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.

Keywords: crickets, pulsed electric fields, proteins, fat, chitin

Highlights

- PEF treatment increased protein and fat yield by 18.62 % and 41.75%, respectively
- PEF enhanced techno-functional properties of the cricket flour
- PEF did not affect chitin yield (10g/100g d.w.)

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, 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 *Hermetia 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 (*Acheta domesticus*) were purchased at an adult age from Tropic-Shop (Nordhorn, Germany) and were stored for 2 h inside a cold room at 4^{o}C to reduce their movement activity. Afterwards they were freeze inactivated at -20^{o}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 100-1000 (energy input between 4.9-49.1 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 Equation 1 (Raso et al., 2016):

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

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 et al., 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. Figure 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 0.2M, pH=5. Crude fat was

150 determined with a Soxhlet apparatus operating for 6 h with n-hexane as solvent at 68^{o} C. Chitin
151 content was determined by measuring the content of glucosamine and N-Acetyl-Glucosamine
152 (Zamani, Jeihanipour, Edebo, Niklasson, & Taherzadeh, 2008). Standard chitin was used for
153 the calibration curve.

154 *2.3.2 Functional properties*

155 *2.3.2.1 Water binding capacity (WBC)*

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

161 WBC (g water/g dw) = $(m_f-m_0)/m_{0,dw}$ (Equation 2)

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

164 *2.3.2.2 Oil binding capacity (OBC)*

165 For determination of the OBC a similar procedure as for the WBC was followed. In summary,
166 0.5 g of cricket powder was weighed and mixed with 2.5 mL of commercial rapeseed oil, which
167 was also weighed. Mixtures were vortexed for 60 s, and then centrifuged at 4000xg for 20 min.
168 After removing the supernatant, the pellet was weighed and OBC was calculated using Eq.2
169 and expressed as g oil/g d.w. (Schwenke et al., 1981).

170 *2.3.2.3 Emulsifying capacity (EC)*

171 One gram of cricket powder was added to 50 mL H_2O and afterwards 50 mL of commercial
172 rapeseed oil was added. Then the mixture was homogenized for 15 min at 9500 rpm (T-25
173 Ultra turrax, IKA, Staufen, Germany). The emulsion was centrifuged at 10000xg for 10 min in
174 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):

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

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, 3200xg 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, 3200xg 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, 7000xg 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 Equation 4:

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

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 3200xg, 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/100g 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 10000xg, 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, 1M (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 10000xg, 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, Baran, 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 10000xg, 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/100g 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/100g 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 sec, 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 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).

As shown in Figure 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 (Figure 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 (Figure 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 Figure 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 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 Figure 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 *Hermetia 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 *Tenebrio 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 *Tenebrio 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 *Acheta 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 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 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 *Tenebrio 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/100g 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 et al. (2019), who reported a yield of 5.14 % in basis of dried weight (Hirsch, Cho, Kim, & Jones, 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 (Figure 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 & 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.

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6. Conflict of Interests

The authors have declared no conflict of interests.

7. References

Alles, M. C., Smetana, S., Parniakov, O., Shorstkii, I., Toepfl, S., Aganovic, K., & Heinz, V. (2020). Bio-refinery of insects with Pulsed electric field pre-treatment. *Innovative food science & emerging technologies, 64*, 102403.

Anand, A. N., & Lorenz, M. W. (2008). Age-dependent changes of fat body stores and the regulation of fat body lipid synthesis and mobilisation by adipokinetic hormone in the last larval instar of the cricket, Gryllus bimaculatus. *Journal of Insect Physiology, 54*(10-11), 1404-1412.

Andersen, S. O., Hojrup, P., & Roepstorff, P. (1995). Insect cuticular proteins. *Insect biochemistry and molecular biology, 25*(2), 153-176.

Andreou, V., Dimopoulos, G., Alexandrakis, Z., Katsaros, G., Oikonomou, D., Toepfl, S., . . . Taoukis, P. (2017). Shelf-life evaluation of virgin olive oil extracted from olives subjected to nonthermal pretreatments for yield increase. *Innovative food science & emerging technologies, 40*, 52-57.

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.

Arrese, E. L., & Soulages, J. L. (2010). Insect fat body: energy, metabolism, and regulation. *Annual review of entomology, 55*, 207-225.

Asif, T., Javed, U., Zafar, S. B., Ansari, A., Qader, S. A. U., & Aman, A. (2019). Bioconversion of colloidal chitin using novel chitinase from Glutamicibacter uratoxydans exhibiting anti-fungal potential by hydrolyzing chitin within fungal cell wall. *Waste and Biomass Valorization*, 1-15.

Bolat, B., Ugur, A. E., Oztop, M. H., & Alpas, H. (2021). Effects of High Hydrostatic Pressure assisted degreasing on the technological properties of insect powders obtained from Acheta domesticus & Tenebrio molitor. *Journal of Food Engineering, 292*, 110359.

Bradford, N. (1976). A rapid and sensitive method for the quantitation microgram quantities of a protein isolated from red cell membranes. *Anal. Biochem, 72*(248), e254.

Buchmann, L., Brändle, I., Haberkorn, I., Hiestand, M., & Mathys, A. (2019). Pulsed electric field based cyclic protein extraction of microalgae towards closed-loop biorefinery concepts. *Bioresource technology, 291*, 121870.

Bußler, S., Rumpold, B. A., Jander, E., Rawel, H. M., & Schlüter, O. K. (2016). Recovery and techno-functionality of flours and proteins from two edible insect species: Meal worm (Tenebrio molitor) and black soldier fly (Hermetia illucens) larvae. *Heliyon, 2*(12), e00218.

Bußler, S., Steins, V., Ehlbeck, J., & Schlüter, O. (2015). Impact of thermal treatment versus cold atmospheric plasma processing on the techno-functional protein properties from Pisum sativum 'Salamanca'. *Journal of Food Engineering, 167*, 166-174.

Canavoso, L. E., Jouni, Z. E., Karnas, K. J., Pennington, J. E., & Wells, M. A. (2001). Fat metabolism in insects. *Annual review of nutrition, 21*(1), 23-46.

Caparros Megido, R., Haubruge, E., & Francis, F. (2017). Small-scale production of crickets and impact on rural livelihoods. *Insects as food and feed: From production to consumption*.

Chen, X., Feng, Y., & Chen, Z. (2009). Common edible insects and their utilization in China. *Entomological research, 39*(5), 299-303.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. t., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical chemistry, 28*(3), 350-356.

Fröhling, A., Bußler, S., Durek, J., & Schlüter, O. K. (2020). Thermal Impact on the Culturable Microbial Diversity Along the Processing Chain of Flour From Crickets (Acheta domesticus). *Frontiers in Microbiology, 11*.

Hirsch, A., Cho, Y.-H., Kim, Y. H. B., & Jones, O. G. (2019). Contributions of protein and milled chitin extracted from domestic cricket powder to emulsion stabilization. *Current Research in Food Science, 1*, 17-23.

Ibitoye, E., Lokman, I., Hezmee, M., Goh, Y., Zuki, A., & Jimoh, A. (2018). Extraction and physicochemical characterization of chitin and chitosan isolated from house cricket. *Biomedical Materials, 13*(2), 025009.

Jonas-Levi, A., & Martinez, J.-J. I. (2017). The high level of protein content reported in insects for food and feed is overestimated. *Journal of Food Composition and Analysis, 62*, 184-188.

Jung, S., Murphy, P. A., & Johnson, L. A. (2005). Physicochemical and functional properties of soy protein substrates modified by low levels of protease hydrolysis. *Journal of food science, 70*(2), C180-C187.

Kamau, E., Mutungi, C., Kinyuru, J., Imathiu, S., Tanga, C., Affognon, H., . . . Fiaboe, K. (2018). Moisture adsorption properties and shelf-life estimation of dried and pulverised edible house cricket Acheta domesticus (L.) and black soldier fly larvae Hermetia illucens (L.). *Food Research International, 106*, 420-427.

Kaya, M., Baran, T., Asan-Ozusaglam, M., Cakmak, Y. S., Tozak, K. O., Mol, A., . . . Sezen, G. (2015). Extraction and characterization of chitin and chitosan with antimicrobial and antioxidant activities from cosmopolitan Orthoptera species (Insecta). *Biotechnology and Bioprocess Engineering, 20*(1), 168-179.

Kaya, M., Bitim, B., Mujtaba, M., & Koyuncu, T. (2015). Surface morphology of chitin highly related with the isolated body part of butterfly (Argynnis pandora). *International Journal of Biological Macromolecules, 81*, 443-449.

Kim, H. W., Setyabrata, D., Lee, Y., Jones, O. G., & Kim, Y. H. B. (2017). Effect of house cricket (Acheta domesticus) flour addition on physicochemical and textural properties of meat emulsion under various formulations. *Journal of food science, 82*(12), 2787-2793.

Klunder, H., Wolkers-Rooijackers, J., Korpela, J. M., & Nout, M. R. (2012). Microbiological aspects of processing and storage of edible insects. *Food control, 26*(2), 628-631.

Lai, Y. S., Parameswaran, P., Li, A., Baez, M., & Rittmann, B. E. (2014). Effects of pulsed electric field treatment on enhancing lipid recovery from the microalga, Scenedesmus. *Bioresource technology, 173*, 457-461.

Laroche, M., Perreault, V., Marciniak, A., Gravel, A., Chamberland, J., & Doyen, A. (2019). Comparison of conventional and sustainable lipid extraction methods for the production of oil and protein isolate from edible insect meal. *Foods, 8*(11), 572.

Liang, R., Cheng, S., & Wang, X. (2018). Secondary structure changes induced by pulsed electric field affect antioxidant activity of pentapeptides from pine nut (Pinus koraiensis) protein. *Food chemistry, 254*, 170-184.

Liu, X., Chen, X., Wang, H., Yang, Q., ur Rehman, K., Li, W., . . . Zhang, J. (2017). Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. *PLoS One, 12*(8), e0182601.

Lucas-González, R., Fernández-López, J., Pérez-Álvarez, J. A., & Viuda-Martos, M. (2019). Effect of drying processes in the chemical, physico-chemical, techno-functional and antioxidant properties of flours obtained from house cricket (Acheta domesticus). *European Food Research and Technology, 245*(7), 1451-1458.

Manditsera, F. A., Luning, P. A., Fogliano, V., & Lakemond, C. M. (2019). Effect of domestic cooking methods on protein digestibility and mineral bioaccessibility of wild harvested adult edible insects. *Food Research International, 121*, 404-411.

Mercer, P., & Armenta, R. E. (2011). Developments in oil extraction from microalgae. *European journal of lipid science and technology, 113*(5), 539-547.

Mohammed, M. H., Williams, P. A., & Tverezovskaya, O. (2013). Extraction of chitin from prawn shells and conversion to low molecular mass chitosan. *Food Hydrocolloids, 31*(2), 166-171.

Nakagaki, B. J., & Defoliart, G. R. (1991). Comparison of diets for mass-rearing Acheta domesticus (Orthoptera: Gryllidae) as a novelty food, and comparison of food conversion efficiency with values reported for livestock. *Journal of Economic Entomology, 84*(3), 891-896.

Ndiritu, A. K., Kinyuru, J. N., Kenji, G. M., & Gichuhi, P. N. (2017). Extraction technique influences the physico-chemical characteristics and functional properties of edible crickets (Acheta domesticus) protein concentrate. *Journal of Food Measurement and Characterization, 11*(4), 2013-2021.

Nyangena, D. N., Mutungi, C., Imathiu, S., Kinyuru, J., Affognon, H., Ekesi, S., . . . Fiaboe, K. K. (2020). Effects of traditional processing techniques on the nutritional and microbiological quality of four edible insect species used for food and feed in East Africa. *Foods, 9*(5), 574.

Otero, P., Gutierrez-Docio, A., Del Hierro, J. N., Reglero, G., & Martin, D. (2020). Extracts from the edible insects Acheta domesticus and Tenebrio molitor with improved fatty acid profile due to ultrasound assisted or pressurized liquid extraction. *Food chemistry, 314*, 126200.

Parniakov, O., Barba, F. J., Grimi, N., Marchal, L., Jubeau, S., Lebovka, N., & Vorobiev, E. (2015). Pulsed electric field and pH assisted selective extraction of intracellular components from microalgae Nannochloropsis. *Algal Research, 8*, 128-134.

Percot, A., Viton, C., & Domard, A. (2003). Optimization of chitin extraction from shrimp shells. *Biomacromolecules, 4*(1), 12-18.

Porusia, M., Rauf, R., & Haryani, F. (2020). Nutritional value of grasshopper and cricket cooked with different methods. *Eurasian Journal of Biosciences, 14*(2), 5369-5374.

Purschke, B., Tanzmeister, H., Meinlschmidt, P., Baumgartner, S., Lauter, K., & Jäger, H. (2018). Recovery of soluble proteins from migratory locust (Locusta migratoria) and characterisation of their compositional and techno-functional properties. *Food Research International, 106*, 271-279.

Ramos-Bueno, R. P., González-Fernández, M. J., Sánchez-Muros-Lozano, M. J., García-Barroso, F., & Guil-Guerrero, J. L. (2016). Fatty acid profiles and cholesterol content of seven insect species assessed by several extraction systems. *European Food Research and Technology, 242*(9), 1471-1477.

Raso-Pueyo, J., & Heinz, V. (2010). *Pulsed electric fields technology for the food industry: fundamentals and applications*: Springer Science & Business Media.

Raso, J., Frey, W., Ferrari, G., Pataro, G., Knorr, D., Teissie, J., & Miklavčič, D. (2016). Recommendations guidelines on the key information to be reported in studies of application of PEF technology in food and biotechnological processes. *Innovative food science & emerging technologies, 37*, 312-321.

Rausch, T. (1981). The estimation of micro-algal protein content and its meaning to the evaluation of algal biomass I. Comparison of methods for extracting protein. *Hydrobiologia, 78*(3), 237-251.

Ravi, H. K., Vian, M. A., Tao, Y., Degrou, A., Costil, J., Trespeuch, C., & Chemat, F. (2019). Alternative solvents for lipid extraction and their effect on protein quality in black soldier fly (Hermetia illucens) larvae. *Journal of Cleaner Production, 238*, 117861.

Rumpold, B. A., & Schlüter, O. K. (2013a). Nutritional composition and safety aspects of edible insects. *Molecular nutrition & food research, 57*(5), 802-823.

Rumpold, B. A., & Schlüter, O. K. (2013b). Potential and challenges of insects as an innovative source for food and feed production. *Innovative food science & emerging technologies, 17*, 1-11.

Schösler, H., De Boer, J., & Boersema, J. J. (2012). Can we cut out the meat of the dish? Constructing consumer-oriented pathways towards meat substitution. *Appetite, 58*(1), 39-47.

Schwenke, K., Prahl, L., Rauschal, E., Gwiazda, S., Dabrowski, K., & Rutkowski, A. (1981). Functional properties of plant proteins. Part 2. Selected physicochemical properties of native and denatured protein isolates from faba beans, soybeans, and sunflower seed. *Food/Nahrung, 25*(1), 59-69.

Shepherd, V. A. (2006). The cytomatrix as a cooperative system of macromolecular and water networks. *Current topics in developmental biology, 75*, 171-223.

Shorstkii, I., Comiotto Alles, M., Parniakov, O., Smetana, S., Aganovic, K., Sosnin, M., . . . Heinz, V. (2020). Optimization of pulsed electric field assisted drying process of black soldier fly (Hermetia illucens) larvae. *Drying Technology*, 1-9.

Shorstkii, I., Mirshekarloo, M. S., & Koshevoi, E. (2017). Application of pulsed electric field for oil extraction from sunflower seeds: electrical parameter effects on oil yield. *Journal of Food Process Engineering, 40*(1), e12281.

Smetana, S., Mhemdi, H., Mezdour, S., & Heinz, V. (2020). Pulsed electric field–treated insects and algae as future food ingredients *Pulsed Electric Fields to Obtain Healthier and Sustainable Food for Tomorrow* (pp. 247-266): Elsevier.

Soon, C. Y., Tee, Y. B., Tan, C. H., Rosnita, A. T., & Khalina, A. (2018). Extraction and physicochemical characterization of chitin and chitosan from Zophobas morio larvae in varying sodium hydroxide concentration. *International Journal of Biological Macromolecules, 108*, 135-142.

Starcher, B. (2001). A ninhydrin-based assay to quantitate the total protein content of tissue samples. *Analytical biochemistry, 292*(1), 125-129.

Tzompa-Sosa, D. A., Yi, L., van Valenberg, H. J., van Boekel, M. A., & Lakemond, C. M. (2014). Insect lipid profile: aqueous versus organic solvent-based extraction methods. *Food Research International, 62*, 1087-1094.

Udomsil, N., Imsoonthornruksa, S., Gosalawit, C., & Ketudat-Cairns, M. (2019). Nutritional values and functional properties of house cricket (Acheta domesticus) and field cricket (Gryllus bimaculatus). *Food Science and Technology Research, 25*(4), 597-605.

Ugur, A. E., Bolat, B., Oztop, M. H., & Alpas, H. (2020). Effects of High Hydrostatic Pressure (HHP) Processing and Temperature on Physicochemical Characterization of Insect Oils Extracted from Acheta domesticus (House Cricket) and Tenebrio molitor (Yellow Mealworm). *Waste and Biomass Valorization*, 1-10.

Van Huis, A. (2013). Potential of insects as food and feed in assuring food security. *Annual review of entomology, 58*, 563-583.

Williams, J., Williams, J., Kirabo, A., Chester, D., & Peterson, M. (2016). Nutrient content and health benefits of insects *Insects as sustainable food ingredients* (pp. 61-84): Elsevier.

Woodring, J., Clifford, C., & Beckman, B. (1979). Food utilization and metabolic efficiency in larval and adult house crickets. *Journal of Insect Physiology, 25*(12), 903-912.

Xue, D., & Farid, M. M. (2015). Pulsed electric field extraction of valuable compounds from white button mushroom (Agaricus bisporus). *Innovative food science & emerging technologies, 29*, 178-186.

Yasumatsu, K., Sawada, K., Moritaka, S., Misaki, M., Toda, J., Wada, T., & Ishii, K. (1972). Whipping and emulsifying properties of soybean products. *Agricultural and Biological Chemistry, 36*(5), 719-727.

Yi, L., Lakemond, C. M., Sagis, L. M., Eisner-Schadler, V., van Huis, A., & van Boekel, M. A. (2013). Extraction and characterisation of protein fractions from five insect species. *Food chemistry, 141*(4), 3341-3348.

Zamani, A., Jeihanipour, A., Edebo, L., Niklasson, C., & Taherzadeh, M. J. (2008). Determination of glucosamine and N-acetyl glucosamine in fungal cell walls. *Journal of Agricultural and food Chemistry, 56*(18), 8314-8318.

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

Figure (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).

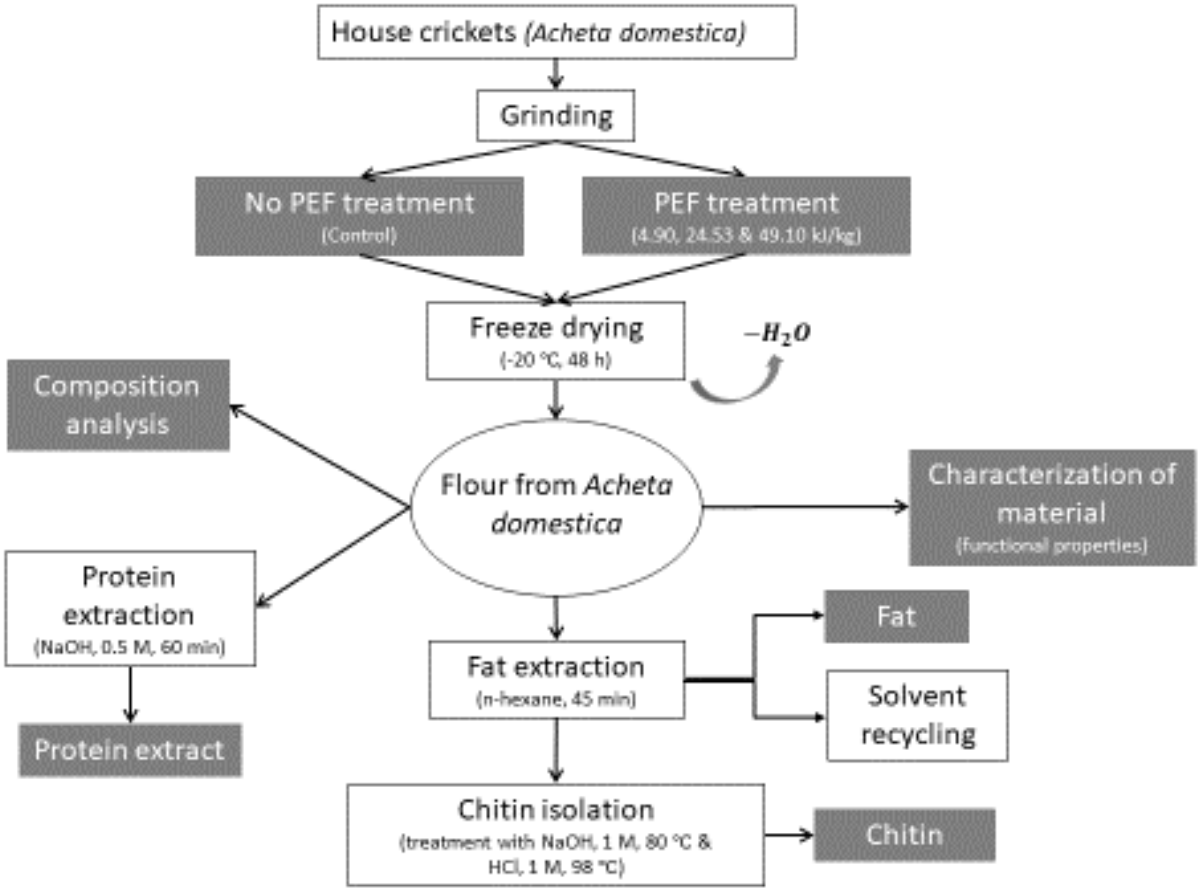
Figure (3): Effect of PEF pretreatment on the fat extraction yield (g fat/100 g d.w.). The error bars indicate the standard errors of measurements. Superscript letters (a,b,c…) indicate the significant differences ($p<0.05$) between the means of the functional properties of PEF-treated samples and the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

Figure (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.

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

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

677



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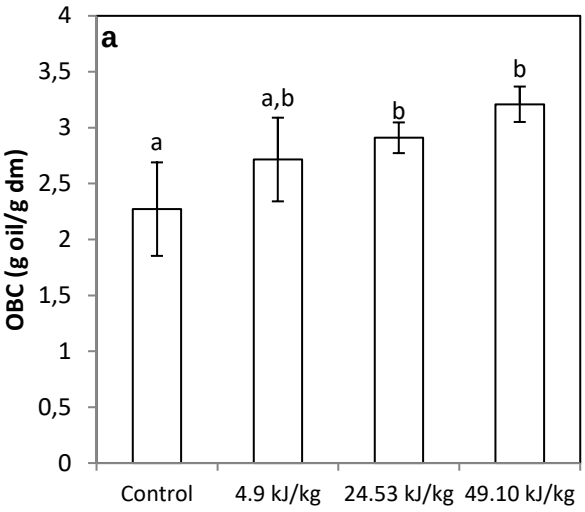
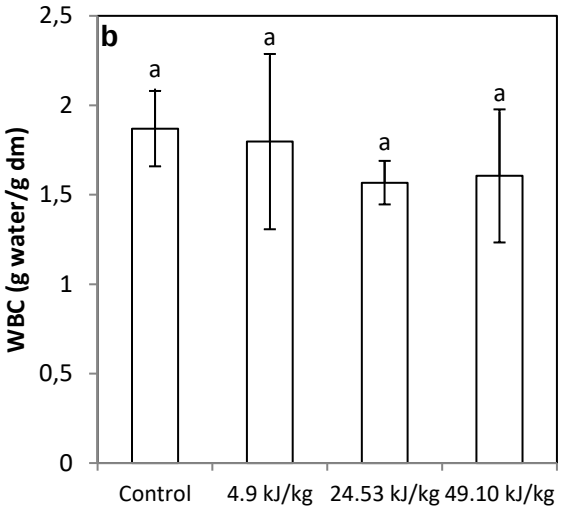
Compounds	g/100 g dry weight
Moisture	10.97 ± 2.41
Proteins	72.45 ± 1.30
Crude fat	18.19 ± 0.63
Ash	3.97 ± 0.96
Carbohydrates	6.64 ± 0.15
Chitin	7.34 ± 0.73

680 Values are presented as mean ± SD.





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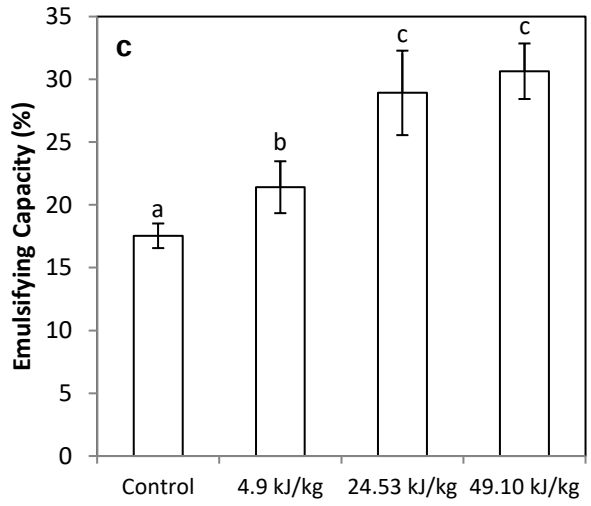

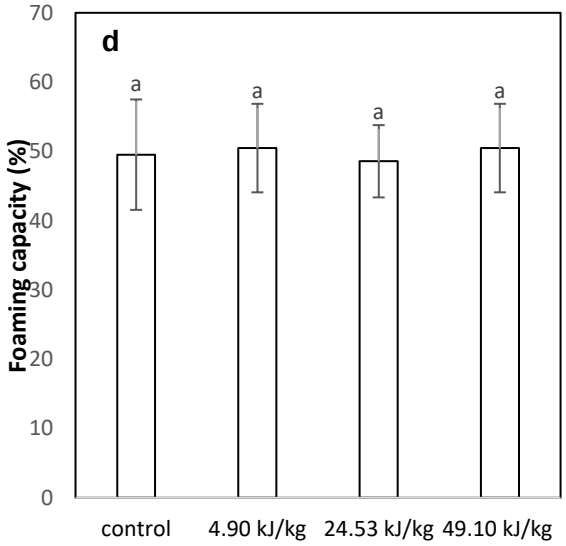

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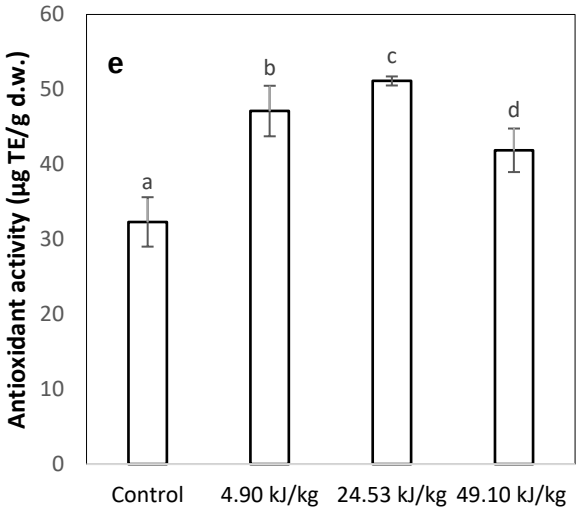

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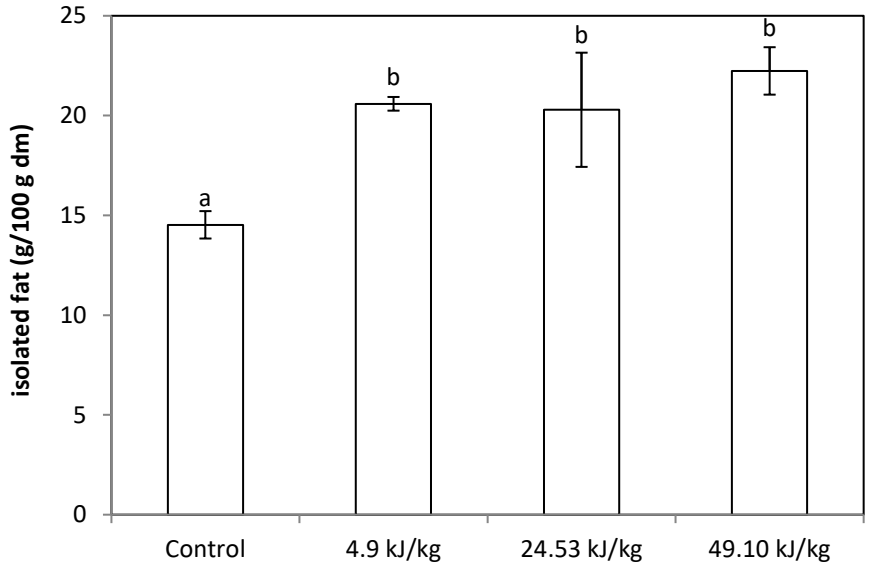
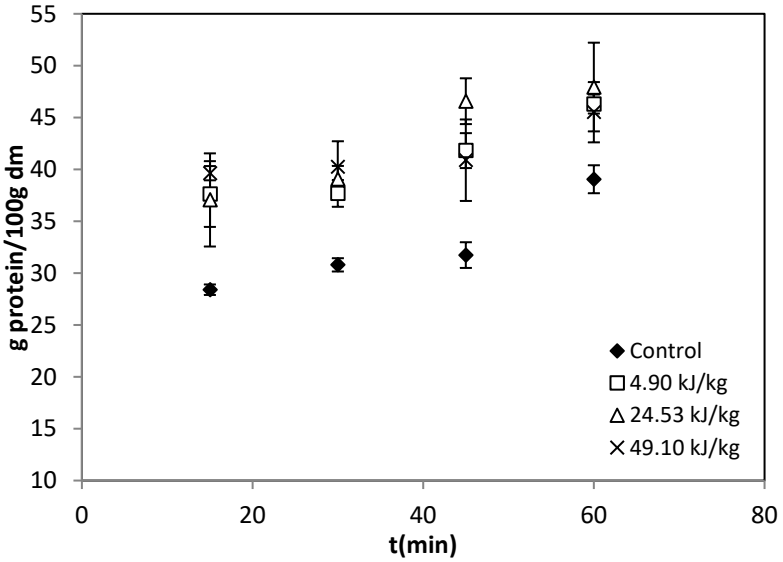

isolated fat (g/100 g dm)
25
20
15
10
5
0
a
b
b
b
Control
4.9 kJ/kg
24.53 kJ/kg
49.10 kJ/kg

684


g protein/100g dm
55
50
45
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35
30
25
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15
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40
60
80
t(min)
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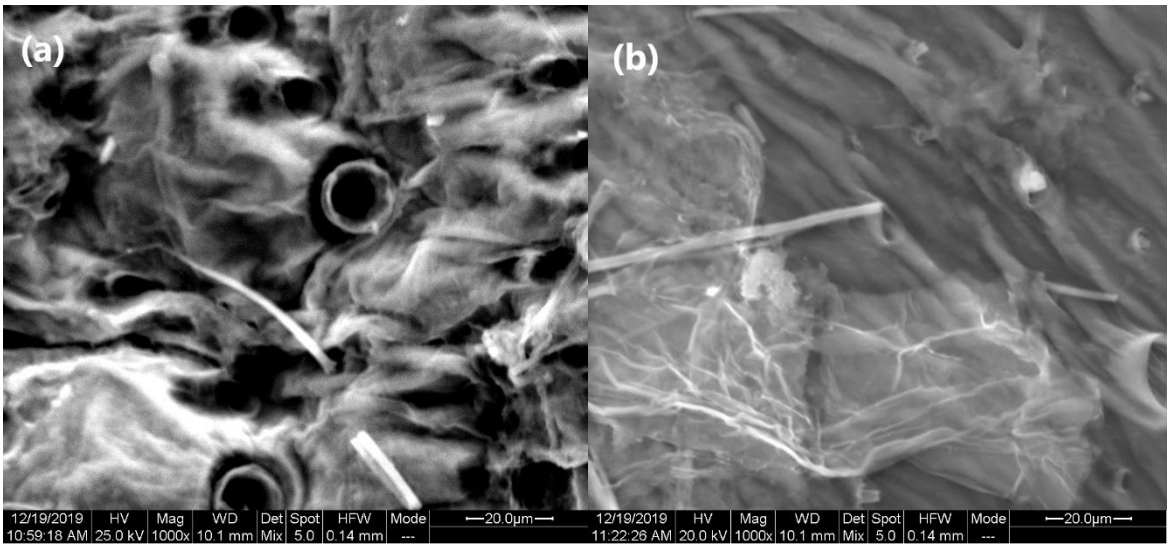
24.53 kJ/kg
49.10 kJ/kg

685


(a)
(b)
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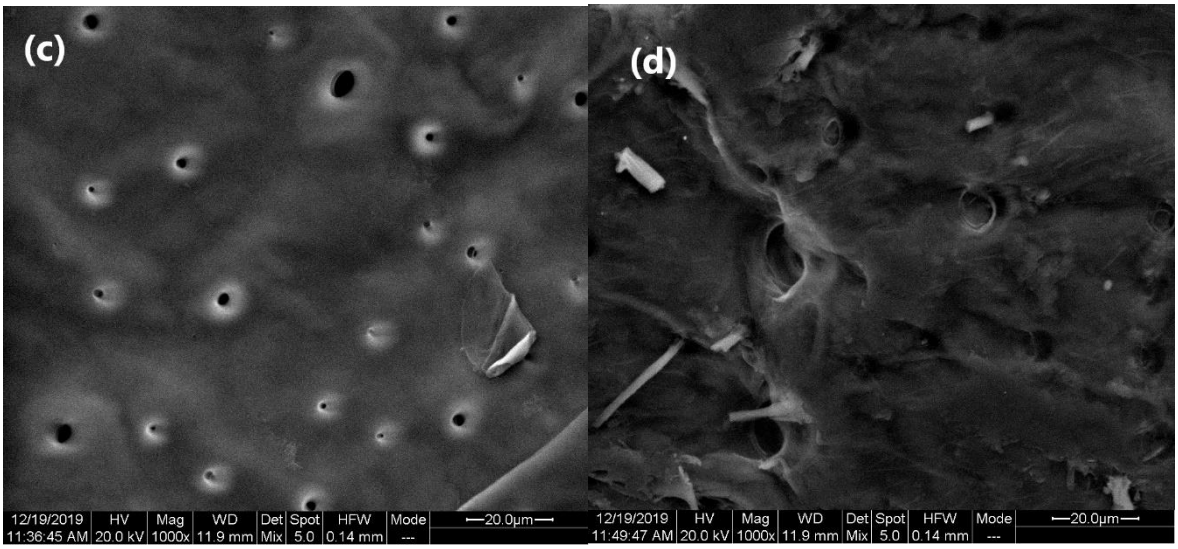
686


(c)
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HV 20.0 kV
Mag 1000x
WD 11.9 mm
Det Mix
Spot 5.0
HFW 0.14 mm
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