil removal from the solid, this effect did not transfer to the protocol applied for chitin isolation. This protocol was on its own exhaustive, since chitin is not extracted from within the cells, but isolated through a procedure that aims to remove the rest of the compounds from the sample. The isolated chitin yield was higher than the one extracted by Hirsch, Cho, Kim, and Jones (2019), who reported a yield of 5.14% in basis of dried weight (Hirsch et al., 2019). Furthermore, the yield of the isolated material seemed to be higher than the chitin content of the material. This was an indication of remaining impurities on the isolated chitin. Although chitin yield was not affected by PEF ($p > 0.05$), the morphology of chitin was influenced by PEF treatment (Fig. 5).

It was observed that the chitin obtained has pores with a various diameter, a smooth surface (Fig. 5a,c) and with evident nanofibers (Fig. 5a,b). A similar morphology of chitin has been observed for *Brachytrupes portentosus* (Ibitoye et al., 2018), *Zophobas morio* (Soon, Tee, Tan, Rosnita, & Khalina, 2018) and *Argynnis Pandora* (Kaya, Bitim, Mujtaba, & Koyuncu, 2015). Furthermore, it appears that the chitin obtained from insect flours that were subjected to the two most intense PEF treatments appear smoother. During the chitin isolation process with the sequential chemical treatments, the linkage of the *N*-acetyl-D-glucosamine monomers that are connected in a fibril network is distorted. This distortion is observed through the appearance of cracks on the surface of chitin, (Asif et al., 2019).

It is important to note that the cuticle of insects consists of an outer layer called epicuticle that consists of mainly proteins and lipids and a thick procuticle that consists mainly of chitin linked to the functional groups of proteins(Andersen, Hojrup, & Roepstorff, 1995; Jonas-Levi &

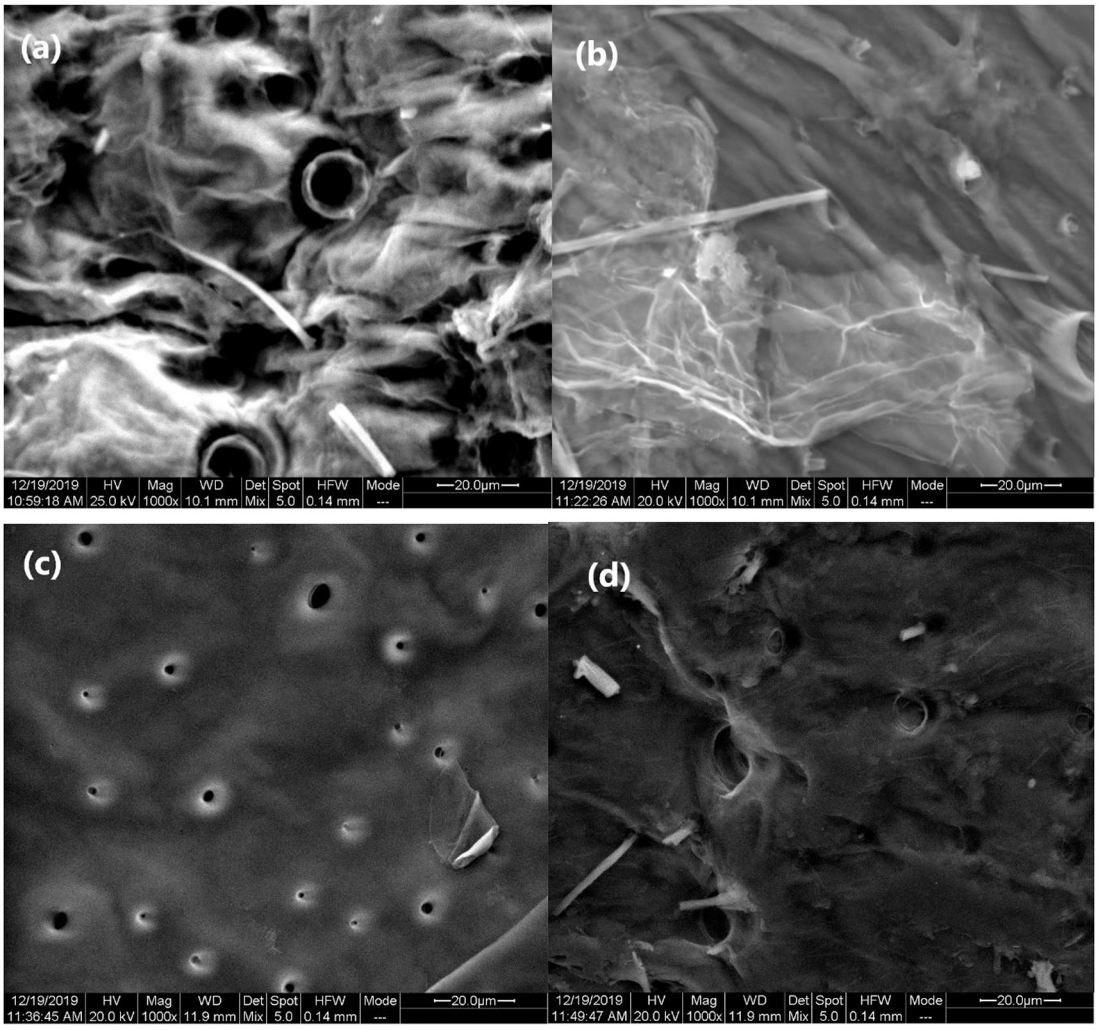

Fig. 5. SEM pictures of isolated chitin from house crickets with an analysis magnification of 1000×. (a): Control, (b): 4.9 kJ/kg, (c): 24.53 kJ/kg, (d): 49.10 kJ/kg.

Martinez, 2017). Therefore, it was assumed that since PEF treatment is shown to enhance protein extraction, it can facilitate the deproteinization process. Consequently, PEF treatment can have a positive effect on the surface structure of the isolated chitin, since the easier removal of proteins can decrease the effect of the alkaline treatment on the chitin. However, further studies are needed to confirm this hypothesis.

4. Conclusions

The results obtained from the present study lead to the conclusion that PEF enhances the functional properties of house cricket flour as a food material and assists the fractionation of valuable compounds (protein, fat and chitin). PEF treatment at 4.90 kJ/kg increased the OBC, EC and antioxidant activity of the cricket flour by 19.53, 22.06 and 45.79%, respectively. PEF treatment also increased the extraction yields of protein and fat by over 18% and 40% respectively. Based on the summary of results, PEF treatment at 4.90 kJ/kg can be considered the most appropriate among the tested PEF treatment conditions. Further, technological advancement, economic viability and sustainability of PEF processing for insects should be considered for further studies. Additionally, the effect of PEF on the bioactivity and properties of the isolated fractions from the crickets should be evaluated since the present study focuses only on the effect of PEF treatment on the extraction yield of the crickets' compounds. In conclusion, PEF can be applied to assist the utilization of house crickets as a food resource (whole flour and extracted fractions), while the sustainability and possible continuous use of PEF could offer a complete procedure for advancing this technology at industrial scale.

Conflict of interests

The authors have declared no conflict of interests.

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6.5 Application of emerging technologies for valorization of biomass from house crickets for food applications: an insect bio-refinery

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Evaluating an emerging technology-based biorefinery for edible house crickets

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Introduction: Edible insects, specifically house crickets, are expected to play an important role in the future food systems due to their rich nutritional profile, low environmental impact and growing consumer acceptance as food. Their content of proteins, lipids, chitin and phenolics offer great potential for the valorization of their biomass into nutritional end products and fractions. Furthermore, emerging food processing technologies and green solvents are relevant for improving the valorization process.

Materials and methods: High pressure (HP) and ultrasound (US) processing were implemented in an insect biorefinery system, where a hexane/methanol/water solvent was used to separate fat, phenolics and a solid fraction containing proteins and chitin. Subsequently, a deep eutectic solvent of betaine and urea (B/U) was used to for protein and chitin isolation.

Results: A maximum of 15% of fat was isolated, with no positive effect from the US or HP treatments. The US treatment enhanced the phenolic extraction yield by 38.69%, while HP negatively affected the antioxidant capacity. B/U was efficient in separating proteins and chitin, resulting in a protein concentrate with a protein content ≥80% and a chitinous fraction with a chitin content ≥70%.

Conclusion: House cricket biomass can be refined into valuable fractions with a quick and simple method, making the process industrially relevant.

KEYWORDS

resilient food processing, ultrasound, high pressure, deep eutectic solvent, extraction, fractionation

1. Introduction

Edible insects are a promising resource for utilization in the food sector due to their high nutritional value (1) and low environmental impact (2). House crickets (*Acheta domesticus*) are particularly interesting since they have a history of being farmed (3) and are consumed as food and feed in some parts of the world (4). Furthermore, they have been accepted as novel food in the EU (5) and have also been proposed as a food ingredient (6, 7).

A biorefinery refers to the conversion of a biomass feedstock to a number of functional or valuable products (8). Biorefineries are processing facilities that convert biomass into value-added products such as biofuels, biochemicals, bioenergy/biopower, and other biomaterials. Various types of biorefineries have been presented in the literature. Most of them are mainly defined based on individual feedstock, such as corn-based biorefinery, wood-based biorefinery, forest-based biorefinery, palm-based biorefinery, and algae-based biorefinery. However, some researchers and technologists defined biorefineries based on the generation of feedstock, which are first-generation biorefinery (energy crop, edible oil seeds, food crops, and animal fats), second-generation biorefinery (lignocellulosic biomass), and third- or fourth-generation biorefinery (algae and other microbes) (9).

House crickets are characterized by high protein and fat contents (1), phenolic contents (10), and chitin, from which chitosan with antimicrobial properties can be produced (11). These compounds underline the potential of the cricket biomass to be refined into food ingredients, biomaterials, and feed (12). The possibility of insect biorefinery has been explored for some species, including *Tenebrio molitor* (13), *Hermetia illucens* (14), and *Bombyx eri larva* (15). Even though there are no known studies focusing on *Acheta domesticus* biorefinery, the potential of house crickets as a base for the extraction of valuable compounds has been explored (16, 17).

Emerging food processing technologies and green solvents have been suggested to enhance the process of isolation or extraction of valuable compounds from edible insects (18). Ultrasound (US) and pulsed electric fields, for instance, have been shown to increase the fat extraction yield from house crickets (19, 20) and black soldier fly larvae (21). High pressure (HP) can increase the extraction yield of phenolics and proteins from olive pomace (22). Deep eutectic solvents (DESs) offer a successful and environmentally friendly approach for chitin extraction from black soldier flies (23).

House crickets have the potential for conversion into valuable ingredients. A rapid, simple, and waste-reducing biorefinery procedure, which is based on two fractionation steps, was applied to house cricket biomass. Ultrasound and high-pressure treatments, as well as DES, were implemented in the biorefinery to reduce the use of materials that are considered hazardous (24) and to improve the yield of each step. Therefore, the overall aim of this study was to introduce an environmental friendly biorefinery system for the valorization of biomass from house crickets.

2. Materials and methods

2.1. Sample preparation

Living crickets (*A. domestica*) were purchased from Tropic Shop (Nordhorn, Germany) and were inactivated by freezing at −20°C. Afterward, they were thawed at 4°C, separated from the frass, washed with water, and oven-dried at 60°C until a constant weight was achieved. Dried insects were milled for 10 s using a laboratory mill from Retsch (Haan, Germany) to obtain cricket flour. All chemicals were purchased from Carl Roth GmbH & Co. Kg (Karlsruhe, Germany) unless stated otherwise. Betaine and the standard mixture of amino acids were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Trolox and 2,2-diphenyl-1-picrylhydrazyl were purchased from Alfa Aesar (Massachusetts, United States).

2.2. Determination of the sample composition

The moisture content of the flour was determined by the gravimetrical difference of the sample after being placed in a drying oven at 105°C for 48 h. The ash content was determined by the gravimetrical difference of the sample after being placed in a furnace at 550°C. For the determination of the protein content, 50 mg of the sample was hydrolyzed with 2 ml of HCl of 6 N at 98°C for 24 h. The hydrolysates were cooled to room temperature and mixed with 2 ml of NaOH of 6 N and 2 ml of phosphate buffer (720 mM, pH = 6.6). Then, they were diluted to 200 ml and subjected to the analysis of free amino nitrogen content. Briefly, 0.5 ml of the sample, after the appropriate dilution, was mixed with 0.25 ml of ninhydrin color reagent (phosphate buffer 720 mM, pH = 6.6, 0.5% w/v ninhydrin, 0.3% fructose) and incubated at 95°C for 20 min. After being cooled down, samples were mixed with 1.25 ml of 0.2% KIO_3 solution in 40% ethanol, and the absorption was measured at 575 nm with a UV/Vis spectrometer. Bovine serum albumin was used for the calibration curve at a concentration range of 10–40 mg/ml, and the results are expressed as g protein/100 g of sample (25).

Fat content was determined with the Folch method. Briefly, 5 g of insects were homogenized with 100 ml of chloroform/methanol (2:1) solvent at room temperature for 1 h. Afterward, the mixture was centrifuged, and the supernatant was collected and mixed with water at a volume of 0.2 times the volume of the supernatant. The mixture was mixed for 30 more minutes at room temperature and then was centrifuged for 10 min at room temperature and 3,900 × g. The lower phase was collected, and the solvent was removed with a rotary evaporator (Büchi R-100, Flawil, Switzerland). Then, the fat content was determined gravimetrically (26). For the estimation of chitin content, 10 mg of samples were hydrolyzed for 90 min with 0.3 ml of 72% sulfuric acid. Afterward, 8.4 ml of water was added, and the samples were further hydrolyzed for 1 h at 121°C. While still warm, 0.5 ml of the samples were taken and mixed with 0.5 ml of $NaNO_2$, 1M solution (A), and another 0.5 ml was mixed with 0.5 ml of water (B). These samples were capped and incubated at room temperature for 6 h followed by overnight incubation without a cap. Then, 0.5 ml of 12% ammonium sulfamate was added to both mixtures A and B, followed by thorough vortexing for 4 min. Next, 0.5 ml of 0.5% MBTH solution was added to both A and B mixtures that were then incubated for 1 h at room temperature. Finally, 0.5 ml of $FeCl_3$ was added to both A and B mixtures, and after 30 min of incubation, the mixtures were diluted appropriately and their absorbance at 650 nm was measured. Standard chitin was used for the calibration curve at a concentration range of 26–130 μg/ml in the cuvette, and the results were expressed as g chitin/100 g sample (27). For the estimation of total carbohydrates, 90 mg of samples were hydrolyzed for 2 h with 2 ml of 12 M sulfuric acid. Afterward, 10 ml of water was added, and the samples were heated at 98°C for 2 h. Then, the samples were cooled down to room temperature, and the hydrolysates were mixed with 6 ml of 10 N KOH and diluted to 100 ml with sodium acetate buffer (200 mM, pH = 5). Total carbohydrate content was estimated on the solutions with the phenol sulfuric acid method (28).

2.3. Fractionation process

The flow chart of the experiments that were performed in the present study is presented in Figure 1. Initially, 5 g of the sample was mixed with 20 ml of distilled water. The US treatment of

Abbreviations: US, ultrasound; HP, high pressure; TPC, total phenolic content; GAE, gallic acid equivalent; TE, Trolox equivalent; FRAP, ferric iron reducing power; DES, deep eutectic solvent; B/U, betaine/urea; AA, amino acid; FTIR, Fourier-transform infrared spectroscopy.

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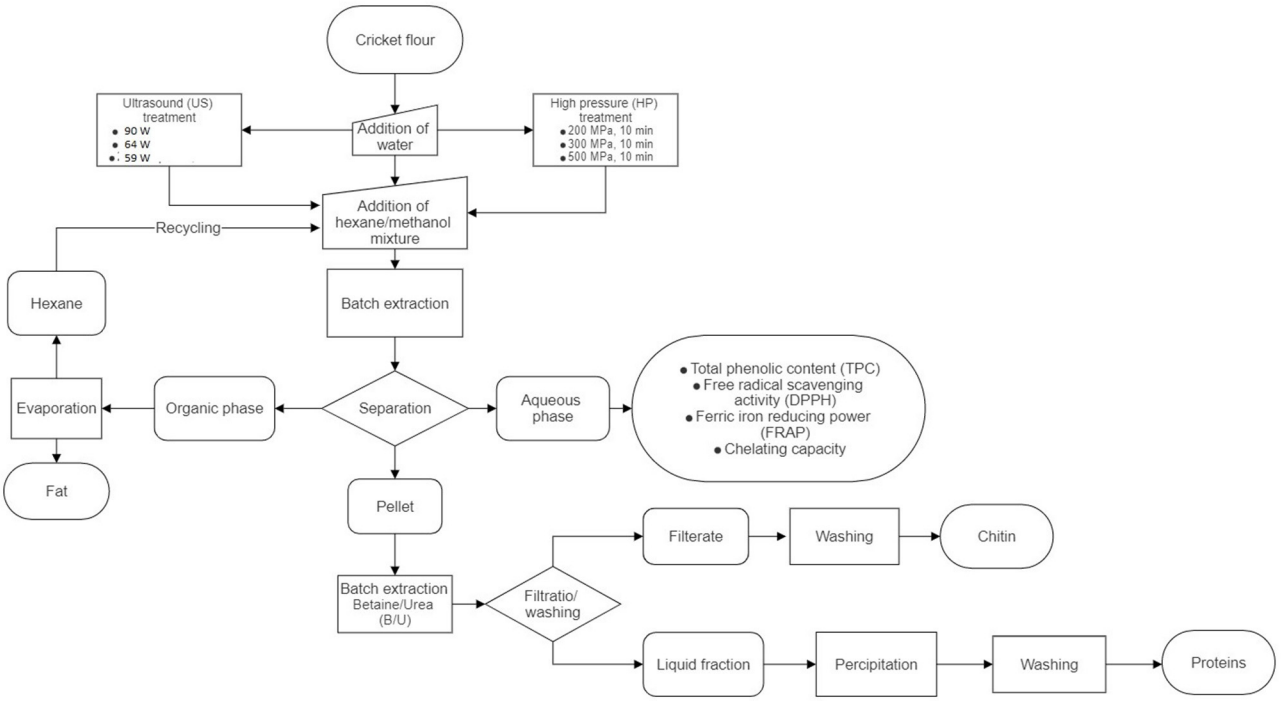

FIGURE 1
Flow chart of the experimental setup.

TABLE 1 Composition (g/100 g dry weight) of the adult house cricket flour. Data are expressed as mean ± SD ($n = 3$).

Compounds	g/100 g dry weight
Proteins	55.58 ± 3.29
Crude fat	17.45 ± 0.62
Ash	4.17 ± 0.34
Carbohydrates	4.52 ± 0.15
Chitin	8.80 ± 1.26

the samples was performed with a UIP1000hdT unit (Hielscher Ultrasonics GmbH, Teltow, Germany) in accordance with the following conditions: (a) 50% amplitude, 5 min, and 90 W; (b) 25% amplitude, 5 min, and 64 W; and (c) 25% amplitude, 10 min, and 59 W. During the treatments, the samples were placed inside a water bath at 4°C to control the temperature increase, which never exceeded 50°C for any treatment. The HP treatment of the samples was performed with a mobile high-pressure system U33 (Institute of High Pressure Physics, Warsaw, Poland) that was connected to a water pump, at 200–500 MPa for 10 min, using water as a pressure transmitting medium. A non-treated mixture of sample and water was used as a control.

Afterward, a hexane/methanol (1:1) solvent was added to each sample at different volumes (25 or 50 ml), and the mixture was stirred at room temperature for 1 h (29). The two different volumes of the solvent were chosen with the aim to reduce the amount of required chemicals as much as possible. The selection of the extraction solvent was based on the Folch extraction method, which included the aqueous fraction to remove impurities from the isolated fat (26), but with hexane replacing chloroform, as it is considered less hazardous (24). Afterward, the mixture was centrifuged at 3,900 × g for 10 min, and three phases were generated. The top layer was the organic phase containing hexane and fat, the middle layer was an aqueous-methanolic extract, and the bottom layer was a protein–chitin-rich pellet. After centrifugation, the three phases were separated.

Afterward, the pellet was subjected to a treatment with a DES, composed of betaine and urea (B/U) for 2 h at 80°C, as it has been shown to be the most efficient DES for chitin isolation (23). The solvent was prepared by mixing betaine and urea at a molar ratio of 1:2 and agitating the mixture while heating until a clear liquid was formed. After the treatment of the pellet with B/U, the mixture was filtered through a mesh of 0.063 mm, and the filtrate containing chitin was washed with water that was heated to 60°C. The filtered liquid that was generated was stored at 4°C for 60 min after its pH was modified to 4.5 in order for proteins to precipitate. Afterward, the proteins were isolated via centrifugation at 3,900 × g, 10 min and washed with water with modified pH at 4.5 twice. Both filtrate and protein precipitates were then dried at 60°C until constant weight.

2.4. Analysis of fractions

2.4.1. Organic phase

The organic phase containing hexane and fat was placed inside a rotary evaporator (Büchi R-100, Flawil, Switzerland) connected

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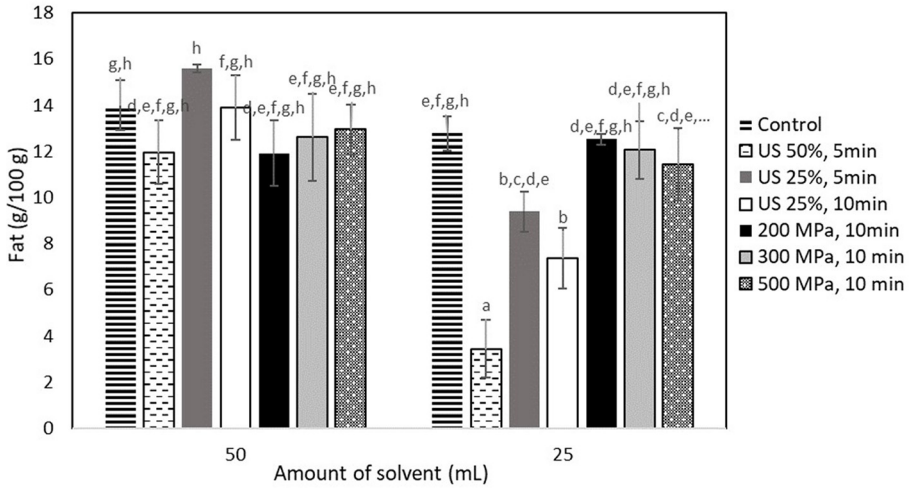

FIGURE 2
Fat extraction yield (g/100 g sample) obtained from samples treated by US and HP at different conditions. Error bars indicate standard errors among replicates of the same process. Superscript letters (a, b, ...) indicate significant differences between the means obtained from different samples.

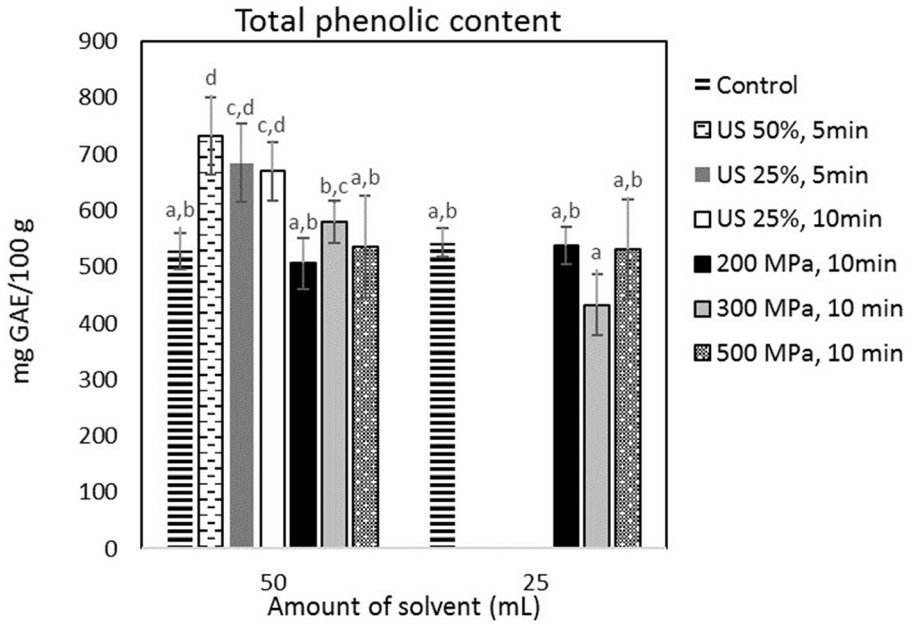

FIGURE 3
TPC (mg GAE/100 g sample) obtained from the samples treated by US and HP at different conditions. Error bars indicate standard errors among replicates ($n = 9$) of the same measurement. Superscript letters (a, b, ...) indicate significant differences between means obtained from different samples.

to an electric vacuum pump (Büchi V-100, Flawil, Switzerland) operating at 40°C and 10 mbar. The hexane was separated from the fat and recycled for further use. The fat extraction yield was determined gravimetrically.

2.4.2. Aqueous phase

2.4.2.1. Total phenolic content

The total phenolic content (TPC) was determined with the Folin–Ciocalteu method. Extracts were diluted appropriately, and then, 0.1 ml of diluted extracts was mixed with 7.9 ml of water. Afterward, 0.5 ml of Folin–Ciocalteu reagent (1 N) was added, and then, 1.5 ml of saturated sodium carbonate solution was added to the mixture. After incubation at 40°C for 30 min in the dark, the samples were cooled down to room temperature, and the absorbance was measured with a UV/Vis spectrometer at 765 nm. Methanol was used as a blank control. A gallic acid solution was used for the calibration curve at a concentration range of 100–1,000 mg/L, and the results were expressed as mg gallic acid equivalent (GAE)/100 g of the initial cricket sample (22).

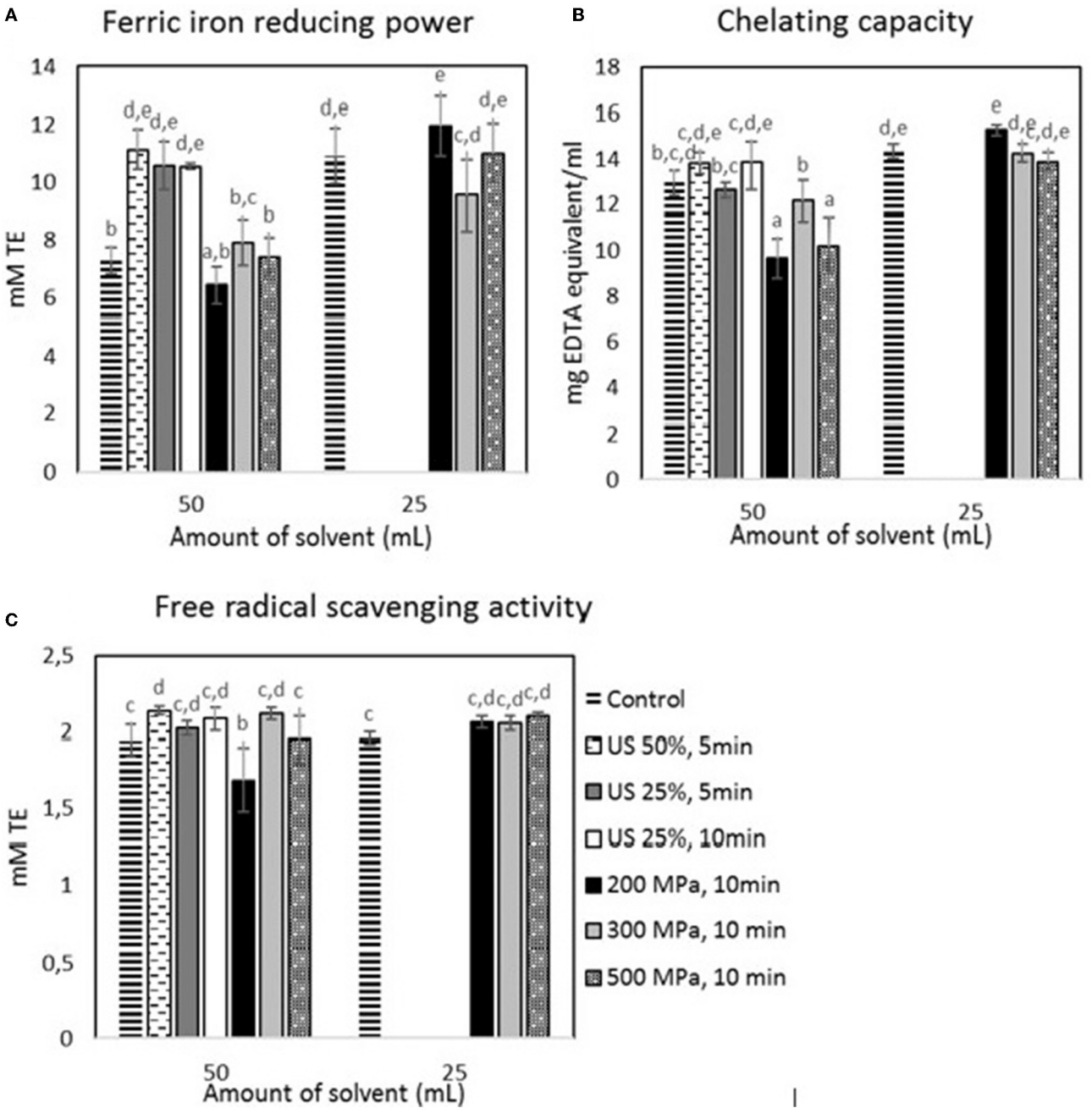

FIGURE 4
Antioxidant activity estimated for the samples treated by US and HP at different conditions: **(A)** FRAP, **(B)** chelating capacity, and **(C)** DPPH. Error bars indicate standard errors among replicates ($n = 9$) of the same measurement. Superscript letters (a, b, ...) indicate significant differences between means obtained from different samples.

2.4.2.2. Free radical scavenging activity (2,2-diphenyl-1-picrylhydrazyl)

The free radical scavenging activity was determined with the DPPH method. Briefly, 0.1 ml of the appropriately diluted extract was mixed with a freshly prepared 6×10^{-5} M DPPH solution and incubated at room temperature in the dark for 15 min. Afterward, the absorbance was measured at 515 nm, using methanol as a blank control. The antioxidant activity is correlated with the difference in absorbance between the blank control and the sample. Trolox was used for the calibration curve at a concentration range of 0.1–1 mM, and the results are expressed as mM Trolox equivalent (mM TE) (30).

2.4.2.3. Ferric iron reducing power

Ferric iron reducing power (FRAP) was estimated by mixing 0.5 ml of an appropriately diluted extract with 0.5 ml of sodium phosphate buffer (0.2 M, pH = 6.6) and 0.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min, and then, 0.5 ml of 10% trichloroacetic acid was added. Afterward, 2 ml of water and 0.4 ml of ferric chloride 0.1% were added. Samples

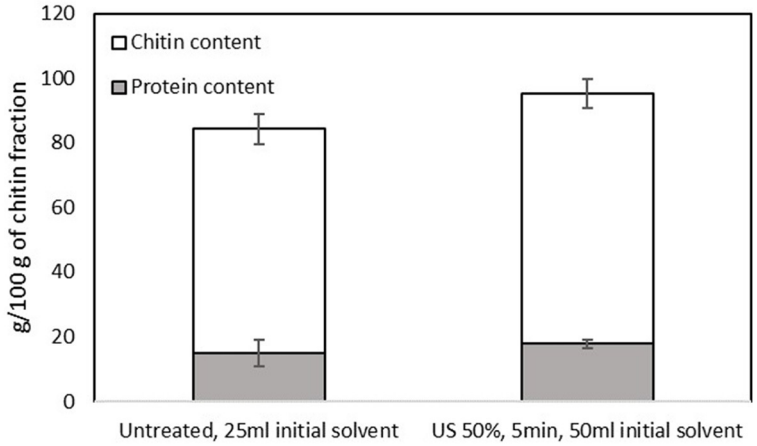

FIGURE 5
Protein and chitin contents of the chitin-rich fraction obtained from the pellet of the untreated sample that was mixed with 25 ml of the initial solvent and the US-treated sample with a 50% of amplitude for 5 min that was mixed with 50 ml of the initial solvent. Error bars indicate standard errors among replicates ($n = 9$) of the same measurement. Significant differences ($p > 0.05$) among means are indicated with the uppercase and lowercase of the same superscript letters (a and A, b and B).

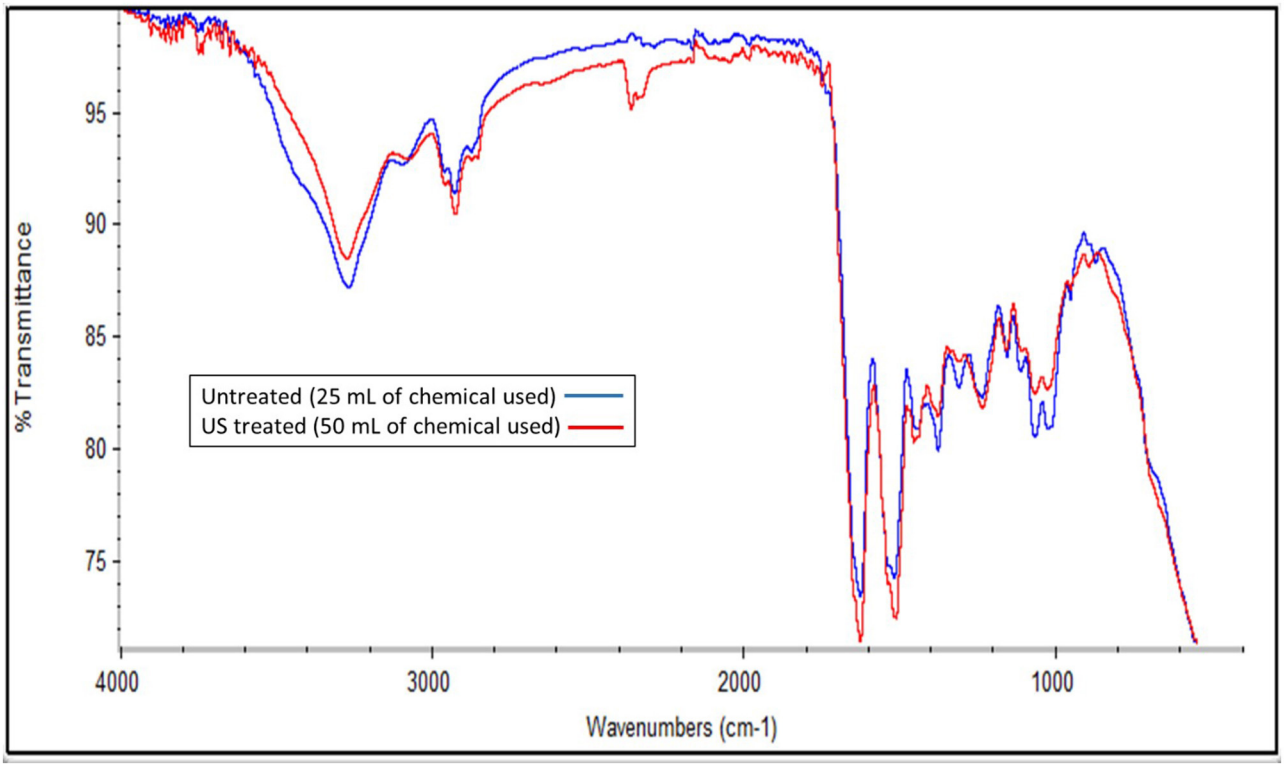

FIGURE 6
FTIR spectra of the chitin-rich fraction obtained from the pellet of the untreated sample that was mixed with 25 ml of the initial solvent and the US-treated sample with a 50% of amplitude for 5 min that was mixed with the 50 ml of the initial solvent.

were vortexed, and the absorbance was measured at 700 nm. A higher absorbance indicates a stronger antioxidant activity. Methanol was used as a blank control, and Trolox was used for the calibration curve at a concentration range of 50–500 μM. The results are expressed as mM TE (31).

2.4.2.4. Chelating capacity

Chelating capacity was estimated by mixing 0.1 ml of an appropriately diluted extract with 3.7 ml of methanol and 0.1 ml of 2 mM $FeCl_2$. After incubating the samples for 3 min at room temperature, 0.2 ml of 5 mM ferrozine was added. The samples

were then incubated for another 10 min at room temperature, and the absorbance was measured at 562 nm. Methanol was used as a blank control. The antioxidant activity is correlated with the difference in absorbance between the blank and the sample. EDTA was used for the calibration curve at a concentration range of 0.25–2 mg/ml, and the results are expressed as mg EDTA equivalent/ml (32).

2.4.3. Filtrate

Regarding the filtrate, the protein and chitin contents were determined with the methods described in Section 2.2. The filtrates were further analyzed directly with FTIR using a Nicolet iS5 spectrometer (Thermo Scientific, US-WI 53711 Madison, USA).

TABLE 2 Characteristic peaks identified for the chitin-rich fractions obtained from the pellet of the untreated sample that was mixed with 25 ml of the initial solvent and the US-treated sample with a 50% of amplitude for 5 min that was mixed with the 50 ml of the initial solvent.

Wavelength (cm^{-1})	Bond
1,025	C–O asym. stretching in the phase ring
1,066	C–O–C asym. stretch in the phase ring
1,157	Asymmetric bridge oxygen stretching
1,309	Waging of CH_2 of amide III
1,376	Symmetrical deformation mode of CH_3
1,446	Bending of CH_2
1,509	N–H bend and N–C stretch
1,625	C=O stretch of amide I
2,925	C–H stretching of CH_2 and CH_2OH groups
3,270	N–H stretching

2.4.4. Protein precipitate

Similarly, the protein and chitin contents of the protein precipitate were analyzed with the methods that are described in Section 2.2. Additionally, the amino acid (AA) profile was estimated as follows: 0.1 g of each sample was hydrolyzed for 24 h at 110°C using an aqueous hydrolysis solution that was prepared as follows: 0.5 g of phenol was mixed with 200 ml of water and then 66 mg of norleucine and 250 ml of 6 N HCl were added. After the hydrolysis, 0.2 ml of the hydrolysate was removed, dried, and mixed with 1 ml of lithium dilution buffer (650-0018, MembraPure GmbH, Hennigsdorf Germany). Then, the samples were injected in an Aracus Classic amino acid analyzer (MembraPure GmbH, Hennigsdorf Germany) with an ion exchange chromatographic column (125×3 mm), an autosampler with 100 μl loop, and a reactor for post-column derivatization with ninhydrin. The samples were detected at 570 nm and 440 nm for proline. The duration of the analysis was 90 min (33).

2.5. Statistical analysis

Each fractionation process was repeated in triplicate, and each analytical method used for each fraction was repeated in triplicate as well. Error bars on the graphs indicate standard errors. Significant differences among the data collected from the samples that were processed with different methods were identified with a one-way analysis of variance (ANOVA), and Tukey's test was applied *post hoc* for mean separation at a confidence level of 0.05. Normality was tested with the Shapiro–Wilk test, and data that did not follow normal distribution were normalized prior to the analysis. All analyses were performed using IBM SPSS Statistics 23 (IBM Corp., Armonk, N.Y., USA).

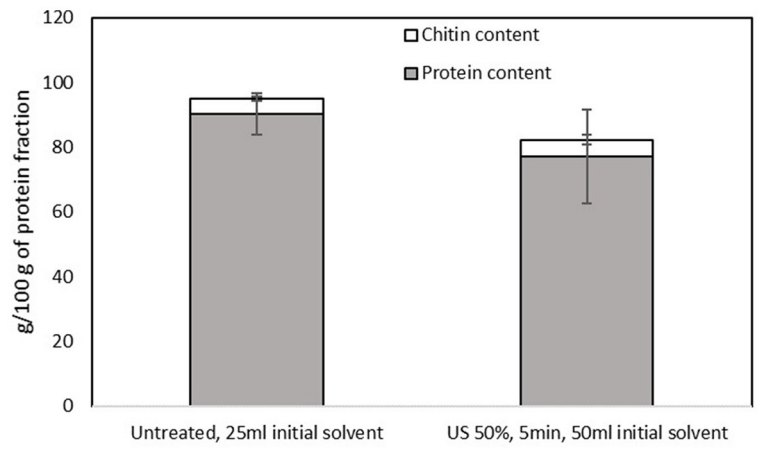

FIGURE 7
Protein and chitin contents of the protein-rich fraction obtained from the pellet of the untreated sample that was mixed with 25 ml of the initial solvent and the US-treated sample with a 50% of amplitude for 5 min that was mixed with 50 ml of the initial solvent. Error bars indicate standard errors among replicates ($n = 9$) of the same measurement. Significant differences ($p > 0.05$) among means are indicated with the uppercase and lowercase of the same superscript letters (a and A, b and B).

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TABLE 3 Amino acids (μmol/l) that were identified in the hydrolysate from the protein-rich fraction obtained from the pellet of the untreated sample that was mixed with 25 ml of the initial solvent and the US-treated sample with a 50% of amplitude for 5 min that was mixed with 50 ml of the initial solvent.

Amino acids (μmol/l)	Untreated (25 ml of chemical used)	US treated (50 ml of chemical used)	*p*-value
Aspartic acid	1,493.81 ± 190.60	1,302.79 ± 95.97	0.196
Threonine	1,032.96 ± 77.01	950.94 ± 76.77	0.261
Serine	1,447.58 ± 185.25	1,275.40 ± 105.99	0.235
Glutamic acid	2,436.31 ± 246.96	2,206.47 ± 77.69	0.199
Proline	2,530.67 ± 155.91	2,355.77 ± 261.48	0.376
Glycine	1,879.11 ± 3.93	1,783.03 ± 122.65	0.247
Alanine	3,012.21 ± 136.98	2,905.84 ± 338.83	0.641
Valine	1,568.94 ± 42.85	1,496.00 ± 162.74	0.484
Cysteine	119.83 ± 25.37	95.57 ± 23.92	0.295
Methionine	316.71 ± 46.63	268.33 ± 12.17	0.119
Isoleucine	1,037.48 ± 79.67	937.20 ± 58.93	0.155
Leucine	1,853.38 ± 125.49	1,677.59 ± 98.22	0.129
Norleucine	203.13 ± 6.57	205.91 ± 6.83	0.637
Tyrosine	895.64 ± 17.56	839.09 ± 68.16	0.236
Phenylalanine	627.99 ± 77.52	552.55 ± 41.85	0.212
Beta-alanine	2,605.11 ± 650.79	4,263.19 ± 804.94	0.171
G-aminobutyric acid	160.90 ± 21.44	163.98 ± 25.43	0.880
Histidine	414.86 ± 19.85	385.08 ± 23.76	0.171
1-Methyl-histidine	15.74 ± 1.08	15.62 ± 4.24	0.965
Tryptophan	22.92 ± 0.44	22.68 ± 4.90	0.938
Carnosine	49.19 ± 5.90	45.45 ± 15.92	0.722
Asparagine	565.04 ± 53.14	292.17 ± 174.72	0.061
Ornithine	7.26 ± 0.42	6.75 ± 4.70	0.860
Lysine	745.89 ± 0.00	745.83 ± 251.81	0.090
Arginine	1,074.67 ± 88.42	965.29 ± 52.08	0.130

Data are presented as mean ± SD (n = 9).

3. Results

3.1. Composition of the material

The estimated composition of the biomass of house crickets (Table 1) confirms their potential for the utilization of their ingredients in the food sector. As has been suggested by Rumpold and Schlüter (1), Psarianos et al. (19), and Udomsil et al. (34), crickets were found to have high protein and lipid contents, as well as a significant amount of chitin. The variation of the values of the insect composition among different studies can be attributed to different rearing procedures and feeding substrates of the targeted insects (35).

3.2. Fat isolation

As shown in Figure 2, neither US nor HP processing had a positive effect on the fat extraction yield. The highest yield obtained from the US treated samples at 90 W (50% amplitude, 5 min) mixed with 50 ml of the extraction medium was 15.61 ± 0.17 g fat/100 g cricket flour, which was still not significantly higher ($p < 0.05$) than the yield obtained from the control sample (14.00 ± 1.07 g fat/100 g cricket flour). A similar trend was also observed for the process pathways that required 25 ml of the extraction medium, with the highest yield being observed for the control sample (12.78 ± 0.74 g fat/100 g cricket flour). The fat yield obtained from the control sample that was mixed with the 50 ml of the extraction medium was 80.23% of the total fat (Table 1). This means that the fat extraction process was quite efficient without any pretreatment with HP or US.

Regarding the untreated samples that were subjected to extraction at different solvent volumes of 25 and 50 ml, no significant difference was observed ($p < 0.05$), with a yield of ∼13 g fat/100 g of cricket flour. However, the US-treated samples that were mixed with 25 ml of the extraction solvent showed a significantly negative yield ($p < 0.05$), with the lower yield being obtained from the one treated with 50% amplitude for 5 min (3.44 ± 1.25 g fat/100 g insect flour).

3.3. Aqueous extract

Figure 3 shows that HP processing had no effect on the extraction yield of phenolic compounds ($p < 0.05$) with the control extraction process using 50 and 25 ml of extraction medium leading to a 528.08 ± 32.49 mg and 543.09 ± 25.75 mg GAE/100 g of cricket flour, respectively. US treatment at all tested conditions did nevertheless lead to a significant ($p < 0.05$) increase of the TPC in the aqueous extract. The highest yield of phenolic compounds was obtained, after treatment with US at 90 W and was equal to 732.38 ± 68.94 mg GAE/100 g cricket flour. In that case, US treatment increased the TPC in the extract by 38.69%, compared to the untreated sample that was mixed with the same amount of solvent. However, the US-treated sample showed the highest TPC compared to every sample.

According to Figure 4, a similar trend to the TPC was observed for the FRAP and free radical scavenging activity, where US treatment did increase the antioxidant activity of the aqueous extracts. In specific, the samples mixed with the higher solvent volume showed a 52.40% ($p < 0.05$) and a 9.81% ($p < 0.05$) increase in their FRAP and free radical scavenging activity, respectively, after the US treatment with 50% amplitude for 5 min. However, the samples subjected to the higher solvent volume showed no significant differences after HP processing, apart from the treatment with 200 MPa for 10 min that reduced the radical scavenging activity by 15.38% ($p < 0.05$). Furthermore, there was no positive effect of US and HP on the chelating capacity. Nevertheless, HP processing significantly ($p < 0.05$) reduced the chelating capacity of the samples treated with 200 and 500 MPa and subjected them to the higher solvent volume. Furthermore, decreasing the solvent volume by 50% without any pretreatment resulted in a significant ($p < 0.05$) increase in the FRAP of the extracts but did not affect the radical scavenging ($p > 0.05$) and chelating activity ($p > 0.05$). Finally, the radical scavenging activity of the US-treated sample at 50% amplitude for 5 min was the highest among all samples.

3.4. Chitin isolation

The treatment with B/U was successful in isolating a chitin-rich fraction from both samples (Figure 5). The chitin fractions have a high chitin content (~70 g/100 g of filtrate) and a low content of protein impurities (<20 g/100 g filtrate). This is considered sufficient since an incomplete deproteinization and thus the existence of protein impurities in chitin does not affect the properties of chitosan (36). The application of NADES for chitin extraction from insects has previously been reported to lead to a chitinous fraction with a purity of 70%−90%, depending on the solvent (23). The chitin content in the filtrate obtained from the US-treated sample was found to be significantly higher ($p < 0.05$) than that of the untreated one (77.44 ± 4.41 and 69.45 ± 4.68 g chitin/100 g filtrate, respectively). The FTIR spectrum of the two materials (Figure 6) is almost identical and has all the characteristic peaks that have been observed for insect chitin (Table 2) (11, 37).

3.5. Protein isolation

The efficiency of the separation of proteins and chitin in the pellet was also evident in the protein fraction. The protein content of the precipitate was high (ranging from 77 to 90 g/100 g of precipitate) for both samples, without significant differences among them ($p > 0.05$), as shown in Figure 7. Regarding the AA profile of the samples that are presented in Table 3, both samples contain both essential and non-essential amino acids, without any significant differences among samples, while ammonia was observed (~1,805.55 ± 58.85 μmol/l), which was considered a by-product of the treatment with urea. The amino acids that are commonly reported in fractions from house crickets (1, 38) were also estimated in the present study. Additionally, it was observed that the protein precipitates contained norleucine, beta-alanine, g-aminobutyric acid, 1-methyl-histidine, carnosine, and ornithine.

4. Discussion

4.1. Fat fraction

The refining process that the present study suggests to fractionate the cricket flour is already quite effective when targeting the fat yield, so US processing could not cause a further increase, as it was reported for other fat extraction methods (20). HP treatment has been reported to negatively affect fat extraction when treated at intense HP conditions, which was attributed to the disruption of triglyceride structure (39). Therefore, a lack of enhancement of the fat extraction due to HP treatment could be expected. However, the potential of HP for the facilitation of the extraction of phenolics was further investigated.

Regarding the samples mixed with 25 ml of the solvent, the negative effect of US could be attributed to the generation of an emulsion that was observed after the US treatment in all cases and that could not be broken by the addition of the chemical solvent at a lower volume. US treatment has been reported to be used for the generation of emulsions and to improve emulsifying properties (40). This is mainly attributed to the breaking of oil and emulsion droplets, generating a fine emulsion (41) or the formation of a micro-jet during cavitation that would push water droplets in the oil phase (42).

Based on these results, it was considered that the fractions of the US-treated samples that were mixed with the 25 ml of the solvent should not be further evaluated, since the treatment had a negative effect on the generation of the fat fraction.

4.2. Aqueous extract

US treatment has previously been reported to enhance the extraction of phenolic compounds from insects (43) and other materials (44, 45), which was also confirmed in the present study. Furthermore, HP treatment has been reported to enhance phenolic extraction in plant oils (46) and TPC in oils extracted from insects (39). However, in the present study, HP did not yield any increase in phenolics in the extract. The different results that were observed by (39) may be attributed to the fact that the increase in the phenolic

yield, which they observed is estimated on the isolated oil and not in an insect-based extract oil. Furthermore, in the present study, the tested HP treatment conditions were milder in terms of operating pressure and treatment time compared to the treatments used by Ugur et al. (39). This could also attribute to no increase of TPC in the present study, since it has been shown that the yield of TPC can be affected by both pressure and treatment time (22).

The enhancement of the antioxidant activity of extracts that are obtained from food materials with the implementation of US treatment was expected due to the increased TPC, since the antioxidant activity can be linked to the TPC (22).

Based on the results that are presented in Section 3.3, HP was considered inappropriate for implementation in the insect biorefinery. HP treatment has been reported to disrupt the structures of triglycerides in house crickets and mealworms, thus having a negative effect on fat extraction (39). In the present study, no negative effect of HP on the extraction yield of the cricket compounds was observed, which could be attributed to the lower pressure and shorter duration of the HP treatment. However, considering that HP did not improve the extraction yield of both lipids and phenolics from the house crickets, the possibility of implementing HP processing in the process of isolation of valuable compounds from crickets was rejected. On the contrary, the application of US treatment showed potential. Regarding the samples subjected to 50 ml of the initial extraction solvent, the one treated with US at 90 W was considered the most promising. Regarding the ones subjected to the 25 ml of the initial solvent, the untreated one was considered the most promising. Therefore, the pellet from these two samples was collected and subjected to the treatment with B/U to obtain chitin and a protein concentrate.

4.3. Chitin fraction

DESs have been successfully implemented in the process of chitin isolation from different materials, including crustacean waste (47) and insects (23). In particular, B/U has been tested for chitin extraction, among a variety of DES, from *H. illucens* and was reported to be the most efficient, with high degrees of demineralization and deproteinization (23). The significantly different chitin contents of the two filtrates ($p > 0.013$) could partially explain the slight differences in the FTIR peak intensities (Figure 6), while the improvement of chitin extraction via US processing has also been observed for squid pens (48).

4.4. Protein fraction

The importance of some of the observed amino acids is related to their properties. Beta-alanine has been reported to positively affect body performance (49), same as ornithine (50), and g-aminobutyric acid has been reported to have various benefits on sleep, stress, and blood pressure reduction (51), while carnosine has been suggested as a functional food ingredient for its antioxidant properties (52).

The ability of B/U to cause the isolation of chitin and the removal of other fractions is related to the structure of the DES (23). The DESs are formed from salts that work as hydrogen bond donors and acceptors forming hydrogen bonds, with a high melting point, which when mixed together at a particular molar ratio lead to a depression of the melting point (53, 54). During the treatment of the sample with the B/U, the hydrogen bond donor and receiver molecules dissolve chitin by disturbing its structure and forming new bonds (55). During filtration and washing, the insoluble chitin remains in the filtrate, while the proteins are mostly in the liquid and can be isolated with precipitation.

5. Conclusion

House cricket flour was found to be an appropriate substrate for insect biorefinery with a simple and quick process. All main fractions (lipids, proteins, and chitin) were recovered successfully, while an antioxidant and a phenolic-rich extract were also generated. Both HP and US were found to be inefficient to increase the fat extraction yield; however, US treatment increased the yield of phenolics and enhanced the antioxidant activity of the aqueous extract. Furthermore, chitin was successfully separated from proteins using DES. No effect of HP on the fat extraction could be attributed to the triglyceride structure (39), while the negative effect of US on the fat extraction of some of the samples was attributed to an observed emulsion that was the result of the US process. However, further studies are needed to explore the potential of these pretreatments on the biomass of house crickets.

Using a lower volume of the initial solvent (25 ml instead of 50 ml) without any pretreatment showed a comparable extraction efficiency when combining a higher volume (50 ml) with US treatment at 90 W. Furthermore, the process pathway implementing the US treatment offered the advantage of higher antioxidant ability of the aqueous fraction. Due to the short duration of each sequential process and the successful isolation of all main fractions from the house crickets, the processing pathway implemented in the present study presents the potential to be applied to an industrial scale.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

MP: research design, data collection and analysis, and writing and review—editing. SO: research design and writing and review—editing. OS: writing and review—editing and project administration. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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6.6 Chitin Isolation and Chitosan Production from House Crickets (Acheta domesticus) by Environmentally Friendly Methods

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Chitin Isolation and Chitosan Production from House Crickets (*Acheta domesticus*) by Environmentally Friendly Methods

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Abstract: Alternative methods were evaluated for chitin isolation from *Acheta domesticus*. Chemical demineralization was compared to fermentation with *Lactococcus lactis*, citric acid treatment, and microwave treatment, leading to a degree of demineralization of 91.1 ± 0.3, 97.3 ± 0.8, 70.5 ± 3.5, and 85.8 ± 1.3%, respectively. Fermentation with *Bacillus subtilis*, a deep eutectic solvent, and enzymatic digestion were tested for chitin isolation, generating materials with less than half the chitin content when compared to alkaline deproteinization. Chitosan was produced on a large scale by deacetylation of the chitinous material obtained from two selected processes: the chemical treatment and an alternative process combining *L. lactis* fermentation with bromelain deproteinization. The chemical and alternative processes resulted in similar chitosan content (81.9 and 88.0%), antioxidant activity (59 and 49%), and degree of deacetylation (66.6 and 62.9%), respectively. The chitosan products had comparable physical properties. Therefore, the alternative process is appropriate to replace the chemical process of chitin isolation for industrial applications.

Keywords: insect; chitosan; enzymes; fermentation; characterization; Resilient food system

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1. Introduction

Edible insects are considered an important food source in many countries with approximately 2100 species consumed as food worldwide [1]. A change in food systems is essential to combat climate change [2], and edible insects are a strong candidate to replace conventional livestock due to their lower environmental impact [3] and good nutritional profile [4]. However, when taking advantage of alternative bioresources, all fractions of the edible insects must be used either for food or non-food applications.

One of the compounds in edible insects is chitin; the amount varies depending on the species, developmental stage, and age of the insect. For example, the chitin content from adult crickets and adult mealworms is estimated to be 67.1 and 137.2 mg/kg on a dry basis, respectively [5]. Chitin is a biopolymer that consists of β-(1-4)-N-acetyl-D-glucosamine [6] and is mainly found in invertebrates and fungal species [7]. Chitosan is a derivative of chitin that is formed through deacetylation (the conversion of chitin acetamide groups into amine groups) and has antioxidant [8], antitumor, and antimicrobial activities [9].

The common method for chitin isolation from insects consists of three major steps: delipidation, deproteinization, and demineralization. Usually, deproteinization and demineralization are performed with an alkaline and an acidic treatment, respectively, combined with heating [10]. This sequential chemical treatment is also applied to other materials, such as crustacean materials [11]. Nevertheless, this method has a major disadvantage of environmental pollution [12].

Several alternative methods have been suggested to replace the chemical treatments in crustacean materials, including fermentation [8], deep eutectic solvents (DES) [13], or less hazardous chemicals [14].

There is a growing interest regarding the house cricket (*Acheta domesticus*), owing to its relatively easy rearing process [15] and valuable nutritional profile [16]. However, there are limited studies aiming to extract chitin and evaluate chitosan from *A. domesticus*, with the exception of studies of the emulsifying properties of a chitin fraction [17] or the lipid binding and antimicrobial capacity of chitosan [18]. Consequently, the present study aims to test some of the alternative processes for chitin isolation from house crickets and to evaluate their applicability. Furthermore, an alternative process line for obtaining a chitinous material is designed and compared to a sequential chemical process, with respect to the properties of the chitosan generated after deacetylation of the chitinous product. Figure 1 presents the experimental setup used to compare and evaluate the different methods of chitin isolation in the present study.

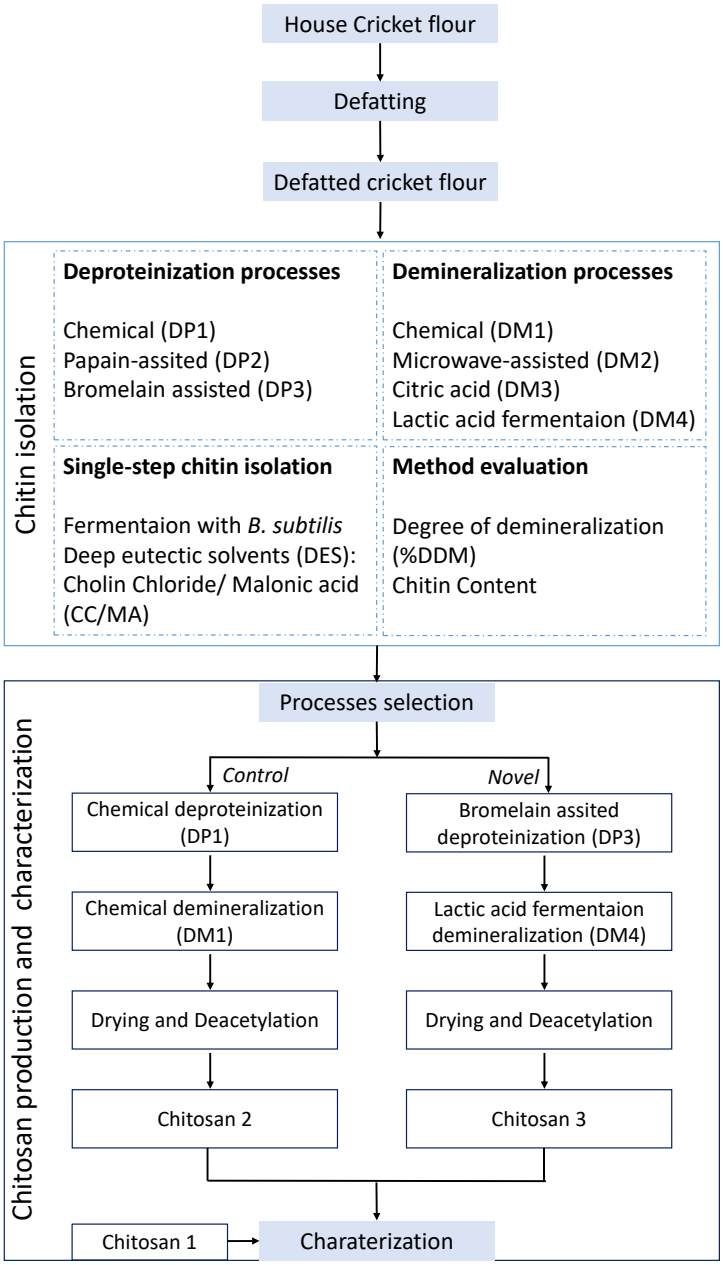

Figure 1. Flow chart of the experimental setup of the present study.

2. Results and Discussion

2.1. Evaluation of Methods for Chitin Isolation

2.1.1. Comparison of the %DDM

As shown in Figure 2, the use of different methods for demineralization of the insect flour had a significant effect ($p > 0.05$) on the efficiency of the process. The %DDM of each sample was significantly different ($p < 0.05$) from the one determined for the other samples. The chemical method resulted in 91.1 ± 0.3% demineralization, while the implementation of microwave treatment and fermentation led to 97.3 ± 0.8% and 85.80 ± 1.3% demineralization, respectively, while the use of citric acid led to only 70.5 ± 3.5% demineralization.

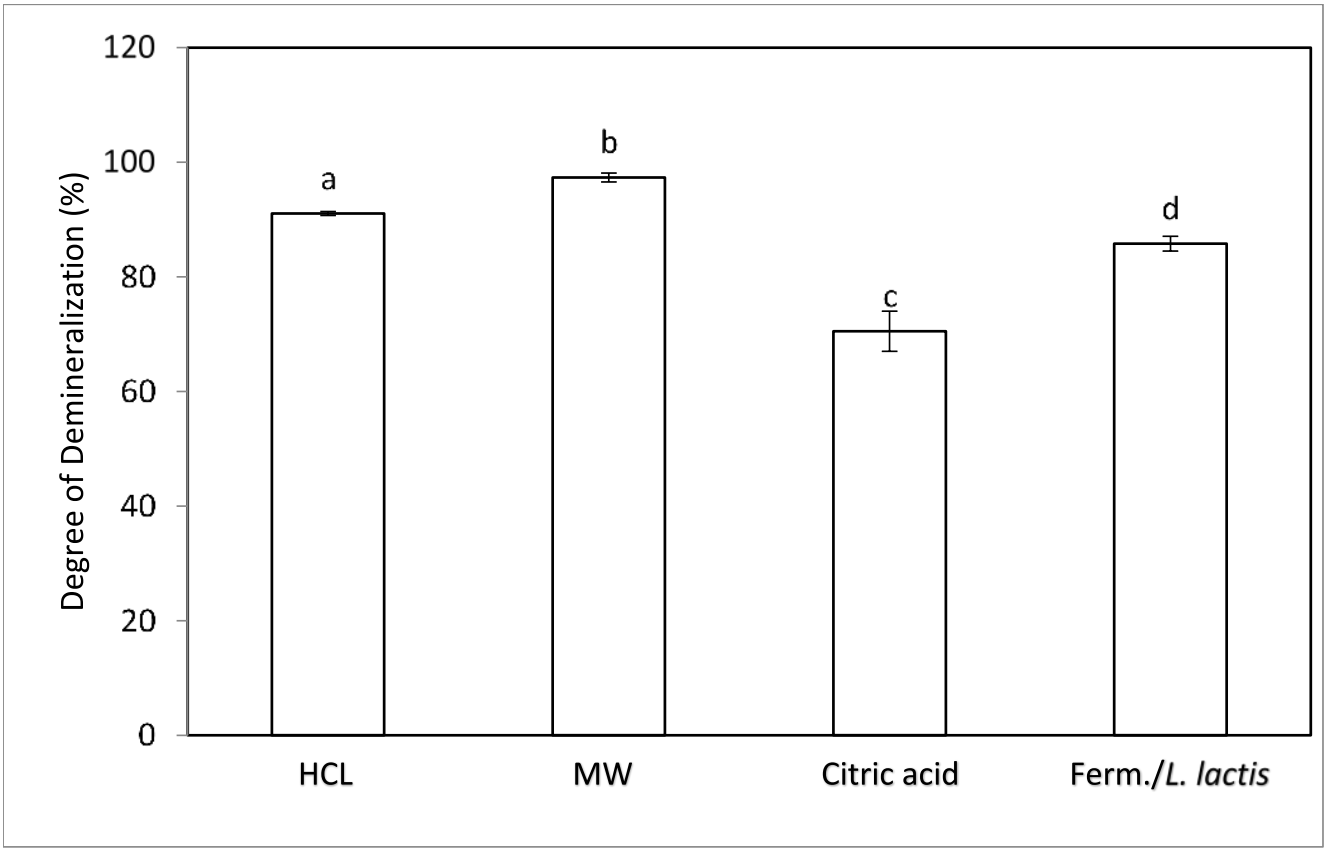

Figure 2. Efficiency of demineralization methods based on the degree of demineralization. Error bars indicate the standard error based on the variation in the %DDM. Different subscript letters indicate significant differences ($p < 0.05$) among the means of the %DDM obtained from each sample treated with a different demineralization process.

During microwave treatment, an oscillating electromagnetic field is initiated. Subsequently, a rotational displacement of polar molecules takes place, leading to the generation of heat and the transfer of ions and electrons [19]. The potential of microwave processing as well as fermentation for enhancing demineralization has also been demonstrated for crustacean materials such as prawn waste and shrimp shells [20,21].

2.1.2. Evaluation of Chitin Content of Materials Subjected to Different Methods for Deproteinization and Chitin Extraction

Figure 3 presents the chitin content (mg/100 mg d.w.) of solid residues obtained after different deproteinization processes and one-step processes of for chitin isolation. The results showed no significant difference when using proteolytic enzymes, DES, or fermentation processes for chitin isolation. However, the material generated after treatment with NaOH had a significantly higher ($p < 0.05$) chitin content (27.1 ± 1.6 mg chitin/100 mg d.w.

of the residue) than the other materials. The average chitin content of the other materials ranged between 6 and 13 mg/100 mg d.w. of the residue, with no significant differences ($p > 0.05$) among the chitin content of the different materials.

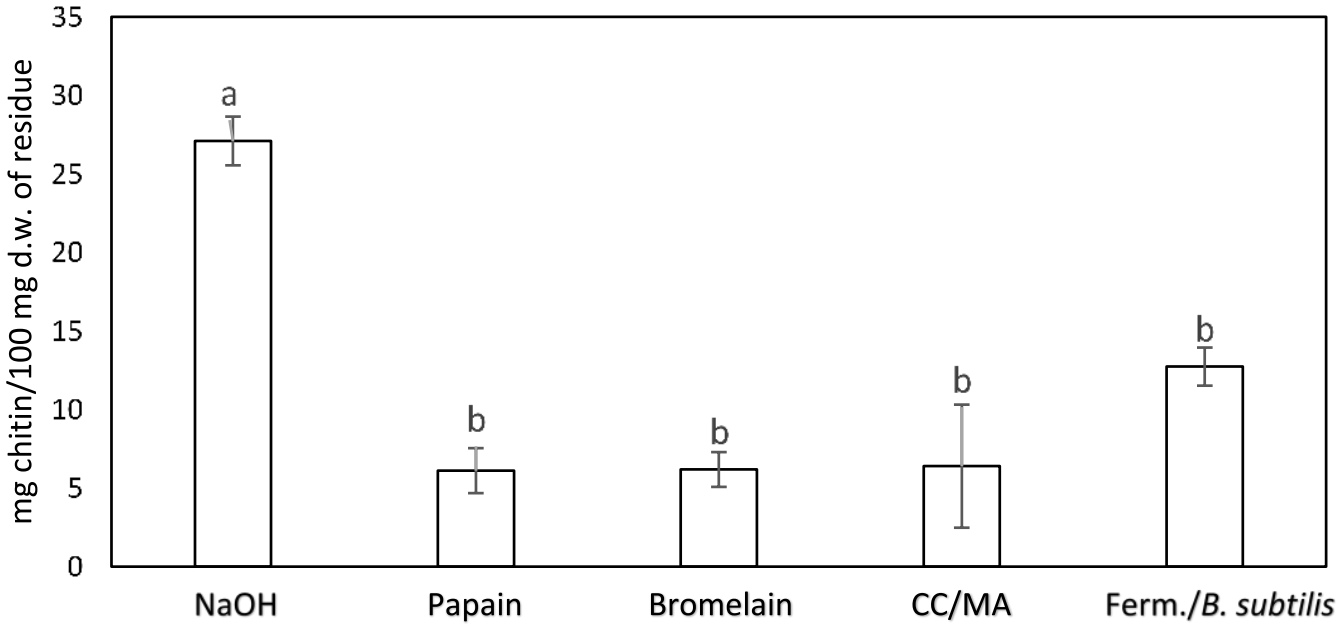

Figure 3. Chitin content (mg/100 mg d.w.) of solid residues obtained after different deproteinization processes or one-step processes for chitin isolation. Error bars indicate the standard error based on the variation in the chitin content. Different subscript letters indicate significant differences ($p < 0.05$) among the means of the chitin content determined for each sample.

The implementation of the treatment of cricket flour with papain, bromelain, *Bacillus* species-generated proteases, and DES was not as successful, possibly because of differences in the nature of raw material compared to the crustacean materials; the chitin content of the material generated after each process was less than half of that of the material produced from the chemical treatment with NaOH.

This difference in efficiency of the methods based on other chitinous material can be attributed to the properties of the proteins present in the crickets, which have low extractability in aqueous solutions [22]. Furthermore, these proteins tend to form stable complexes with chitin, making complete deproteinization more difficult [23]. Da Silva, Brück & Brück (2017) [24] also reported residual impurities from fermented mealworm, despite a high degree of demineralization, which they did not identify as chitin, but rather as a chitinous material. However, enzymatic hydrolysis and DES have been successfully introduced to isolate chitin from *Tenebrio molitor* [24] and *Hermetia illucens* [25], respectively.

2.2. Selection of an Alternative Process for Chitin Isolation

Regarding the alternative method for chitin isolation, microwave-assisted demineralization was rejected to avoid the use of chemicals. The implementation of the DES, citric acid, and fermentation with *Bacillus subtilis* methods was considered inefficient. Finally, of the two enzymatic treatments, the one containing bromelain was considered more appropriate due to the shorter treatment time. The deproteinization step was performed before the demineralization, since it is reported that this sequence leads to a higher purity of chitin due to the removal of a protein layer that leaves chitin unprotected [26].

The chitin content of the isolated materials obtained through the chemical and the biological methods was 73.5 ± 0.2 and 56.3 ± 0.5 mg chitin/100 mg d.w. of isolated material, respectively. It is important to note that the chemical method, which is very

commonly implemented for chitin isolation [10], did not lead to the isolation of pure chitin, as observed also by Kaya et al. (2015). Specifically, they argued that a degree of acetylation of chitin higher than 100%, which is reported for chitin obtained from other organisms, such as crab, shrimp, or bumblebee, is an indication of protein or mineral residues in the isolated chitin [27]. Regarding edible insects, there are only a few studies exploring the potential of an alternative and sustainable chitin isolation process. Fermentation has been applied to mealworms for the isolation of chitin with a high degree of demineralization (>90%) but also the presence of residual impurities [28].

2.3. Characterization of Chitinous Materials and Chitosan

2.3.1. Properties of Chitosan

The properties of the produced chitosan materials are presented in Table 1. The chitosan content of Chitosans 1 and 2 appears to be high (81.9 and 88.9 mg chitin equivalent/100 mg d.w. of Chitosans 2 and 3, respectively), even though the chitin content of the solid materials that were isolated by both the chemical and biological extraction methods was lower. This is attributed to the extraction of protein residues during the deacetylation process, which would lead to the production of chitosan at higher purity than the chitin [29]. Further, the chitosan content of Chitosan 2 was significantly lower ($p < 0.05$) than that of Chitosan 3, which can be attributed to the partial dissolution during the acidic treatment [30]. Furthermore, the DD% of chitosan 1 is 75.1% and is higher than the chitosans produced from cricket flour (66.6 and 62.9% for chitosan 2 and 3, respectively), which both had a very similar value of DD%.

Table 1. Properties of obtained chitosan samples. Chitosan 1 refers to the chitosan produced from commercial chitin, Chitosan 2 to the chitosan produced from chitin isolated with the chemical method from cricket flour, and Chitosan 3 to the chitosan produced from the chitinous material isolated by the biological method from cricket flour. Data are presented as mean ± SD. Superscript letters (a, b, c) indicate significant differences ($p < 0.05$) between means of the same property of different chitosan samples.

Sample	Chitosan Content (mg Chitin Equivalent/100 mg)	Degree of Deacetylation (DD%)	Antioxidant Activity (%)	Molar Mass × 10^3 (g/Mole)
Chitosan 1	-	75.1	35.00 ± 3.6^{a}	471.2 ± 2.5^{a}
Chitosan 2	81.9 ± 0.5^{a}	66.6	59.0 ± 0.6^{b}	103.4 ± 2.4^{b}
Chitosan 3	88.0 ± 0.1^{b}	62.9	49.3 ± 5.2^{b}	86.8 ± 3.1^{c}

The chitosan that was produced from commercial chitin has four times greater ($p < 0.05$) molar mass compared to the chitin produced from crickets. Additionally, the chitosan samples obtained from the crickets have a low molecular weight, with that of Chitosan 3 being significantly lower ($p < 0.05$). The lower molar mass of the chitosan obtained from the biological process is attributed to the depolymerization of chitinous materials by bromelain. However, the low molar mass is a desirable property for chitosan, since lower molar mass indicates enhanced antioxidant, antimicrobial, and antitumor activities [31].

This indication was confirmed regarding the antioxidant activity. Specifically, Chitosan 1 had significantly lower ($p < 0.05$) antioxidant activity (%) than Chitosans 2 and 3. This result is attributed to the difference in molar mass between the samples. This result suggests a further advantage of implementing the biological method to generate the final chitosan product from the cricket flour.

2.3.2. Characterization of Structure (FTIR)

The main bands identified from the spectra of the materials (Figure 4) were: N-H stretching at 3270 cm^{-1} (A), C-H stretching at 2880 cm^{-1} (B) of CH, CH_2, and CH_3 groups, C-O stretch of amide I at 1620 (C) and 1653 cm^{-1}, N-H bend and N-C stretch at 1559 cm^{-1} (D), bending of CH_2 and stretching vibration of C-N at 1420 and 1315 cm^{-1} (F), respectively, symmetrical deformation mode of CH_3 at 1380 cm^{-1} (E), and anti-symmetric stretching the C-O-C bridge at 1156 cm^{-1} (G) [24,27,32].

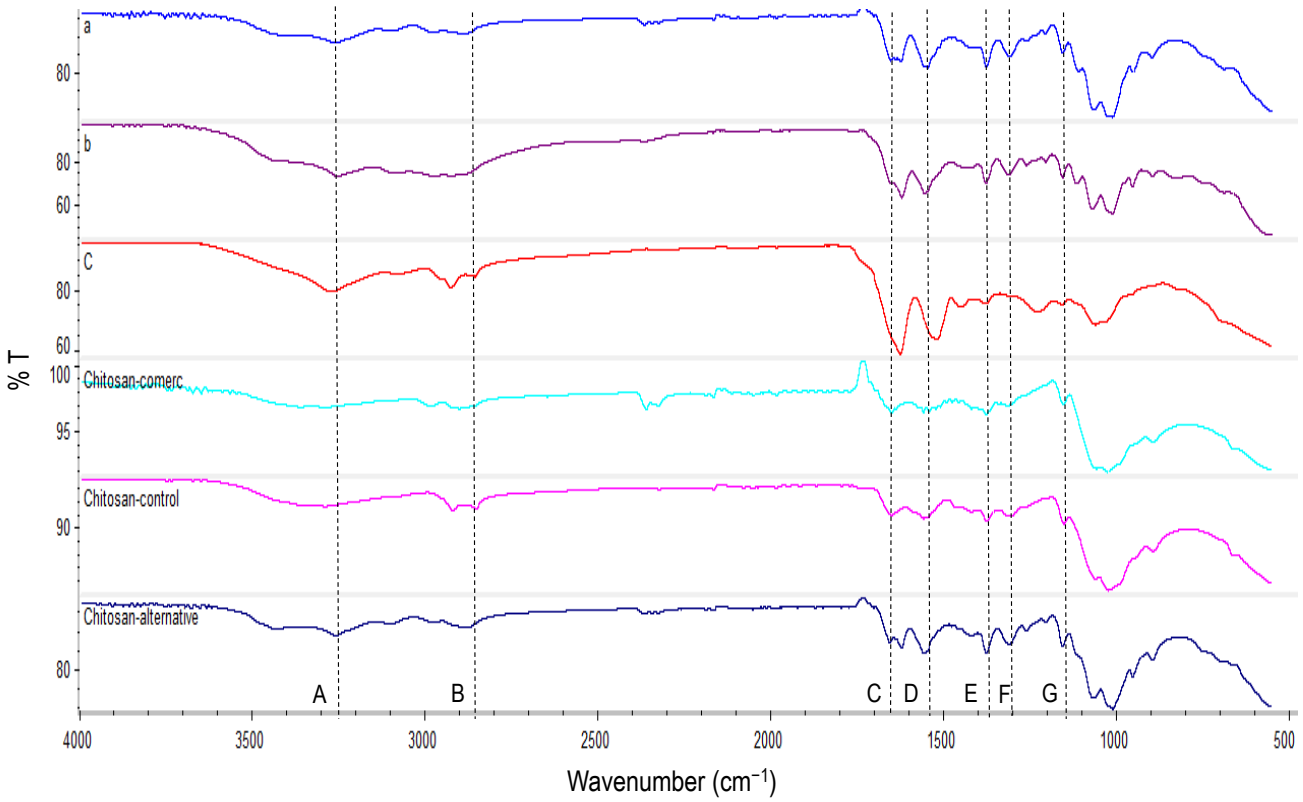

Figure 4. FTIR spectra of commercial chitin (a), chitin obtained from cricket flour by the chemical method (b), chitinous material obtained from cricket flour by the biological method (c), chitosan produced from commercial chitin (chitosan-commerc.), chitosan produced from chitin extracted from the cricket flour by the chemical method (Chitosan-control) and chitosan produced from chitin extracted from the cricket flour by the biological method (Chitosan-Alternative). A–G are the characteristic bands.

Regarding the chitin isolated from the cricket flour by the chemical method, it was observed that due to the formed hydrogen bonds, the carbonyl groups (-C=O) and (-NH-) of amide I and II, respectively, appear at 1650 cm^{-1}, and the one between the CH2OH and the carbonyl group (-C=O) appears at 1620 cm^{-1}, meaning the band of amide I is split in two peaks. This indicates that the chitin in house crickets is α-chitin [32]. It is also observed that the O-H and N-H stretching bands that appear usually at 3450 and 3270 cm^{-1}, respectively, overlap due to the water content and the peaks are not well separated [33]. Although the spectra of the chitosans are similar, the chitinous material that was isolated by the biological method appears different from the other chitinous fractions, with the peaks of amide I, N-H bend, and N-C stretch being higher due to the protein residues of the material.

2.3.3. Thermal Stability (TGA)

For Chitosan 1, the TGA suggests that the decomposition takes place between 280 and 400 °C with a 45.2% mass loss (Figure 5a). Before the decomposition starts, the observed mass loss is 11.62%, which comprises a 6.5% mass loss between 0 and 68.8 °C and another 5.2% between 69 and 300 °C. For Chitosan 2 (Figure 5b), the decomposition takes place between 280 and 400 °C with a 46.3% mass loss. Before the decomposition starts, between 0 and 150 °C, there is a mass loss of 10.7%. For Chitosan 3 (Figure 5c), the decomposition takes place between 280 and 400 °C with a 49.6% mass loss. Before decomposition, the mass loss takes place between 0 and 150 °C and there is a 7.4% mass loss and then another 3.4% between 200 and 300 °C. The residual mass of all three samples was approximately 30%.

The output of the TGA of the three studied chitosan products is very similar to the one previously reported for chitosan. The mass change between 0 and 150 °C has been attributed to the evaporation of water and is usually less than 10%, while the decomposition has been reported to take place between 300 and 400 °C. However, a mass loss between 150 and 300 °C is less common and suggests a partial decomposition within that range of temperature as well. The residual mass has been reported to be approximately 30% at 600 °C [27,34,35]. The three samples had similar thermal stability.

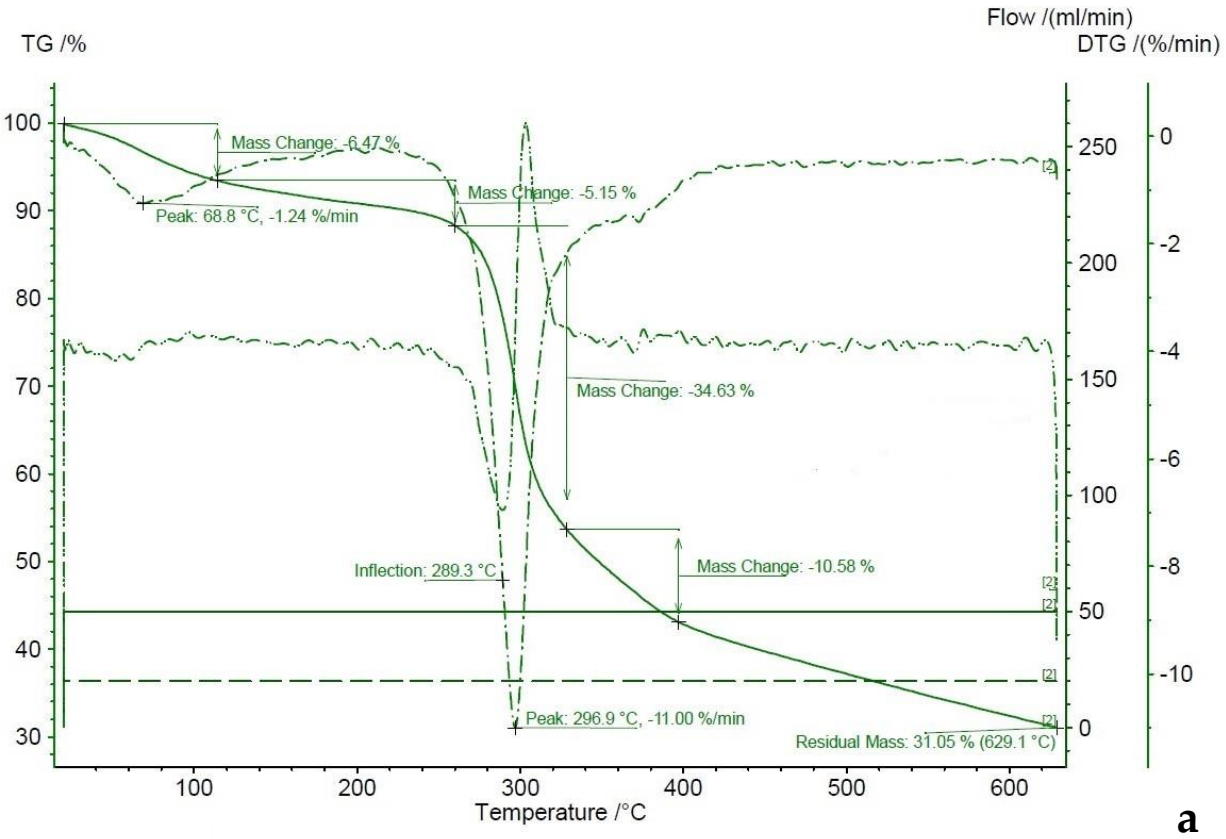

Figure 5. *Cont.*

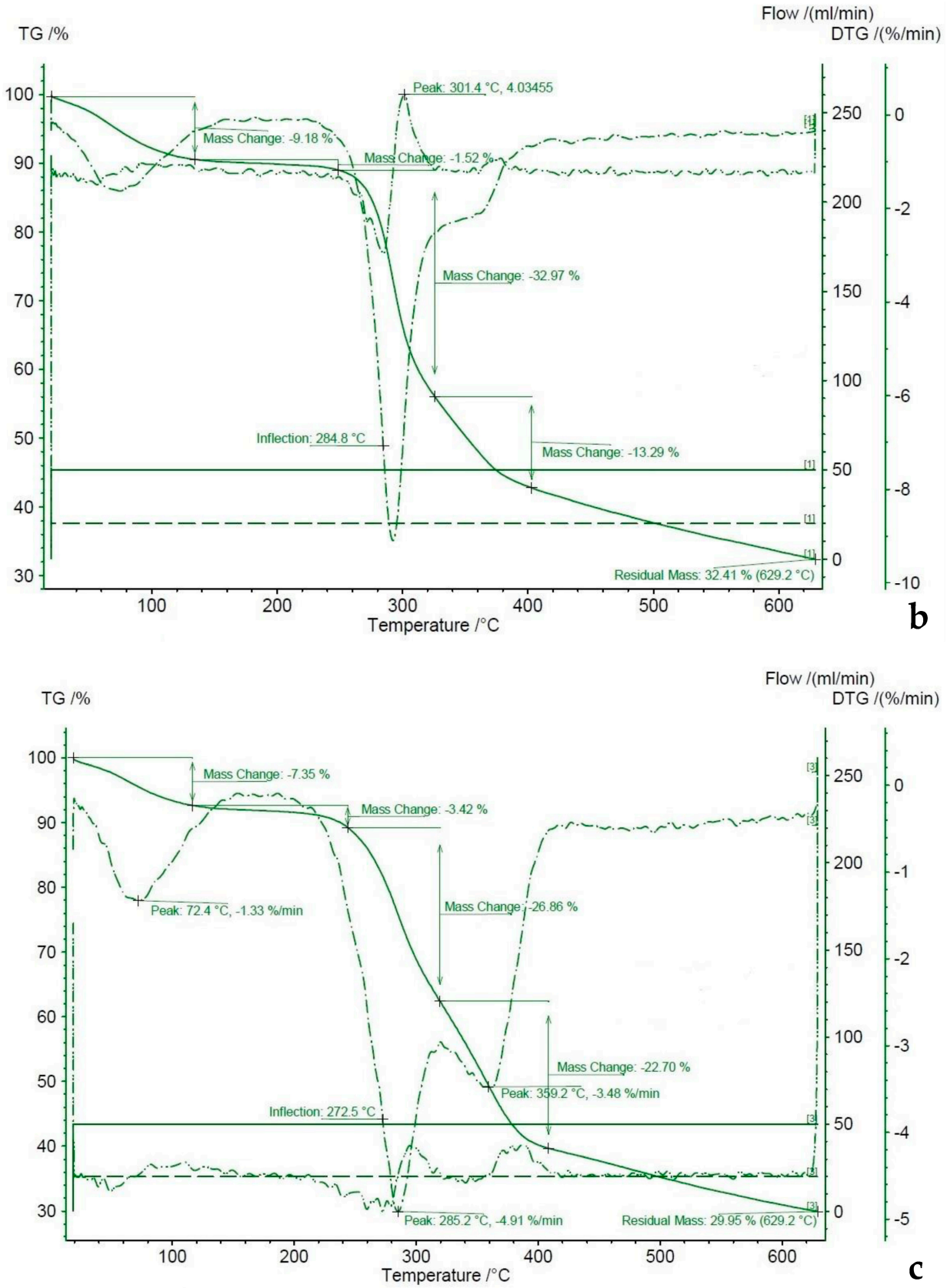

Figure 5. TGA thermographs and DTA curves of chitosan produced from commercial chitin (**a**), chitosan produced from chitin extracted from the cricket flour by the chemical method (**b**), and chitosan produced from chitin extracted from the cricket flour by the biological method (**c**).

2.3.4. Morphology (SEM)

As seen in Figure 6, the two materials obtained from cricket flour have a smooth surface with pores of various diameters. The surface of the chitin obtained by the chemical method (Figure 6b) appears smoother than the material obtained by the biological method (Figure 6c). A similar morphology of chitin has been observed for *Zophobas morio* [36] and some body parts of *Argynnis Pandora* [37].

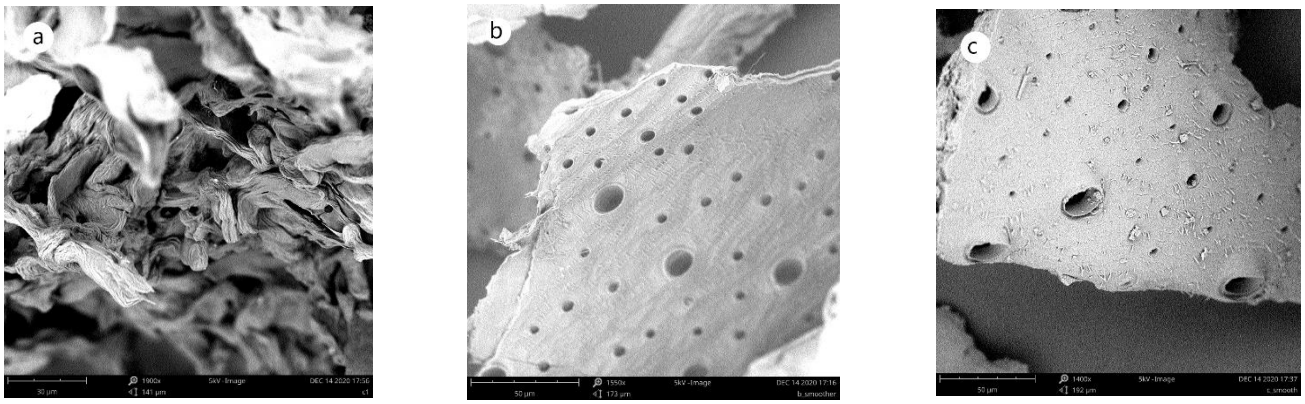

Figure 6. Morphology of chitin and chitinous material analyzed by SEM at 5 kV. (**a**) Commercial chitin, (**b**) chitin obtained from *Acheta domestica* by the chemical method, (**c**) chitinous material obtained from *Acheta domestica* by the biological method.

Chitosan 1 (Figure 7a) had a different morphology than the chitosan obtained from the isolated chitin from cricket flour (Figure 6b,c). Chitosan 1 had a flake-like structure. The chitosan produced from the cricket flour had a smooth surface, while some small pores appeared on the surface of Chitosan 3. The smooth surface of chitosan has been observed for chitosan from other species, such as cicada and grasshoppers [38], while a similar surface as the one depicted in Figure 7c has been observed for *Zophobas morio* [36].

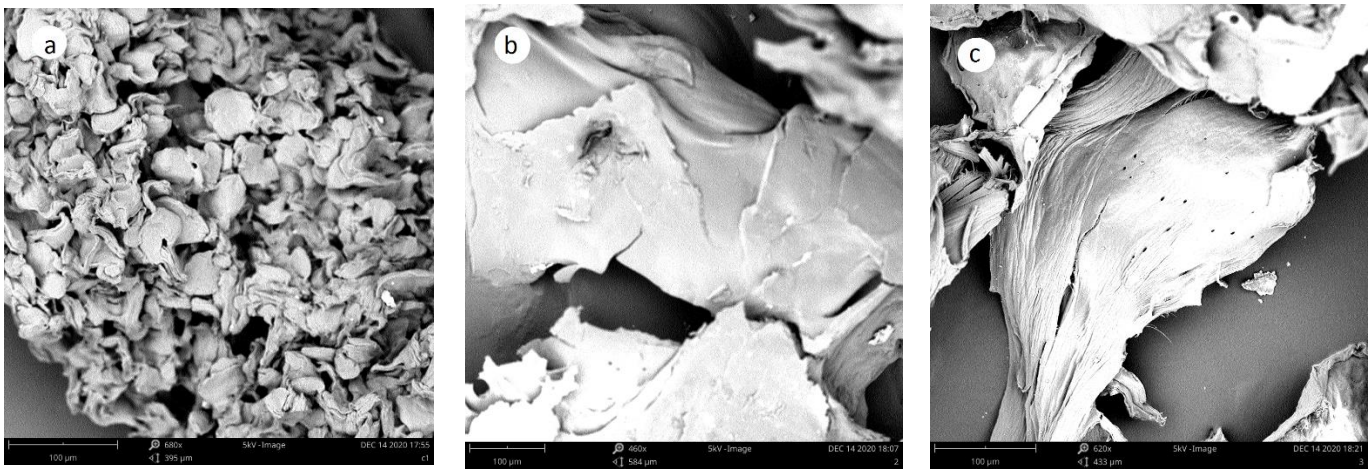

Figure 7. Morphology of chitosan analyzed by SEM at 5 kV. (**a**) Chitosan produced from commercial chitin, (**b**) chitosan produced from chitin obtained from *Acheta domestica* by the chemical method, (**c**) chitosan produced from chitinous material obtained from *Acheta domestica* by the biological method.

3. Materials and Methods

3.1. Insect Flour and Defatting

A commercial cricket flour (Thailand Unique, Udon Thani, Thailand) was used for this study. The flour was initially defatted with n-hexane for 2 h. The amount of removed

fat (17.7 ± 0.8 g fat/100 g d.w.) was determined gravimetrically, after removing the solvent with a rotary evaporator (Buchi R-100, Flawil, Switzerland). All chemicals were purchased from Carl Roth GmbH & Co. Kg (Karlsruhe, Germany), unless otherwise stated.

3.2. Chitin Isolation and Purification

3.2.1. Deproteinization Processes

Chemical treatment and proteolytic enzymes were tested for deproteinization of defatted cricket flour.

Chemical Deproteinization

Briefly, the chemical deproteinization (DP1) was performed by mixing the flour with a 1 M NaOH solution at s/l ratio of 1:50 and agitating the mixture at 80 °C for 24 h [39,40].

Papain-Assisted Deproteinization

For the deproteinization with papain (DP2), the flour was mixed with a 5 mM cysteine solution (s/l = 1:20) and was digested through the enzymatic solution with an enzyme/substrate ratio of 1:100 (mg/mg). The pH was adjusted to 6.5 and the process was performed for 24 h at 60 °C [41]. The pH and temperature conditions were based on the specification provided by Carl Roth GmbH & Co. Kg (Karlsruhe, Germany) for commercial papain.

Bromelain-Assisted Deproteinization

The treatment with bromelain (DP3) was performed by mixing the flour with water (s/l = 1:20) and digesting it through the enzymatic treatment with an enzyme/substrate ratio of 2% w/w for 5 h at 60 °C after adjusting the pH value to 5.5 [42]. The pH and temperature conditions were based on the specification provided by Carl Roth GmbH & Co. Kg (Karlsruhe, Germany) for commercial bromelain.

3.2.2. Demineralization Processes

Demineralization of defatted cricket flour was carried out using four different methods: chemical demineralization), microwave treatment, citric acid, and fermentation with Lactococcus lactis (DSMZ Braunschweig Germany, DSM 20729).

Chemical Demineralization

The chemical demineralization (DM1) refers to the mixing of the sample with 1 M HCl (s/l ratio 1:30) and then agitating for 2 h at 98 °C [27].

Microwave-Assisted Demineralization

For the microwave-assisted demineralization (DM2), the samples were added to a 1 M HCl solution (s/l ratio= 1:30) and then processed via microwave heating for 8 min at 500 W [20].

Citric Acid Demineralization

The demineralization with citric acid (DM3) was performed with 0.5 M citric acid (s/l ratio 1:30) and agitation for 2 h at room temperature [14].

Lactic Acid Demineralization

Fermentation with *L. lactis* spp. (previously stored in the form of lyophilized culture) was performed for demineralization (DM4). Bacterial strains of *L. lactis* were supplied by the library of the Department of Bioengineering and the Microbiology Lab of the Leibniz Institute for Agricultural Engineering and Bioeconomy, respectively. Sterile MRS Bouillon Lactobacillus broth acc. (Merck KGaA, Darmstadt, Germany) was mixed with the lyophilized culture that was cultivated at 30 °C for 48 h. The culture was composed of 10%

w/*v* cricket flour. The amount of inoculant was <1% v/v. The fermentation lasted 7 days at 30 °C with agitation at 150 rpm [21].

3.2.3. Single-Step Chitin Isolation

Fermentation with *B. subtilis*

Bacterial strain of *B. subtilis* was supplied by the library of the Department of Horticultural Engineering and the Microbiology Lab of the Leibniz Institute for Agricultural Engineering and Bioeconomy, respectively. The fermentation process with *B. subtilis* was performed in the following medium (g/L): peptone 10, yeast extract 5, and NaCl 5, with the pH adjusted to 7. The culture was composed of 5% (w/v) cricket flour and 5% (w/v) of glucose. The fermentation was performed for 5 days at 37 °C on a rotary shaker (150 rpm). The amount of inoculant was <1% *v*/*v* [8].

Eutectic Solvents

For chitin isolation by DES, choline chloride (Alfa Aesar, Massachusetts, United States) and malonic acid were used at a molar ratio of 1:1 with mixing for 2 h at 100 °C. Cricket flour was mixed with DES (3 g of flour with 50 g of CC/MA) and stirred at 80 °C for 3 h. Then, 100 mL of water was added and the mixture was stirred for another 30 min [13]. At the end of each process, the liquid phase was removed by centrifugation at 3200× *g*, 10 min, after being cooled to room temperature in a water bath. Then, the supernatant was removed and the pellet was washed with water, heated at 60 °C, and then centrifuged again. The washing was repeated until the pH of the water became neutral; then, the pellet was dried at 60 °C until it reached a constant weight, when it was retained for further analysis.

3.2.4. Degree of Demineralization

The efficiency of each demineralization process was evaluated by estimating the ash content of the pellet and the initial material and calculating the degree of demineralization (DDM %) using Equation (1):

$$\%\text{DDM} = \frac{[(M_O \cdot O) - (M_R \cdot R)] \cdot 100}{(M_O \cdot O)} \quad (1)$$

where M_O and M_R are the ash content (g/100 g d.w.) before and after demineralization, respectively, and O and R are the dry weight (g) of the material before and after the demineralization, respectively [8]. The ash content was determined gravimetrically by measuring weight before and after the samples were placed at 550 °C.

3.2.5. Determination of Chitin Content

The efficiency of the deproteinization methods was evaluated by measuring chitin content of the isolated solid fraction [43]. Standard chitin was used for the calibration curve and the results were expressed as mg chitin/100 mg d.w.

3.3. Chitosan Production and Characterization

3.3.1. Selected Processes for the Isolation of a Chitin-Rich Fraction

Two of the aforementioned processing pathways were selected for the production of a chitinous material. The first was the sequential chemical treatment of the defatted flour with NaOH (deproteinization) and HCl (demineralization), with washing steps in between; this was considered as the control. The second method was the sequential combination of the fermentation of the flour with *L. lactis* spp. and the proteolytic solution containing bromelain, with washing steps in between. Both processes were performed on a large scale with washing steps in between. The fermentation was performed in a 2L BIOSTAT B bioreactor (Sartorius AG, Germany) and continuous monitoring of the pH, which was initially equal to 5.9, dropped to 4.3 in the beginning of the 6th day, and remained stable

until the end of the process. The obtained chitinous products were dried at 60 °C until constant weight. All treatments were performed as described in the previous section.

3.3.2. Deacetylation of Products

Deacetylation of the two obtained chitinous materials, as well as of commercial chitin, was performed with a 50% NaOH solution for 3 h at 130 °C. The samples were cooled to room temperature and filtered. Then, the chitosan was washed with water until the washing water had a neutral pH [44]. Standard chitin was deacetylated similarly and chitosan was obtained from commercial chitin to be used as a reference for comparison.

3.3.3. Characterization of Chitin-Rich Fractions

Chitosan Content

The chitin content of the generated materials, was determined spectrophotometrically, as described in Section 3.2.5 [43].

Fourier-Transform Infrared Spectrometry (FTIR)

The chitinous materials were analyzed directly by Fourier-transform infrared spectrometry (Nicolet iS5, Thermo Scientific, US-WI 53711 Madison, Waltham, MA, USA).

Scanning Electron Microscopy (SEM)

The morphology of the chitinous materials was analyzed by scanning electron microscopy (Phenom Elektronenmikroskop, Phenom-World BV, NL-5652 AM Eindhoven, The Netherlands).

3.3.4. Characterization of the Produced Chitosan Materials

Chitosan Content

The chitosan content was determined as described for the chitin-rich fractions.

Fourier-Transform Infrared Spectrometry (FTIR)

The chitosan samples were analyzed as described for the chitin-rich fractions. The degree of deacetylation was calculated based on the FTIR signal from the following equations [45]:

$$DD\% = 100 - DA\% \quad (2)$$

$$A_{1320}/A_{1420} = 0.3822 + 0.03133 \cdot DA \quad (3)$$

Thermogravimetric Analysis (TGA)

The thermal stability of the obtained chitosan materials was determined by thermogravimetric analysis (TGA) with a STA449 F3 Jupiter instrument (NETZSCH-Gerätebau GmbH, D-95100 Selb, Germany) and a 10 °C/min temperature change from 25 to 650 °C in N_2 [46].

Scanning Electron Microscopy (SEM)

The morphology of the obtained chitosan was analyzed as described for the chitinous materials.

Antioxidant Activity

The antioxidant activity of chitosan was determined with the DPPH radical scavenging assay. Specifically, 2,2-diphenyl-1-picrylhydrazyl (Alfa Aesar, Haverhill, MA, USA) was solubilized in methanol to obtain a DPPH solution of $6 \cdot 10^{-5}$ M and the three chitosan products were solubilized in a 1% acetic acid solution to obtain chitosan solutions of 4.5 mg/mL. Then, 1 mL of chitosan solution was mixed with 3 mL of the DPPH solution and incubated at room temperature for 30 min in the dark. The absorbance was measured at 515 nm with a UV/Vis spectrometer (Spectronic Unicam UV1, Thermo Fisher Scientific,

Waltham, MA, USA); 1 mL of the 1% acetic acid solution was used as a blank. Antioxidant activity was calculated from:

$$\text{Antioxidant activity\%} = (A_0 - A)/A_0 \quad (4)$$

where, A_0 is the absorbance of the blank and A is the absorbance of the sample [27].

Determination of Molecular Weight

Viscometry was used to determine molecular weight. Chitosan solutions were prepared by stirring for 24 h at room temperature using a 0.1 M acetic acid/0.2 M NaCl solution with a concentration range of 0.2–1.5 g chitosan /L. The viscosity of the solutions was measured. Intrinsic viscosity [η] was calculated using Equation (5) and the molar mass was calculated using the Mark–Houwink equation (Equation (6)) [47]:

$$[\eta] = (\eta_{SP}/C)_{C\to 0} = (\eta_{reduced})_{C\to 0} \quad (5)$$

$$[\eta] = K \cdot M_w^a \quad (6)$$

where $K = 1.81 \times 10^{-3}$ mL/g and $\alpha = 0.93$ [48].

3.4. Statistical Analysis

Each experiment was repeated at least in triplicate. Significant differences among data obtained for samples generated by different processes were investigated with one-way analysis of variance (ANOVA). Tukey's post hoc test was applied to separate means with significant differences ($p \leq 0.05$). Data that did not follow a normal distribution were normalized before the analysis. The software used was IBM SPSS Statistics 23 (IBM Corp., Armonk, NY, USA).

4. Conclusions

The present study demonstrates that implementing alternative methods for chitin extraction from *Acheta domesticus* was efficient in removing minerals and less efficient in removing proteins compared to conventional treatments. Two chitinous materials were isolated using the conventional process and a biological process (digestion with bromelain solution and fermentation with *L. lactis*) and deacetylated into chitosan with comparable properties. Therefore, the biological process was appropriate for replacing the chemical process to generate a chitinous material as a basis for chitosan production from the cricket flour, with the advantage of non-hazardous waste. The successful large-scale production of chitosan following the biological method makes it applicable for industry. Regarding future perspectives, it is important to further investigate the properties of cricket-derived chitosan and explore its various properties and applications. In terms of the improvement of the extraction methods and the properties of the products, a variety of enzymes and fermentation techniques can be explored, as well as the effect of emerging technologies such as microwave treatment.

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Conflicts of Interest: The authors declare no conflict of interest.

Sample Availability: Samples of the compounds are not available from the authors.

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6.7 Methylene blue adsorption and bioactivity of chitosan produced from house crickets with novel methods

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Methylene blue adsorption and bioactivity of chitosan produced from house crickets with novel methods

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Abstract

Edible insects are an important source of chitin. Chitosan is produced via deacetylation of chitin and offers a variety of applications due to its bioactive properties and wastewater treatment abilities. The process to isolate chitin from insects is usually time consuming and environmentally hazardous. Different methods, including use of proteases, fermentation and microwave treatment, have been proposed to replace the conventional chitin isolation methods. Chitosan was produced from house crickets from chitinous materials that were isolated via the conventional method, a biological process that combines fermentation with *Lactococcus lactis* and digestion with bromelain and a microwave-assisted chemical method. All chitosans were evaluated for their purity, functional properties, antioxidant and antimicrobial activity and methylene blue adsorption properties. All three methods were sufficient in generating a chitosan with a purity higher than 85%. Furthermore, all cricket-derived chitosans showed a significant level of antioxidant activity with an effective concentration of 5 mg/mL or lower and an antimicrobial activity against *Escherichia coli, Staphylococcus aureus* and *Salmonella enterica ssp. Enterica Serovar Typhimurium*. Finally, all chitosans exhibited methylene blue adsorption ability. The one produced from chitinous material that was extracted with the biological method had a maximum adsorbing capacity of 643.899 mg/g. Methylene blue adsorption was best described by the Langmuir isotherm and followed a pseudo-second order kinetics. It was concluded, that the biological chitin extraction method leads to the generation of a chitosan material with high potential for application on different sectors.

Keywords: chitosan, crickets, fermentation, enzyme, bioactivity, methylene blue

Highlights

- Biological and microwave assisted-chitin extraction were applies to house crickets
- Chitosan was produced with high purity, antioxidant and antimicrobial activity
- Biologically extracted chitosan has a high methylene blue adsorption capacity

1. Introduction

Edible insects have attracted considerable popularity in the recent years due to their rich nutritional profiles and low environmental impact [1]. They have lower feed [1] and land requirements [2] and produce lower gas emissions compared to conventional livestock [1]. The house crickets (*Acheta domesticus*) are particularly interesting, because of their easy rearing process [3], high reproduction rate [4] and rich nutritional profile [5]. Furthermore, house crickets are being already consumed as food and feed in different parts of the world [6] and they have been recently accepted as novel food pursuant to Regulation (EU) 2015/2283 [7].

Apart from proteins and lipids, insects contain also chitin [8] Chitin is a polysaccharide that is abundant on fungal species and invertebrates [9] and consists of chain of β-(1-4)-N-acetyl-D-glucosamine [10]. It can be found on the exoskeleton of insects [9] and its content on insects varies, based on the insect species and life stage [8]. The substitution of the N-acetyl group of chitin with an amine group is called deacetylation and describes the reaction of production of chitosan from chitin [11].

A variety of edible insects species has been tested for chitin extraction and chitosan production [12]. The insect-derived chitosan has exhibited some of the bioactive properties that are commonly reported for crustacean-derived chitosan, such as antioxidant activity [13] and antimicrobial activity [14]. Chitin is usually isolated from insects via processing pathways that include sequential delipidation, deproteinization, demineralization and decolorization, which often require the use of hazardous chemicals, such as HCl and NaOH at elevated temperatures, long durations [12] and are not considered environmentally friendly [15].

A number of alternative and more environmentally friendly methods including fermentation, proteolysis and deep eutectic solvents have been proposed to replace the conventional treatments for chitin extraction [16, 17]. Furthermore, microwave processing has been shown to facilitate the conventional method of chitin isolation by significantly reducing the treatment time [18]. These methods have also been underlined for the potential, when applied to edible insects for chitin extraction [19, 20]. A chitinous material can be isolated from mealworms with

fermentation [21]. Furthermore, proteolysis has been successfully applied to mealworm for chitin isolation [22]. Furthermore, deep eutectic solvents have been successful in chitin extraction from black soldier flies [17]. Moreover, a combination of fermentation with *Lactococus lactis* and digestion with bromelain has been proposed to isolate a chitinous material from house crickets that can be used for chitosan production [23].

Chitosan offers potential for a variety of application in the medicine, cosmetic, food and agricultural sector [24]. Chitosan has been characterized with antioxidant activity [25], antitumor activity [26] and antimicrobial activity [27]. Furthermore, chitosan has been reported to be effective in adsorption of ionic dyes, as a means of purifying wastewater [28]. Methylene blue is one of the most commonly used dyes, especially in the textile industry as colorant [29]. Chitosan has shown potential for methylene blue removal, both as a part of composite materials [30] and being used directly after being isolated from shrimp [31].

The present study aims to compare the method that was applied by Psarianos et al. [23] with the conventional method and a microwave-assisted method for chitin extraction and chitosan production and evaluate the properties of the produced chitosans in terms of functionality, bioactivity and methylene blue adsorption capacity.

2. Materials and Methods

2.1 Sample preparation

Living crickets (*Acheta domestica*) were purchased from Tropic Shop (Nordhorn, Germany) and were inactivated by freezing at -20 ^{o}C. They were thawed at 4 ^{o}C, separated from frass, washed with water and dried at 60 ^{o}C until constant weight. The dried insects were milled for 10 s with a laboratory Retsch mill (Haan, Germany) and were defatted using hexane (solid/liquid ratio 1:20) for 1 h at room temperature. The hexane was recycled using a rotary evaporator (Büchi R-100, Flawil, Switzerland) and the defatted cricket biomass was stored for further use. Standard chitosan was used as reference for all experiments. All chemicals were purchased from Carl Roth Gmbh & Co. Kg (Karlsruhe, Germany), apart from 2,2-diphenyl-1-picrylhydrazyl (Alfa Aesar, Massachusetts, United States).

2.2 Chitin extraction

2.2.1. Chemical method

The samples were deproteinized using a 1 M NaOH solution (solid/liquid ratio 1:50) for 24 h at 80ºC. Afterwards they were demineralized with a 1 M HCl solution (solid/liquid ratio 1:30) for 2 h at 98ºC. After both deproteinization and demineralization, the sample was washed with warm distilled water through a 0.063 mm filter, until the water reached a neutral pH. Afterwards, it was dried at 60ºC until constant weight [32, 33].

2.2.2. Biological method

The biological method was adapted from the work of Psarianos et al.[23]. Briefly, the cricket biomass was fermented with *Lactococcus lactis* (Library of the Department of Microbiom Biotechnology of the Leibniz Institute for Agricultural Engineering and Bioeconomy) for 7 d at 30 ºC, 150 rpm, using sterile MRS Bouillon Lactobacillus broth acc. with an inoculant of <1% v/v and a 10% w/v of cricket biomass. Afterwards, the samples were digested with bromelain, after being mixed with water (solid/liquid ratio 1:20), for 5 h at 60 ºC, pH=5.5 and an enzyme/substrate ratio of 2% w/w. Between both steps the samples were washed and afterwards dried, as described in section 2.2.1. [34, 35].

2.2.3. Microwave-assisted method

The microwave-assisted method was adapted from the work of Knidri et al. (2019) [18]. The samples were deproteinized with a 1 M NaOH solution (solid/liquid ratio 1:50) with a microwave treatment for 8 min at 500 W. Afterwards the samples were demineralized with a 1 M HCl solution (solid/liquid ratio 1:30) with a microwave treatment for 8 min at 500 W . Between each steps the samples were washed and in the end, they were dried, as described in section 2.2.1.

2.3 Deacetylation

Deacetylation of all materials was performed with a 50% NaOH solution at 130 ºC for 2 h. Afterwards, the samples were washed with water through a 0.063 mm filter until neutrality of the water and dried at 60ºC until constant weight [36].

2.4 Properties of chitosan

2.4.1. Chitosan content (g chitosan/100 g)

The chitosan content of all chitosan samples was estimated by measuring glucosamine content [37]. Standard chitosan was used for the calibration curve at a concentration range of 2.93-11.72 µg/ml and the results were expressed as percentage of chitosan (%).

2.4.2. Solubility (%)

Solubility was determined after homogenizing 0.1 g of chitosan with 10 mL of an acetic acid 1% solution for 1 h at room temperature inside a falcon. Afterwards, the mixture was centrifuged at 3900 rpm for 10 min and the supernatant was decanted. Solubility was estimated as:

$$\text{Solubility (\%)} = (M_1-M_2)/(M_1-M_0) \times 100 \quad \text{(Eq. 1)}$$

where M_0, M_1 and M_2 are the weight of the empty falcon and the weight of the falcon together with the sample before and after the process, respectively [38].

2.4.3. Oil binding capacity (g oil/g chitosan)

The oil binding capacity (OBC) was determined after homogenizing 0.5 g of chitosan with 10 mL of commercial rapeseed oil and the mixture was vortexed for 60 s and then centrifuged at 3900 rpm for 10 min. The supernatant was decanted and the OBC was estimated as:

$$\text{OBC (g oil/ g chitosan)} = (m_f-m_0)/m_0 \quad \text{(Eq. 2)}$$

where m_f and m_0 are the final and initial weight of the sample, respectively [38, 39].

2.4.4. pH

A chitosan stock solution was prepared at a concentration of 5 mg/mL by mixing 500 mg of chitosan with 100 mL of acetic acid 1% for 24 h at room temperature [23]. Afterwards, the solution was stored in at 4 ^{o}C for maximum a week to avoid destabilization [40]. The pH of the solution was measured directly with a pH meter (Lab 850, SI Analytics GmbH, Rhineland-Palatinate, Germany).

2.4.5. Polydispersity index (PDI) and Zeta Potential (mV)

For determination of the PDI and the zeta potential, the stock solution that is mentioned in the section 2.4.4 was diluted to a concentration of 1 mg/mL and was placed inside a folded capillary cuvette and analyzed directly with a zetasizer (Malvern Panalytical, Malvern, UK).

2.5 Bioactivity

2.5.1. Antioxidant activity

For the determination of the antioxidant activity, the stock solution (section 2.4.4) was diluted to a concentration range of 0.5-5 mg/mL. An ascorbic acid solution at the same concentration levels was used as a positive control.

Free radical scavenging activity (2,2-diphenyl-1-picrylhydrazyl)

The free radical scavenging activity was determined by mixing 1 mL of the chitosan solution at different concentrations with 3 mL of a DPPH solution of $6 \cdot 10^{-5}$ M and incubating the mixture at room temperature in the dark for 30 min. Then the absorbance of the mixture was measured with a UV/Vis spectrometer at 515 nm. 1% Acetic acid was used as blank. Scavenging activity was expressed as:

$$\text{Scavenging activity (\%)} = (A_0 - A_1)/A_0 \times 100 \quad \text{(Eq. 3)}$$

The concentration of each solution that can result in 50% of scavenging activity (IC50) was determined from a linear regression of the concentration of the solution and the scavenging activity [41].

Ferric iron reducing power (FRAP)

The ferric iron reducing power was estimated as follows: 0.5 mL of each solution at different concentrations was mixed with 0.5 mL of sodium phosphate buffer (0.2 M, pH=6.6) and 0.5 mL of potassium ferricyanide 1%. The mixture was incubated at 50 ^{o}C for 20 min and then was mixed with 0.5 mL of trichloroacetic acid 10%. Then 2 mL of water was added to the mixture and finally, 0.4 mL of ferric chloride 0.1%. The mixture was vortexed and the absorbance was

measured with a UV/Vis spectrometer at 700 nm. A higher absorbance indicates a higher antioxidant activity. The effective concentration (EC50), which is the concentration that can lead to an absorbance of 0.5, was estimated with linear regression of the concentration of the solution and the absorbance [42].

2.5.2. Antimicrobial activity

The antimicrobial activity of chitosan was tested using the stock solution (section 2.4.4) at different concentrations against four bacterial strains: *Escherichia coli, Staphylococcus aureus* and *Salmonella enterica ssp. Enterica Serovar Typhimurium.* The strains were stored at -80 ^{o}C prior to use. Each strain was streaked on to a plate count agar plate and incubated for 18 h at 37 ^{o}C. A single colony was removed from each plate, inoculated in tubes containing 25 mL of sterile Mueller–Hinton Broth (MHB), and incubated for 22 h at 37^{o}C. The overnight cultures were shaken and aliquots (10^{7}-10^{8} cells/ml) were diluted in sterile MHB to produce solutions of approximately 10^{6} cells/ml. All solutions (100 µL) were subjected to a minimum inhibitory concentration (MIC) antimicrobial test at serial dilutions of 1:2 by mixing with 50 µL of the bacterial culture of all strains for 24 h at 37^{o}C and then adding 50 µL of Iodonitrotetrazolium chloride (INT) solution and incubating for 1 h using a 96-well micro plate. The MIC of each solution was determined as the lowest concentration at which the pink color did not occur. MHB was used as a blank and gentamycin sulphate 0.2 mg/mL was used as a positive control [43].

2.6 Methylene blue (MB) adsorption capacity

The methodology to evaluate the adsorption of MB from chitosan was based on the work of Marzban et al. (2021) [44]. In order to obtain the kinetic data, 5 mL of a 10-ppm MB solution was mixed with 5 mg of chitosan for 0-20 min. The mixture was then centrifuged at 3900 rpm for 10 min. The absorbance of the supernatant was measured with a UV/Vis spectrometer at 610 nm to determine the concentration of the dye. A MB solution at different concentrations (0-10 ppm) was used for the calibration curve. The adsorption capacity of chitosan q (mg/g) was estimated as follows:

$$q = V \cdot (c_i - c_f)/M \quad \text{(Eq. 4)}$$

where c_f and c_i are the initial and final concentrations of MB (ppm), respectively, V (L) is the volume of the solution and M is the weight (g) of chitosan .

The adsorption of MB was modelled with the adsorption time (min) using the pseudo-first-order (Eq. 5) and the pseudo-second-order (Eq. 6) models [45]:

$$\ln(q_e - q_t) = \ln q_e - k_1 \cdot t \quad \text{(Eq. 5)}$$

where q_e and q_t (g/kg) are the adsorption capacities at equilibrium and at any time interval t (min), respectively and k_1 (1/min) is the pseudo-first-order constant rate.

$$1/q_t = 1/(k_2 \cdot q_e^2) + 1/q_e \quad \text{(Eq. 6)}$$

where k_2 (g/mg·min) is the pseudo-second-order constant rate.

Furthermore, the same procedure was followed, using different concentrations of the MB solution (20-320 ppm) for the time duration (min) that is required to reach equilibrium. The adsorption mechanism was studied using the Langmuir (Eq. 7) and Freundlich (Eq. 8) isotherm models [44, 45]:

$$C_e/q_e = c_e/q_m + 1/k_L \cdot q_m \quad \text{(Eq. 7)}$$

where q_e and q_m (mg/g) are the equilibrium and maximum adsorption capacity, respectively, k_L (L/mg) is the equilibrium constant and c_e (mg/L) is the MB concentration at equilibrium.

$$\ln q_e = \ln k_F + \ln c_e/n \quad \text{(Eq. 8)}$$

where k_F and n are Freundlich constants.

2.7 Statistical analysis

Each method used to produce chitosan was replicated three times. Every analytical test was performed for each replicate of production of each material at least twice. Data were analyzed with an analysis of variance (ANOVA) and Tukey's post hoc test to separate significantly

different means (p≤0.05). The analysis was performed with IBM SPSS Statistics 23 (IBM Corp., Armonk, N.Y., USA).

3. Results and Discussion

3.1 Characterization of chitosan

Table 1: Properties of chitosan that was commercially purchased or generated from the crickets from chitin isolated through the chemical (Chitosan_C), biological (Chitosan_B) and microwave-assisted method (Chitosan_M).

	Commercial chitosan	Chitosan_C	Chitosan_B	Chitosan_M
Chitosan content (%)	-	89.18 ±3.12^{a}	87.96 ±5.33^{a}	87.11 ±6.82^{a}
Solubility (%)	97.34 ±0.48^{a}	70.73 ±6.69^{b}	55.07 ±4.40^{c}	42.56 ±0.52^{d}
OBC (g oil/g chitosan)	411.70±13.98^{a}	1078.62 ±184.68^{b}	885.30 ±163.03^{b}	860.92 ±280.29^{b}
pH	3.32 ±0.00^{a}	3.50 ±0.12^{a}	3.78 ±0.04^{b}	3.43 ±0.15^{a}
PDI	0.43 ±0.07^{a}	0.73 ±0.21^{ab}	0.84 ±0.22^{ab}	1.15 ±0.28^{b}
Zeta Potential (mV)	27.56 ±2.20^{a}	38.86 ±3.41^{ab}	50.58 ±10.94^{b}	52.35 ±5.64^{b}

Data are expressed as mean ±SD. Different superscript letters (a,b…) indicate significant differences ($p<0.05$) among the means of the properties of samples that were generated with different methods.

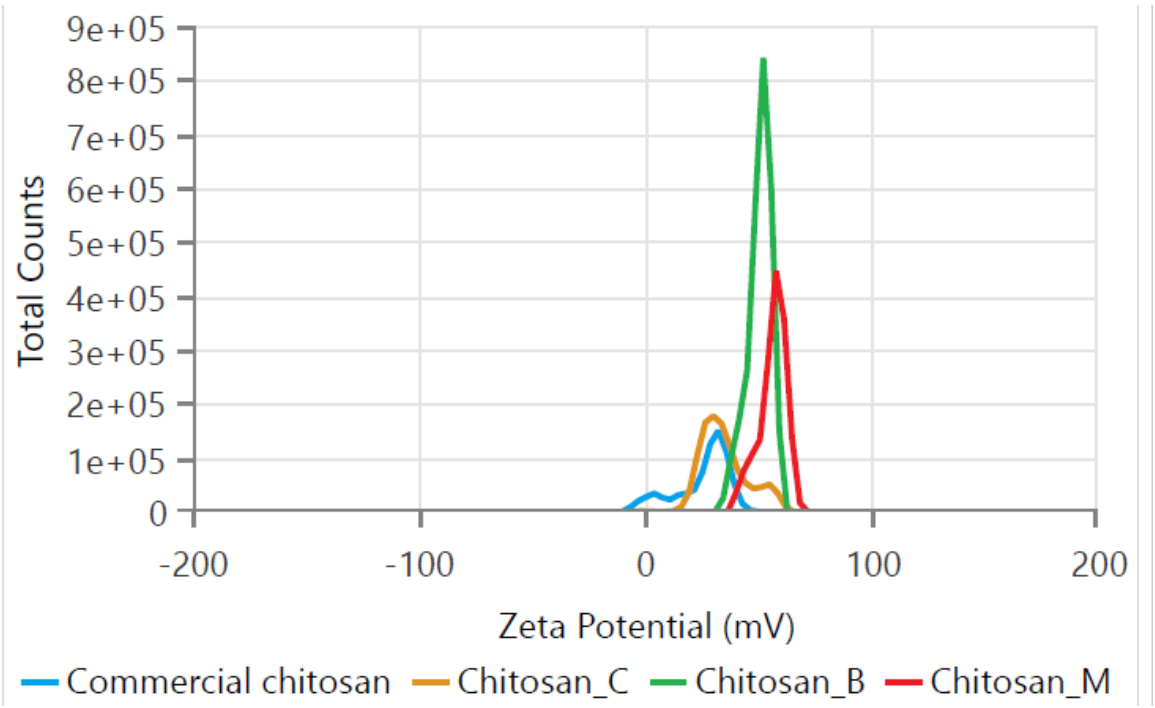


Fig. 1: Zeta potential (mV) of of chitosan that was commercially purchased or generated from the crickets from chitin isolated through the chemical (Chitosan_C), biological (Chitosan_B) and microwave-assisted method (Chitosan_M).

All chitosans produced from the insect biomass showed a high level of purity with a chitosan content higher than 87% for all samples, without significant differences ($p>0.05$). A high chitosan content (%) was expected for all samples, since the biological method has been successfully applied to house crickets to produce chitosan [23]. Furthermore, the positive effect of microwave treatment has also been reported for shrimp chitosan [18].

The chitosans showed a significantly different OBC ($p<0.05$), with the chitosan being produced from the crickets demonstrating over twice OBC, compared to commercial chitosan. The OBC was not affected by the method used to isolate chitin. The OBC was high (>800 g oil/g chitosan) for all samples that were generated from the insects. The differences in the OBC can be attributed to possible differences in crystallinity, salt forming groups and protein residues in the samples [46]. A high value of OBC has also been reported for chitosan isolated from other insect species including cicada, silkworm and mealworm [12].

The solubility of the chitosans also showed significant differences ($p<0.05$). The commercial one had the highest solubility and the ones that were generated from the crickets showed a low solubility. Chitosan solubility is affected by several factors, including pH, temperature, solvent, ionic strength and degree of acetylation (DA%) [47]. The low solubility of the chitosans obtained from the crickets could be explained by a reduced hydrophility due to a DA% that was reported to be within the range of 20% and 50% [23, 48]. It is also important to consider the values of the PDI, since the chitosans that are produced from the insects show values higher than 0.5, which indicate a higher heterogenity [49]. Furthermore, Chitosan_B and Chitosan_C showed significantly higher ($p<0.05$) zeta potential values than the commercial one (Table 1 and Fig. 1), which is an indication of a higher colloidal stability and resistance to aggregation [50].

3.2 Bioactivity of chitosan

3.2.1. Antioxidant activity

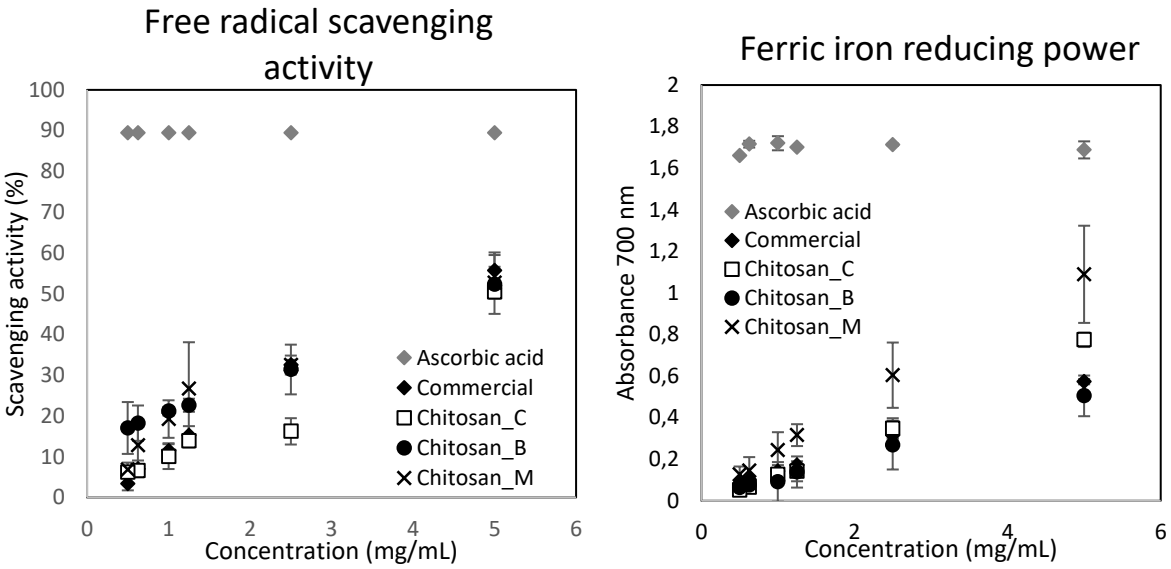


Fig. 2: Free radical scavenging activity (left) and ferric iron reducing power (right) of chitosan that was commercially purchased or generated from the crickets from chitin isolated through the chemical (Chitosan_C), biological (Chitosan_B) and microwave-assisted method (Chitosan_M). Error bars represent the standard deviation of each value based on multiple replications of the measurement.

All chitosans were found to have both free radical scavenging activity and ferric iron reducing power. The free radical scavenging activity of all chitosans ranged between 10% and 60%, with ascorbic acid showing an approximately 90% of scavenging activity. The absorbance at 700 nm that indicates FRAP was found for all chitosan to range between 0.1 and 1. The absorbance obtained for ascorbic acid was approximately 1.7. Higher concentration of chitosan in the solution led to a higher antioxidant activity of the solution.

Both free radical scavenging activity and ferric iron reducing power were observed for all chitosan samples at a concentration range of 0.5-5 mg/mL. This concentration range has been reported also for insect-derived chitosan from different species, including *C. barbarous, O. decorus, Musca domestica* and *Chrysomya megacephala* [13, 41, 51].

Table 2: IC50 and EC50 of chitosan that was commercially purchased or generated from the crickets from chitin isolated through the chemical (Chitosan_C), biological (Chitosan_B) and microwave-assisted method (Chitosan_M).

	IC50 (mg/ml)	EC50 (mg/ml)
Commercial chitosan	4.36 ± 0.28^{a}	4.26 ± 0.11^{ab}
Chitosan_C	$5,31 \pm 0.47^{b}$	3.37 ± 0.25^{b}
Chitosan_B	4.62 ± 0.55^{a}	5.06 ± 1.13^{a}
Chitosan_M	4.46 ± 0.37^{a}	2.27 ± 0.54^{c}

Data are expressed as mean ±SD. Different superscript letters (a,b…) indicate significant differences ($p<0.05$) among the means obtained for samples that were generated with different methods.

Regarding the scavenging activity, Chitosan_B and commercial chitosan showed no significant differences ($p>0.05$) of the IC50, while Chitosan_C was significantly higher ($p<0.05$). In contrary, regarding the EC50, the solution of Chitosan_C showed a significantly lower EC50 ($p<0.05$). Commercial chitosan and Chitosan_B solutions showed no significant differences ($p>0.05$), meaning that regarding both the scavenging activity and the reducing power, Chitosan_B could replace the commercial one.

Furthermore, the effective concentration of solutions of chitosan of crab [25] or insect origin [41, 52] has been reported to be much higher as the one reported by the present study. The lower effective concentration indicates a stronger antioxidant activity and is attributed to the lower molecular weight that has been reported for chitosan produced from house crickets with the methods described in section 2.2 [23]. It has been shown, that a chitosan with lower molecular weight exhibits stronger bioactive properties [26]. Additionally, insect-derived chitosan has been shown to require higher effective concentrations for reducing power than free radical scavenging [41]. This was not the case for the chitosans produced from the house crickets.

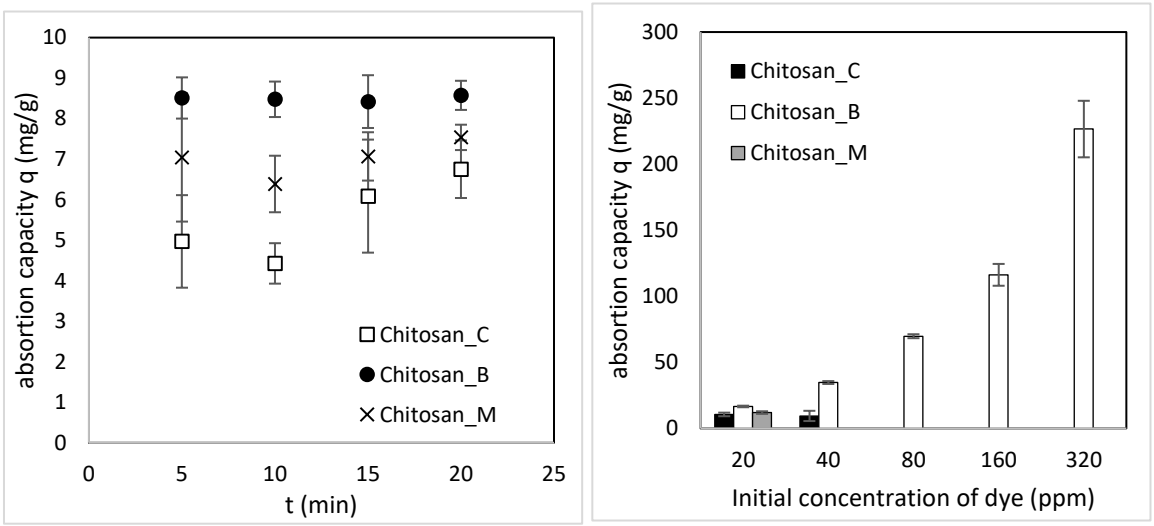
3.2.2. Antimicrobial activity

Table 3: Minimum inhibitory concentration (mg/ml) of chitosan that was commercially purchased or generated from the crickets from chitin isolated through the chemical (Chitosan_C), biological (Chitosan_B) and microwave-assisted method (Chitosan_M).

Minimum inhibitory concentration for antimicrobial activity (mg/ml)			
	Escherichia coli	*Staphylococcus aureus*	*Salmonella enterica*
Commercial chitosan	0.14	0.56	0.28
Chitosan_C	0.14	1.13	0.28
Chitosan_B	0.56	1.13	1.13
Chitosan_M	0.28	0.56	1.13

According to the results presented on Table 3, all chitosans showed an antimicrobial activity that is comparable but lower to the one of the commercial chitosan. Chitosan_C and Chitosan_B have been produced from house crickets and reported to have a low molecular weight [23]. This leads to a stronger antimicrobial activity for Gram negative strains, while the opposite is observed for Gram positive strains [26]. This would explain, why the MIC of the chitosans against *Staphylococcus aureus* is higher, compared to the other strains. Specifically for *Staphylococcus aureus*, it has been suggested that chitosan forms a membrane around the cell surface, not allowing nutrients to enter the cell, while for *Escherichia coli*, the antimicrobial activity of chitosan is pervasion-based [27]. The antimicrobial properties of the chitosans produced from house crickets were similar the ones reported by Malm and Liceaga (2021), who suggested an effective concentration of higher than 0.5 mg/mL [14].

298 *3.2.3. Adsorption of methylene blue*



299

300 **Fig. 3: Methylene blue adsorption capacity of chitosan that was commercially purchased or**
301 **generated from the crickets from chitin isolated through the chemical (Chitosan_C), biological**
302 **(Chitosan_B) and microwave-assisted method (Chitosan_M). Error bars represent the standard**
303 **deviation of each value based on multiple replications of the measurement.**

304 According to Fig. 3, all chitosans that were produced from the crickets showed a methylene
305 blue adsorbtion capacity for water at a concentration level of 10 ppm. In specific, Chitosan_C
306 reached an equilibrium of 6.09±1.39 mg/g of adsorption capacity, after 15 min of treatment.
307 Chitosan_B and Chitosan_M reached an equilibrium of 8.51±0.51 and 7.04±1,58 mg/g,
308 respectively, after 5 min of treatment. At higher levels of dye concentration (<40 ppm), only
309 Chitosan_B had adsorbtion capacity, while when treating a water sample with a 320 ppm of
310 dye, the adsorption capacity was equal to 226.59±21.39 mg/g. Commercial chitosan exhibited
311 no adsorption capacity at all dye concentration levels.

312 **Table 4: Kinetic parameters of adsorption of MB from chitosan that was commercially purchased**
313 **or generated from the crickets from chitin isolated through the chemical (Chitosan_C), biological**
314 **(Chitosan_B) and microwave-assisted method (Chitosan_M).**

	Pseudo-first-order kinetic model			Pseudo-second-order kinetic model		
	q_e (mg/g)	k_1 (1/min)	R^2	q_e (mg/g)	k_2 (g/mg/min)	R^2

Chitosan_C	6.22	0.24	0.918	6.94	0.07	0.934
Chitosan_B	8.56	0.67	0.995	8.77	0.22	0.999
Chitosan_M	7.07	0.66	0.978	7.63	0.14	0.992

According to Table 4, both pseudo-first and second-order kinetic models showed a good fitting to the experimental data. However, the pseudo-second-order kinetic model showed a higher regression coefficient for all chitosans. This has been reported for other materials, such as chitosan-lignin blends [30], chitin nanoparticles [53] and sodium alginate-kaolin beads [44].

Table 5: Isotherm parameters of adsorption of MB from chitosan that were generated from the crickets from chitin isolated through the biological method (Chitosan_B).

Langmuir isotherm			Freundlich isotherm		
qm (mg/g)	k_L (L/mg)	R^2	n	k_F (L/mg)	R^2
643.899	0.009	0.988	1.312	7.79	0.954

The isotherm parameters of the MB adsorption of Chitosan_B are presented on Table 5. According to the regression coefficient, the Langmuir isotherm describes better the adsorption of the MB from Chitosan_B. The Langmuir isotherm is known for describing a monolayer adsorption [53]. The maximum adsorption capacity of Chitosan_B was high, compared to other chitin- or chitosan-based materials, as presented on Table 6.

Table 6: Maximum MB adsorption capacity of chitin- and chitosan-based materials

Material	qm (mg/g)	Reference
Chitosan_B	643.899	Present study
Clay-chitosan composite 50%	330	[54]
Chitosan–montmorillonite/ polyaniline nanocomposite	111	[55]

Lignin-chitosan extruded blend	36.250	[30]
Shrimp chitosan	11.04	[31]
α-chitin	6.900	[53]
Chitosan (Indian marine foods limited)	0.00943	[56]

4. Conclusions

A variety of processing pathways used for chitosan production from other resources, are applicable to the house cricket biomass. Microwave processing, as well as, the combination of biological processes, such as fermentation and digestion, are efficient in replacing the conventional process for chitosan production based on its properties.

Chitosans that were produced from the crickets were found to have significant bioactive properties. In specific, they showed a high antioxidant activity and were quite efficient as antimicrobial agents against some commonly encountered pathogenic bacterial strains.

Additionally, chitosans produced from house crickets exhibited potential for wastewater purification due to their methyle blue adsorption capacity. The chitosan produced from the chitinous material that was isolated with the biological processes exhibited the highest methylene blue adsorption capacity, with a maximum adsorption capacity of 643.899 mg/g.

The findings reported by the present study underline the applicability of alternative and more environmentally friendly methods for chitin isolation to house crickets, in order to produce a chitosan with various possible applications.

Conflict of Interest

The authors declare no conflict of interest.

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Author contributions

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7. List of publications

Journal publications

1. Andreou, V., **Psarianos, M**., Dimopoulos, G., Tsimogiannis, D., & Taoukis, P. (2020). Effect of pulsed electric fields and high pressure on improved recovery of high-added-value compounds from olive pomace. *Journal of food science*, *85*(5), 1500-1512.
2. Ojha, S., Bußler, S., **Psarianos, M**., Rossi, G., & Schlüter, O. K. (2021). Edible insect processing pathways and implementation of emerging technologies. *Journal of Insects as Food and Feed*, *7*(5), 877-900
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6. **Psarianos, M.**, Fricke, A., Ojha, S., Baldermann, S., Schreiner, M., & Schlüter, O. K. (2022). Effect of Narrowband UV-B Irradiation on the Growth Performance of House Crickets. *Foods*, *11*(21), 3487.
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8. Fricke, A., **Psarianos, M.**, Sabban, J., Fitzner, M., Reipsch, R., Vogt, J., ... & Baldermann, S. Composite materials for innovative urban farming of alternative food sources (macroalgae and crickets). *Frontiers in Sustainable Food Systems*, 613.
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10. **Psarianos M**, Ojha S and Schlüter OK (2023) Evaluating an emerging technology-based biorefinery for edible house crickets. Front. Nutr. 10:1185612
11. **Psarianos, M**., Iranshahi, K., Rossi, S., Gottardi, D., & Schlüter, O. (2023). Quality evaluation of house cricket flour processed by electrohydrodynamic drying and pulsed electric fields treatment. Food Chemistry, 138276.
12. **Psarianos, M**., Fricke A., Altuntas H., Baldermann S., Schreiner M., Schlüter O. Effect of different illumination conditions on the production of house crickets *(under review)*
13. **Psarianos M**., Marzban N., Schneider R., Ojha S., Schlüter O. Methylene blue adsorption and bioactivity of chitosan produced from house crickets with novel methods *(under review)*
14. **Psarianos M**., Schneider R., Dimopoulos G., Taoukis P., Schlüter O. Production of bioethanol from insect excreta: a case study on frass from house crickets (*under review)*

Invited book chapters

1. Rossi, G., **Psarianos, M.**, Schlüter, O. K., & Ojha, S. (2023). Protein from insects—a new biosphere of opportunity. In Future Proteins (pp. 173-194). Academic Press.

2. Rossi, G., **Psarianos, M**., Schlüter, O. K., & Ojha, S. (2024). Other applications of insects in the agrifood sector. In Insects as Food and Food Ingredients (pp. 215-235). Academic Press.
3. **Psarianos, M.,** Baliota, G., Rumbos, C. I., Athanassiou, C. G., Ojha, S., & Schlüter, O. K. (2024). Insect processing for chitin production. In Insects as Food and Food Ingredients (pp. 129-143). Academic Press.

Oral presentations

1. Andreou V., Dimopoulos G., **Psarianos M**., Dermesonlouoglou E., Taoukis P., Study and Application of Pulsed Electric Fields for the yield increase of tomato products and the valorisation of tomato by-products, (2019). 12th National Conference of Chemical Engineering of Greece, Athens, Greece

2. **Psarianos M.,** Dimopoulos G., Shikha O., Cavini Moreno A. C., Bußler S., Taoukis P., Schlüter O., Pulsed electric field (PEF) treatment of house crickets: effect on drying and protein extraction, (2021). 6th International ISEKI-Food Conference

3. **Psarianos M.**, Dimopoulos G., Bußler S., Cavini Moreno A.C., Ojha S., Taoukis P., Schlüter O., Pulsed electric field assisted extraction of proteins and fat from house crickets (*Acheta domesticus*): Effects on yield and technofunctional properties (2020). 34th EFFoST International Conference 2020

4. **M. Psarianos**, A. Fricke, S. Ojha, O. Schlüter, Exploration of the effect of UV illumination on the production of house crickets (*Acheta Domesticus*) (2022). Insects to Feed the World 2022, Quebec City, Canada

5. **Psarianos M**., Marzban N., Shikha O., Schneider, R., Schlüter O. Functional properties, bioactivity and ionic dye absorption of chitosan produced from house crickets with novel methods (2021). Insects to Feed the World 2022, Quebec City, Canada

6. **Psarianos M.**, Fricke A., Ojha S., Baldermann S., Schreiner M., Schlüter O., Implementation of LED/ narrow band UV-B irradiation regime on the production of house crickets (*Acheta Domesticus*) (2022). Insecta2022 International Conference

7. **Psarianos M**., Ojha S., Schlüter O., Application of innovative technologies for valorisation of biomass from house crickets (2022). 36th EFFoST International Conference 2022

8. **Psarianos M.**, Fricke A., Ojha S., Baldermann S., Schreiner M., Schlüter O., Effect of UV-B illumination on the production of edible crickets for their introduction in an urban co-cultivation system (2022), AgEng-Land.Technik 2022 International Conference

9. **Psarianos M**., Iransahi K., Rossi S., Defraye T., Schlüter O., Effect of non-thermal assisted processing on the quality of house crickets: pulsed electric fields and electrohydrodynamic drying (2023). International Congress on Engineering and Food (ICEF14)

10. Monika Schreiner, Susanne Baldermann, Maria Fitzner, Anna Fricke, Julia Vogt, **Marios Psarianos**, Oliver Schlüter, Holger Kühnhold & Andreas Kunzmann. Alternative food sources under UVB - for a nutritious and sustainable diet. Presentation at UV4Pigments Workshop of UV4Plants, 17 April 2023, Szekszard, Hungary.

11. **Psarianos M**., Ojha S., Schlüter O., Exploring the potential of house crickets (Acheta domesticus) in future agri-food systems, 37th EFFoST International Conference 2022, Valencia, Spain

Poster presentations

1. Andreou V., **Psarianos M**., Dimopoulos G. Taoukis P., Effect of High Pressure and Pulsed Electric Fields on improved recovery of high-added value compounds from olive pomace, (2018). Effost2018, Nantes, France
2. **Psarianos M**., Andreou V., Dimopoulos G. Taoukis P., Effect of High Pressure and Pulsed Electric Fields on improved recovery of high added-value compounds from olive pomace, (2019). 8th Annual National Conference: Recent Trends in the Department of Lipids, Greek Lipid Forum, Member of Euro Fed Lipid, Athens, Greece
3. **Psarianos M**., Dimopoulos G., Bußler S., Taoukis P., Schlüter O., Effect of PEF pretreatment on the isolation of valuable compounds from house crickets (*Acheta domesticus*), (2020). Conference IFT20 Annual Event & Food Expo, Chicago IL, USA
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5. **Psarianos M.,** Shikha O., Schneider R., Schlüter O., Alternative processes for the production of chitosan from house crickets (*Acheta domesticus*), (2021), Insecta 2021 International Conference 2021, Magdeburg, Germany
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7. **Psarianos M**., Ojha S., Schlüter O., Utilization of biomass from house crickets into valuable ingredients, (2022). Insecta2022 International Conference, Giessen, Germany
8. **Psarianos M.,** Schlüter O., Mathematical modeling of the production of house crickets for food purposes (2023). Model-IT 2023 VII International Symposium on Applications of Modelling as an Innovative Technology in the Horticultural Supply Chain, Potsdam, Germany
9. **Psarianos M**., Schneider R., Schlüter O., Utilization of house cricket frass for bioethanol production, Insecta 2023 International Conference 2023, Magdeburg, Germany