LWT - Food Science and Technology 224 (2025) 117876

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Effect of pulsed electric field on *Zophobas morio* flour: evaluation of lipid extraction, techno-functional properties, and protein characterization

Michelle Fernández-Salas [a,1], Johanan Espinosa-Ramírez [a], Mariana Morales-de la Peña [b], Luis Eduardo Garcia-Amezquita [a], Sandra Teresita Martín-del-Campo [c], Viridiana Tejada-Ortigoza [a,*,1]

[a] Tecnologico de Monterrey, Escuela de Ingeniería y Ciencias, Ave. Eugenio Garza Sada 2501, Monterrey, N.L., 64849, Mexico
[b] Department of Food Technology, Science and Engineering, University of Lleida, Rovira Roure Ave. 191, Lleida, 25198, Spain
[c] DRIFT-FOOD Research Centre, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, Prague–Suchdol, 16500, Czech Republic

ARTICLE INFO

Keywords:
Superworm
Amino acids
fatty acids
Digestibility
SDS-PAGE

ABSTRACT

New food alternatives, such as edible insects, must be considered for the population's nutritional needs. *Zophobas morio* (ZM), an edible insect rich in unsaturated fatty acids, is susceptible to oxidative reactions. Pulsed electric field (PEF) has been proven to enhance the extractability of compounds while maintaining product quality. This research aimed to evaluate the effect of PEF on ZM flour and determine its characterization. PEF was applied at 1–5.6 kV/cm; 10 and 200 pulses; 4 μs; 200 Hz, and the lipid extraction yield, techno-functional properties, and protein characterization were assessed. PEF-treated flours were compared to control (untreated) ZM flour. The highest lipid extractions were achieved at 5.6 kV/cm (200 and 10 pulses), and 3 kV/cm (200 pulses) resulting in 77.60, 64.38, and 64.48% increased extraction, respectively. Water holding capacity was increased by 19% (3 kV/cm and 10 pulses) and the oil holding capacity was decreased by 11% (5.6 kV/cm and 10 pulses). PEF and the defatting increased the techno-functionality of ZM: foaming capacity up to 84%. The digestibility after treatment was 78.68–79.86%, and no molecular weight differences were observed on SDS-PAGE. Using PEF on ZM flours improved the extractability of lipids and may be used to tailor the techno-functionality of ZM.

1. Introduction

The world population is expected to grow to 9.7 billion by 2050 (de Carvalho et al., 2020; FAO, 2017). To meet its needs, the food and feed sectors will need to produce 70–80% more between 2012 and 2050 (Oonincx & de Boer, 2012). Moreover, a reduction of greenhouse gas emissions and increased food production is needed (Mrówczyńska-Kamińska et al., 2021). To decrease the environmental impact, novel protein sources have been proposed as a control action (Oonincx & de Boer, 2012).

Insects have been suggested as being environmentally friendly compared to livestock (Ramos-Elorduy, J., 2005). They require 43% of the land destined to produce 1 kg of animal protein, and 10% of the land for beef production (Oonincx & de Boer, 2012). Their nutritious profile turns them into a suitable option for the population's food demand (de Carvalho et al., 2020). *Zophobas morio* (ZM), also known as superworm or giant mealworm is already eaten by ethnic groups in Mexico (Ramos-Elorduy, 2009), and it has an interesting nutritious profile because of its high protein (20.7–46.8%), lipid (16.0–44.5%), crude fiber (9.1%), and vitamins contents (vitamin D2 and E: 531 and 163.0 IU/kg, vitamin C and B2: 101.0 and 7.5–11.2 mg/kg) (de Carvalho et al., 2020; Finke, 2015; Soares Araújo et al., 2019; Yi et al., 2013).

Despite the benefits, insect consumption is a challenge since, generally, people consider entomophagy disgusting (van Huis, 2013). Nonetheless, it can be decreased when insects or their protein extracts are added to commonly consumed food by drying and grinding them (Caparros Megido et al., 2016; Liceaga, 2021; Yi et al., 2013). Considering that one of their largest components is proteins, understanding the techno-functionality of the flours or defatted flours could help incorporate them into food formulations (Liceaga, 2021; Mishyna et al.,

This article is part of a special issue entitled: Nonthermal processing technology published in LWT.

* Corresponding author.
E-mail address: viri.tejada@tec.mx (V. Tejada-Ortigoza).
[1] Authors are part of Bio-Foods Research Lab.

https://doi.org/10.1016/j.lwt.2025.117876
Received 16 July 2024; Received in revised form 25 April 2025; Accepted 1 May 2025
Available online 3 May 2025

2021). However, one of the obstacles is that insects, such as ZM, are rich in unsaturated fatty acids, which are susceptible to oxidation, limiting their processing and conservation (Nascimento et al., 2022; van Huis, 2013).

Different conventional methods (mechanical pressing or solvent extractions) have been used for defatting (Bakhshabadi et al., 2018). However, when extracting through hot pressing, the remaining lipids might be of low quality due to elevated temperatures, and with cold pressing, the extraction yield is low since no heat is applied (Bakhshabadi et al., 2018). Novel techniques such as pulsed electric field (PEF) have been studied as nonthermal technologies for their effectiveness in the extraction of several compounds (lipids, proteins, phenolic compounds, etc.) and retention of food quality (Ahmmed et al., 2022; Bakhshabadi et al., 2018; Naliyadhara et al., 2022). Combining it with other technologies might be beneficial for extraction purposes.

The electric rupture theory has been proposed as the mode of action of PEF (Jeyamkondan et al., 1999). For instance, after PEF treatment, an enhanced lipid extraction has been demonstrated in microorganisms (*Auxenochlorella protothecoides)* and vegetable matrices (black cumin seeds). After treatment, *A. protothecoides* yielded 31% of extracted lipids (in contrast to 1–4% in control) (Silve et al., 2018); a two-times higher extraction yield (≈82%) when compared to control sample (≈45%) was obtained for black cumin seeds (Bakhshabadi et al., 2018). Insects, like *Acheta domesticus*, have also been treated with PEF, which increased the extracted lipids by ≈ 57% compared to control (Psarianos et al., 2022).

To the best of our knowledge, this is the first study to apply pulsed electric field (PEF) technology to *Zophobas morio* (ZM) flour. Existing literature on ZM as a food ingredient is limited and mostly focuses on its

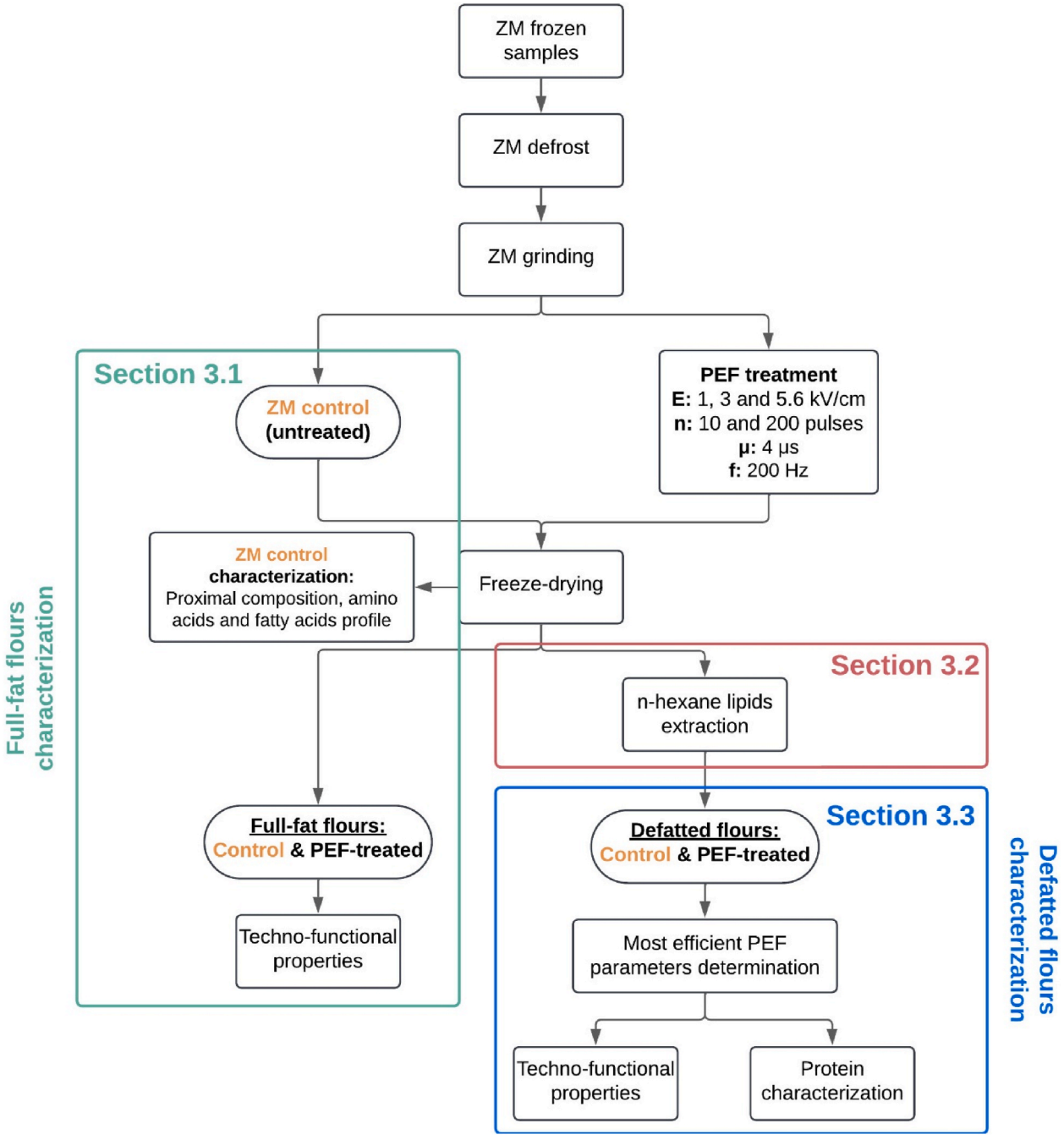

Fig. 1. Flowchart of the PEF processing and characterization of ZM
ZM: *Zophobas morio*; PEF: Pulsed Electric Field; *E*: field strength; n: number of pulses; ŋ: number of pulses; f: frequency ZM control: untreated sample.

use as a partial meat substitute in baked goods and sausages (Aguanta et al., 2023; Scholliers et al., 2020; Sriprablom et al., 2022). No prior research has evaluated the impact of PEF on ZM or explored the techno-functional properties of its defatted flour. The novelty of this work lies in demonstrating how PEF can enhance lipid extraction and modify techno-functional characteristics, thereby broadening the potential of ZM as a sustainable and functional food ingredient. Therefore, this research aimed to apply PEF to ZM flour and to evaluate the yield of extracted lipids. The techno-functional properties and protein characterization of the treated and defatted flours were also determined.

2. Material and methods

Fig. 1 shows the flowchart of the processing of ZM. To gain a comprehensive understanding of the raw material under study, a complete composition analysis was performed in the ZM control sample. *Flours* refer to the full-fatted ZM samples (control and PEF-treated), while *defatted flours* refer to the defatted insect (control and PEF-treated). Analyses of the defatted flour were performed on the most efficient lipid extraction. Control samples have no PEF treatment.

2.1. ZM handling and preparation

ZM larvae were provided by Zofo® (Yucatán, Mexico). According to the supplier, ZM were reared for 3–4 months on wheat bran and lettuce. Zofo® fasted the insects for 8 h and froze them with water on a 1:1 ratio at −20 °C until PEF treatments were performed. Right before the PEF treatment, ZM was thawed at room temperature overnight. Then, ZM was manually separated from the water and ground with a blender (SV-MOL-300, Mexico; 28,000 rpm, 5 min) to obtain a ZM slurry. The thawed water was stored in a capped glass container at 4 °C for ≈1 h for posterior use in the PEF treatments.

2.2. Pulsed electric Field (PEF) treatment

This section addresses the characterization of ZM control flour and the effect of PEF on ZM flour. After thoroughly mixing the thawed water and the ZM slurry (1:1 ratio), the mixture was treated on a PEF system (EPULSUS®-LPM1A-10-System) inside a parallel chamber using stainless steel electrodes. Treatments were carried out at 1, 3, and 5.6 kV/cm; pulse width and frequency were kept constant at 4 μs and 200 Hz, and 10 and 200 pulses (monopolar square-wave pulses). These conditions were based on preliminary experiments. The temperature of the sample was measured before the treatment (21 °C) and never exceeded, once treated, 35 °C. Because water soluble components might be released into the media during PEF processing, the whole mixtures (ZM slurry + treatment water) were then frozen at −80 °C for 6 days, and freeze-dried (−63 °C, 0.135 Torr; FreeZone® Triad® Benchtop Freeze Dryers, USA). Because samples were already ground before drying, samples were manually handled with a spatula from the freeze-drying flask already as powders (PEF-flours). A sample with no PEF treatment was defined as ZM control. After PEF treatments, lipid extraction, techno-functional properties, and protein characterization analysis were done. The research structure can be seen in Fig. 1. Table 1 shows the sample codes of the treatment parameters. Each procedure was performed in duplicate.

Table 1
Sample codes of the PEF treatments.

Sample code	*E* (kV/cm)	η
Control	0	0
T1-10	1	10
T1-200	1	200
T3-10	3	10
T3-200	3	200
T5.6-10	5.6	10
T5.6-200	5.6	200

E: electric field intensity; η: number of pulses.

2.2.1. Proximate analysis

Proximate analysis was conducted according to the AOAC International (2022) methods on the ZM control (untreated) flour: moisture (930.15); protein (920.152) using micro-Kjeldahl with a nitrogen-to-protein-conversion factor (Kp) of 4.76 (Janssen et al., 2017); crude fat (ether extract, 960.39) on a Goldfish equipment using petroleum ether for 6 h; dietary fiber (2011.25); and ashes (940.26). Nitrogen-free extract (NFE) was determined by difference. Analyses were performed in duplicate.

2.2.2. Amino acids and fatty acids profile

The amino acids (AA) profile was determined through the 982.30 method of AOAC. The limiting AA score and the protein digestibility corrected AA score (PDCAAS) were determined following the Joint FAO/WHO/UNU Expert Consultation on Protein (2007) protocol (Eq. (1) and Eq. (2)), considering the AA requirements of infants from 1 to 2 years old.

$$Limiting\ AA\ score = \frac{mg\ AA\ in\ 1\ g\ protein}{mg\ AA\ in\ requirement\ pattern} \quad \text{Eq. 1}$$

$$PDCAAS = (\%\ digestibility \times limiting\ AA\ score)/100 \quad \text{Eq. 2}$$

For the fatty acids (FA) profile, methylation of the ZM control flour followed the Corzo-Ríos et al. (2022) methodology with modifications. Lipids extraction was carried out through Goldfish (6 h, using petroleum ether). Then, 50 mg of the lipids were dissolved in 1 mL of HPLC-grade hexane and 2 mL of sodium methoxide (0.5 M) in HPLC-grade methanol. Further steps after incubation followed the Corzo-Ríos et al. (2022) methodology. The final mixture was centrifuged (6000 rpm, 10 min, 4 °C) and placed on vials for further analysis. Fatty acid methyl esters (FAME) identification was executed following the Ruiz Haddad et al. (2022) procedure. Results were expressed as g FAME/100 g of lipids. The sample was analyzed in duplicate.

2.2.3. Techno-functional properties

The water and oil-holding capacity (WHC and OHC), water and oil emulsion capacity (WEC and OEC), and foaming capacity (FC) were determined following the Ruiz Haddad et al. (2022) methodologies on the ZM control and PEF-treated (flours and defatted flours). Triplicates of all samples were evaluated.

Once the most efficient PEF treatments were settled (Fig. 1), the emulsion activity index (EAI) was determined on the defatted flours (ZM control PEF-treated). Because of the shortcomings due to the equipment, method, or emulsion system when assessing the EC, the turbidimetric method presented by Pearce and Kinsella (1978) was followed with modifications and calculations made by Espinosa-Ramírez and Serna-Saldívar (2016).

2.3. Lipid extraction

The lipid extraction after PEF treatments followed the Psarianos et al. (2022) methodology with slight modifications, where flours turned into defatted flours (for ZM control and PEF-treated). Briefly, insect flour was added to n-hexane (>95%) in a 1:40 (g:mL) proportion. The mixture was stirred (45 min, room temperature), centrifuged (3200 g, 10 min, 15 °C), and the supernatant saved for further lipid quantification. The remaining pellet (ZM control defatted flour and PEF-treated defatted flour) was transferred to an oven (60 °C, 24 h). Defatted flours were used to evaluate techno-functionality and protein characterization.

The supernatant was transferred to a SpeedVac (Savant SpeedVac SPD1010, USA; room temperature, 10 Torr, 2 h) to obtain the extraction yield. The results were expressed as g lipid/100 g of sample (d.b.), and the highest extraction yields determined the most efficient PEF

treatments. The most efficient PEF treatments were selected for the analysis of their techno-functionality (WHC, OHC, FC, and EAI) and protein characterization (protein solubility, *in vitro* digestibility, and SDS-PAGE). Lipid extractions were performed in triplicate.

2.4. Protein characterization

2.4.1. Protein solubility

Protein solubility was determined following the Stone et al. (2019) methodology with modifications on the defatted flours. The pH was not adjusted but rather measured before stirring the mixture. Triplicates of each sample were performed.

2.4.2. In vitro Protein Digestibility (IVPD)

The IVPD was evaluated with modifications according to Hsu et al. (1977), on the defatted flours. Instead of using porcine intestinal peptidase, 1.3 mg/mL of protease *(Streptomyces griseus*, 15 units/mg) was used. Soy protein concentrate was used as a vegetable control. Analyses were made in triplicate.

2.4.3. SDS-Page

The electrophoretic pattern of the defatted flours was determined through sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) following the Espinosa-Ramírez and Serna-Saldívar (2016) procedure using reducing and non-reducing conditions. Except, the electrophoretic reducing sample buffer consisted of 100 mM Tris-HCl pH 6.8, 4% SDS, 0.2% bromophenol blue, and 20% glycerol. And the sample was centrifuged (12,500 RCP, 1 min) before putting it into boiling water. Protein extracts with 15 μg of protein were loaded. Gel images were captured using an iBright CL1500 Imaging System (Invitrogen, Singapore).

2.5. Statistical analysis

Statistical analysis was carried out using Minitab Statistical Software, version 19 (State College, USA). Significance among samples was evaluated by analysis of variance (ANOVA) and mean comparisons through Fisher's least significance difference test. Statistical significance was $p<0.05$ for the mean values of the samples.

3. Results and discussion

The following section is divided into three main sections: 3.1 *Flours characterization*, where the composition analysis (proximal composition, amino acids and fatty acids profile) of the untreated *flour* (control) and the techno-functional properties of the control and PEF-treated *flours* are presented*;* 3.2 *Lipid extraction*, where PEF was used to enhance the extraction of lipids, and once extracted, *flours* turned into *defatted flours*; and 3.3) *3.3 Defatted flours characterization*, in which the *defatted flours* analysis (control and PEF-treated) in terms of techno-functional properties and protein characterization is presented. This can also be observed on Fig. 1.

3.1. Flours characterization

3.1.1. Composition analysis

The composition analysis of ZM control flour is presented in Table 2. Comparison among authors was considering the dry basis (d.b.). The protein content was within the protein range (37.27–42.34%) reported by Dragojlović et al. (2022). The difference in contrast to ZM control flour may be attributed to the diet and harvest time of ZM. Nascimento et al. (2022) and Soares Araújo et al. (2019) reported a higher protein content (42.60 and 46.8%) than ZM control flour (37.72 g/100 sample). However, they used a Kp of 6.25 in contrast to the Kp of 4.76 used by Dragojlović et al. (2022). The first Kp is used for protein determination in common food matrices, but in insects, it overestimates the protein content due to nonprotein nitrogen (chitin, phospholipids, and ammonia derived from excretions). Therefore, as Janssen et al. (2017) suggested, a Kp of 4.76 may be used for the determination of whole larvae.

Table 2
Proximal composition of ZM control flour (g/100 g sample (d. b., except for moisture).

Parameter	g/100 g sample
Proteins	37.72 ± 0.41
Lipids	34.48 ± 0.43
NFE	15.52 ± 0.05
Total dietary fiber	8.85 ± 0.34
Insoluble fiber	7.27 ± 0.00
Soluble fiber	1.58 ± 0.34
Ashes	3.46 ± 0.04
Moisture	2.55 ± 0.92

d.b.: dry basis; NFE: Nitrogen Free Extract.
Mean and standard deviation values of two replicates.

The lipid content of ZM control flour was within that reported by Dragojlović et al. (2022) (32.35–44.48%). ZM control flour had lower lipid content (34.48 g/100 g sample) compared to the obtained by Nascimento et al. (2022) and Soares Araújo et al. (2019) (45.58, and 43.64%). Regarding fiber, which is mainly found in the chitin of the exoskeleton (Nascimento et al., 2022), the dietary fiber obtained in control flour (8.85 g/100 g sample) was similar to the crude fiber reported by Dragojlović et al. (2022) and Nascimento et al. (2022) (9.12% and 8.63%), even though the crude fiber determination might underestimate the dietary fiber content (Moron et al., 1997). The NFE of the control flour was more than double the carbohydrates obtained by Nascimento et al. (2022). These authors also calculated the carbohydrate content by difference; therefore, molecules such as soluble fiber were considered for the carbohydrate content. The ash content (3.56 g/100 g sample) was similar to the value obtained by Nascimento et al. (2022) (3.04%) and Soares Araújo et al. (2019) (4.08%). As Nascimento et al. (2022) stated, protein, lipid, and fiber content differences may be attributed to either the modulation of biosynthetic pathways or the selective uptake of fatty acids from the diet. Hence, the nutritious profile of ZM is affected by the diet, rearing conditions, environment, and harvest time (Rumbos & Athanassiou, 2021).

3.1.2. Amino acids (AA) and fatty acids profile

Table 3 shows the AA profile and the limiting AA score of ZM control flour. The latter corresponds to the sulfur AA (methionine and cysteine). This result was consistent with the research presented by Rumbos and Athanassiou (2021), where ZM is specified to be deficient in methionine content. Also, Oibiokpa et al. (2018) obtained methionine and cysteine as the limiting AA in insects *Macrotermes nigeriensis* (0.47) and *Cirina forda* (0.51). A similar score of 0.83 on the same sulfur AA as ZM control flour was found by Stone et al. (2019) on *T. molitor*, although it was not the limiting AA score. The PDCAAS of ZM was similar to *Gryllodes sigillatus* (0.64), higher than *T. molitor* (0.54), *C. forda* (0.42), and *M. nigeriensis* (0.42), and lower than *Gryllus assimilis* (0.73). Showing that among the insects, ZM control flour and *G. assimilis* had the best protein quality.

The fatty acids profile of ZM control flour is presented in Table 4. Regarding the saturated fatty acids (SAT), hexadecanoic acid was the most prominent SAT, followed by octadecanoic acid. These results were similar to the SAT obtained by Zhang et al. (2019) on ZM, where hexadecanoic and octadecanoic acid were the main SAT (16.7 and 3.15%). Although hexadecanoic and octadecanoic acids were predominant in the SAT content of ZM obtained by Soares Araújo et al. (2019), both of their values were more than double (30.7% and 7.95%, respectively) in contrast to ZM control flour.

The monounsaturated fatty acids (MUFA) were predominant in the evaluated sample, where the cis-9-octadecenoic acid was the main MUFA. While cis-9-octadecenoic acid has been reported as the main

Table 3
Amino acids profile of ZM control flour (g/100 g lipids).

Amino acid	g/100 g protein
Essential amino acids (EAA)	
Leucine	7.58
Valine	6.81
Lysine	6.13
Isoleucine	4.95
Phenylalanine	4.73
Threonine	4.30
Histidine	3.48
Tryptophan	1.43
Methionine	1.23
Non-essential amino acids (NEAA)	
Glutamic Acid	12.54
Aspartic Acid	8.62
Tyrosine	8.36
Alanine	7.32
Proline	5.96
Arginine	5.71
Glycine	5.15
Serine	4.14
Cysteine	0.97
Hydroxylysine	0.35
Ornithine	0.12
Taurine	0.09
Hydroxyproline	0.06
Protein quality parameters	
∑EAA	49.95
∑NEAA	59.38
Limiting AA score (Met + Cys)	0.84
PDCAAS	0.67

EAA: Essential Amino Acids; NEAA: Non-essential Amino Acids.
AA: amino acids; PDCAAS: Protein digestibility corrected amino acid score.
Limiting Sulfur amino acids: Methionine + Cysteine.

Table 4
Fatty acids profile of ZM control flour (g/100 g lipids).

Fatty Acid	Code	g/100 g lipids
Saturated Fatty Acids (SAT)		
Octanoic acid	08:0	0.05 ± 0.07
Decanoic acid	10:0	0.02 ± 0.00
Dodecanoic acid	12:0	0.03 ± 0.00
Tetradecanoic acid	14:0	0.13 ± 0.01
Pentadecanoic acid	15:0	0.09 ± 0.00
Hexadecanoic acid	16:0	13.71 ± 0.00
Heptadecanoic acid	17:0	0.33 ± 0.05
Octadecanoic acid	18:0	3.40 ± 0.40
Eicosanoic acid	20:0	0.02 ± 0.00
Monounsaturated Fatty Acids (MUFA)		
cis-9-Hexadecenoic acid	16:1n7	1.02 ± 0.12
cis-9-Octadecenoic acid	18:1n9	69.57 ± 7.66
Polyunsaturated Fatty Acids (MUFA)		
9,12-octadecadienoic acid	18:2n6	10.72 ± 1.18
9,12,15-octadecatrienoic acid	18:3n3	0.90 ± 0.10
∑SAT	–	17.79 ± 0.55
∑MUFA	–	70.59 ± 7.78
∑PUFA	–	11.62 ± 1.27

SAT: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids.
Mean and standard deviation values of two replicates.

MUFA present in ZM, previous works reported contents from 33.81 to 38% (Nascimento et al., 2022; Soares Araújo et al., 2019; Zhang et al., 2019), while ZM control flour content is two-fold higher. The less abundant group was the polyunsaturated fatty acids (PUFA), where the 9,12-octadecadienoic acid was the most abundant. A content of 15.60–36.50% has been reported (Nascimento et al., 2022; Soares Araújo et al., 2019; Zhang et al., 2019). Differences in the fatty acids profile of the authors in comparison to the analyzed herein may be attributed to the feed and rearing conditions (Dragojlović et al., 2022).

Overall, unsaturated fatty acids prevailed over SAT. The consumption of PUFA prevents arthritis, cardiovascular diseases, and diabetes (Dragojlović et al., 2022). For example, 9,12-octadecadienoic acid consumption is related to the reduction of inflammatory and coronary diseases (Aguilar & dos, 2021). Nonetheless, the abundance of unsaturated fatty acids in ZM and its food products tend to oxidize rapidly, limiting its conservation (Nascimento et al., 2022; Soares Araújo et al., 2019; Zhang et al., 2019). Therefore, defatting its flour may avoid oxidation and rancidity (Sharma, 2021). The obtained lipids may be used for frying, cooking, improvement of flavor and texture, or the preparation of products such as mayonnaise (Aguilar & dos, 2021).

3.1.3. Techno-functional properties of the flours

Table 5 shows the techno-functional properties (WHC, OHC, WEC, OEC, FC) of the ZM control and PEF-treated flours. The ZM control flour had the lowest WHC (1.90 g/g), while PEF treatment increased the WHC of virtually all of the treated samples (T3-10 and T5.6-10, had a higher increase of 2.27 g/g and 2.24 g/g, respectively). As for OHC, most of the samples maintained a similar OHC (2.85–2.98 g/g). However, the OHC of T3-200 decreased significantly ($p<0.05$) to 2.64 g/g compared to the ZM control flour (2.95 g/g). Minimal information is available on the effect of PEF on the techno-functionality of edible insects. It is important to note that the OHC is generally higher than the WHC in ZM. Furthermore, Psarianos et al. (2022) obtained different results after applying PEF on *A. domesticus*, where WHC exhibited no significant difference ($p>0.05$) between the control and the treated samples (≈1.5–1.8 g/g) while OHC was increased after PEF treatments (up to 3.2 g/g), in contrast to control (≈2.3 g/g).

Literature on the effect of PEF on WHC and OHC on a food matrix indicates that the hydrophobic groups of the proteins tend to be exposed, increasing OHC and decreasing or with no significant effect on WHC (Li et al., 2007; Psarianos et al., 2022; Taha et al., 2022). However, this is somehow contradictory to the obtained results. These results may be explained by one of the mechanisms of action of PEF, electroporation, through which two possible effects could occur. The first involves the natural presence of hydrophobic pores in the lipid membrane. When an electric field is applied, a conformational reorientation of membrane lipids may occur, leading to the expansion of existing pores and the formation of stable hydrophilic pores. This transition happens when the pore radius exceeds a critical threshold, at which point maintaining a hydrophilic pore becomes energetically favorable (Jeyamkondan et al., 1999). The increased presence of hydrophilic pores could account for the observed increase in WHC values and the trend toward reduced OHC values. The second possibility is that electroporation facilitates the release of lipidic material from within the cells, thereby reducing the material's affinity for oil.

Regarding WEC, OEC, and FC, there were no significant changes ($p>0.05$) in either of the PEF treatments. Psarianos et al. (2022) reported that the PEF treatment had a significant increase ($p<0.05$) on the EC (50% water/50% oil) of all *A. domesticus* samples. This disparity could be explained by differences in the ratio of water to oil used, exposing the proteins differently to the water-in-oil or oil-in-water solutions. Finally, FC values had minor changes (50–55%). These were similar to the values reported by Psarianos et al. (2022) (≈50%), where no significant difference ($p>0.05$) was found among on *A. domesticus* after PEF treatment. Different results have been reported for PEF-treated canola seeds by Zhang et al. (2017), where an increase in EC and FC

Table 5
Techno-functional properties of ZM control flour and PEF-treated flours.

Flour	WHC (g_{water}/g_{sample})	OHC (g_{oil}/g_{sample})	WEC (%)	OEC (%)	FC (%)
ZM control	1.90 ± 0.05 ^{d}	2.95 ± 0.07 ^{a,b,c}	66.67 ± 0.00 ^{a}	95.56 ± 3.85 ^{a}	53.33 ± 5.77 ^{a}
T1-10	2.03 ± 0.10 ^{b,c}	2.85 ± 0.05 ^{c}	67.78 ± 2.72 ^{a}	97.78 ± 3.44 ^{a}	55.00 ± 5.48 ^{a}
T1-200	2.10 ± 0.08 ^{b}	2.98 ± 0.03 ^{a,b,c}	71.11 ± 3.44 ^{a}	97.78 ± 3.44 ^{a}	50.00 ± 0.00 ^{a}
T3-10	2.27 ± 4 0.03 ^{a}	2.91 ± 0.08 ^{b,c}	68.89 ± 3.44 ^{a}	96.67 ± 3.65 ^{a}	53.33 ± 5.16 ^{a}
T3-200	1.94 ± 0.14 ^{c,d}	2.64 ± 0.19 ^{d}	67.78 ± 2.72 ^{a}	97.78 ± 3.44 ^{a}	53.33 ± 5.16 ^{a}
T5.6-10	2.25 ± 0.07 ^{a}	3.06 ± 0.08 ^{a}	68.89 ± 3.44 ^{a}	96.67 ± 3.65 ^{a}	54.00 ± 5.48 ^{a}
T5.6-200	1.97 ± 0.04 ^{c,d}	3.03 ± 0.02 ^{a,b}	68.89 ± 3.45 ^{a}	97.78 ± 3.44 ^{a}	55.00 ± 5.48 ^{a}

WHC: Water Holding Capacity; OHC: Oil Holding Capacity; WEC: Water Emulsion Capacity; OEC: Oil Emulsion Capacity; FC: Foaming Capacity.
Means not sharing common superscript letter are significantly different ($p<0.05$) within each column.
Mean and standard deviation of three replicates.

values was observed after higher PEF conditions than the ones used in the present work. The control exhibited 56.47% of EC and 119.67% of FC, while the PEF-treated canola seeds significantly increased ($p<0.01$) these techno-functional properties (61.4% and 161.56%, respectively). Hence, higher intensities or number of pulses than the evaluated herein may enhance the EC and FC on the ZM control flour. Further investigation is needed on the effect of PEF on the techno-functional properties of ZM with higher PEF conditions.

3.2. Lipid extraction

The lipid extraction yield of each PEF treatment can be observed in Fig. 2. The results of ANOVA showed that the interaction of the electric field intensity and the number of pulses is significant in lipid extraction. While control flour and T1-10 had the lowest extraction yield (30.72 and 27.34%, respectively), in T1-10, the low extraction yield might be due to the reversible pore formation due to the low intensity and short number of pulses applied (Kumari et al., 2018). Additionally, T3-10 and T1-200 had no significant differences ($p>0.05$) within them. Lastly, the highest extraction yields were achieved by T5.6-200 (54.56%), T5.6-10 (50.50%), and T3-200 (50.53%), with no significant differences ($p<0.05$) within them, leading to a 77.60, 64.38, and 64.48% extraction increase compared to control flour. These results might be associated with irreversible pore formation due to more intensive PEF treatments. Thus, as the electric field intensity and the number of pulses increase, the lipid extraction is improved. This could be explained by the high-energy input the cell membrane is exposed to, causing its rupture, in contrast to a low-energy input (Naliyadhara et al., 2022). Regarding the use of PEF in insects, Smetana et al. (2020) reported that this technology is useful for cell permeabilization and also to improve the extraction yield of intracellular compounds such as lipids. Psarianos et al. (2022) also reported the use of PEF in *A. domesticus* led to a significant increase ($p<0.05$) in the lipid extraction yield among the PEF treatments, where even the lowest energy-consuming treatment (4.90 kJ/kg) resulted in a 41.75% increase compared to the control. While the present work followed the extraction methodology of Psarianos et al. (2022), differences in the yield of extraction may be attributed to the matrix and PEF conditions.

Based on the lipid extraction yield results, T3-200, T5.6-10, and T5.6-200 (Fig. 2, red rectangle) were settled as the most efficient PEF treatments. The techno-functionality and protein characterization of these protein-rich fractions were evaluated. For the following section, these samples will be referred to as *defatted flours*.

3.3. Defatted flours characterization

3.3.1. Techno-functional properties of the defatted flours

Once the PEF treatments that led to an increased lipid extraction were defined (Fig. 2), the techno-functionality (WHC, OHC, FC, and EAI) of the ZM control and the PEF-treated defatted flours were evaluated.

Fig. 3 (A, **solid color)** shows that T3-200-defatted flour had the highest WHC. The other PEF-treated defatted flours had no significant difference among them compared to the ZM control defatted flour. In comparison to flours (Table 5), the defatted flours increased their WHC. As seen in Fig. 3 (A), the OHC of the PEF-treated defatted flours presented no significant change ($p>0.05$) among them but decreased

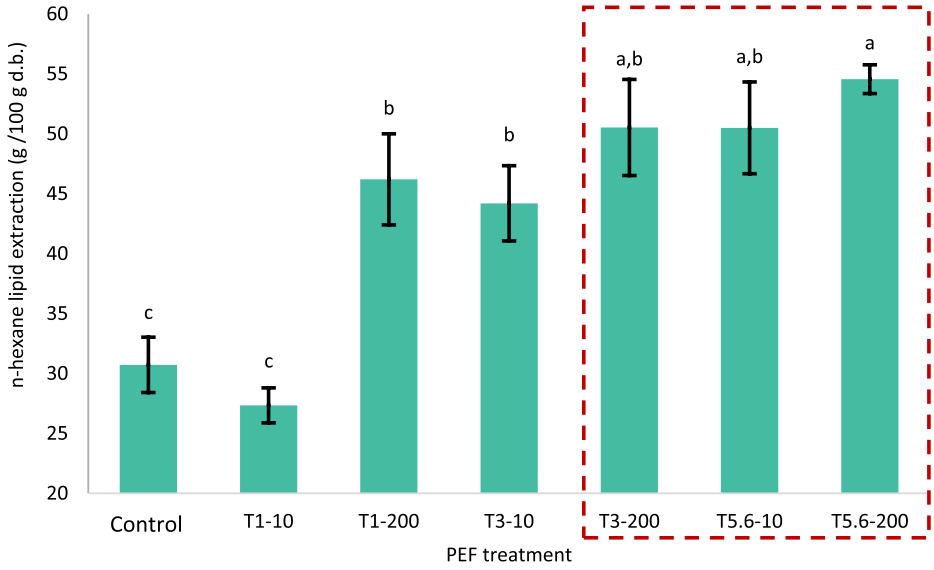

Fig. 2. Lipid extraction yield of ZM control flour and PEF-treated flours.
Error bars represent the standard deviation of three replicates. Means not sharing a common superscript letter are significantly different ($p<0.05$).

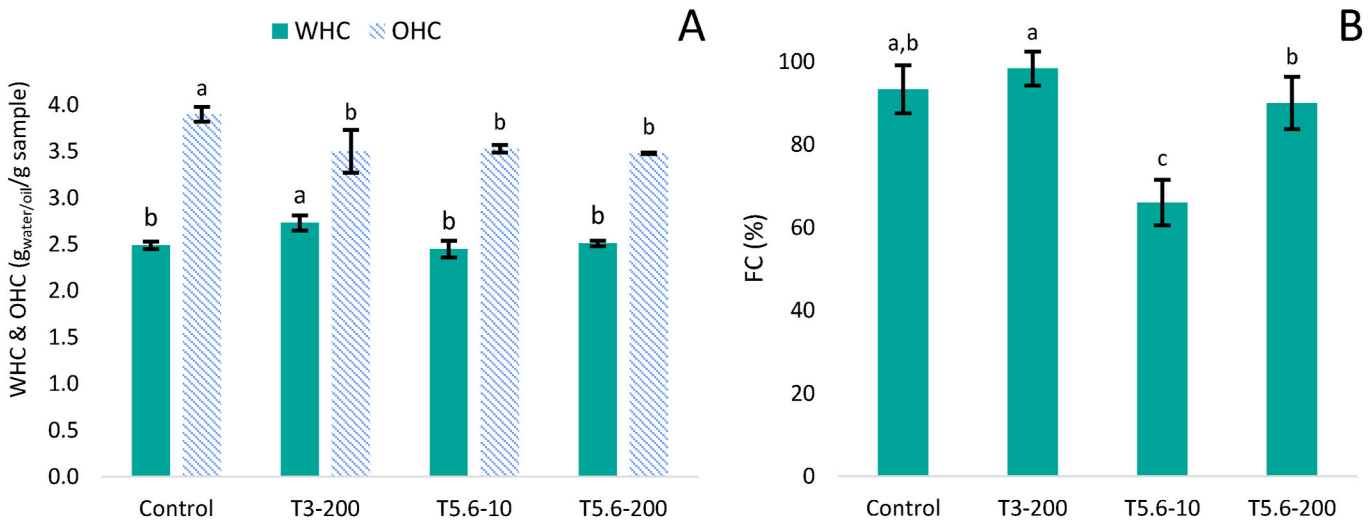

Fig. 3. Techno-functional properties of ZM control defatted-flour and PEF-treated defatted-flours
A) WHC: Water Holding Capacity; **B)** OHC: Oil Holding Capacity; **C)** FC: Foaming Capacity Error bars represent the standard deviation of three replicates. Means not sharing a common superscript letter are significantly different ($p<0.05$) within the techno-functional property.

significantly ($p<0.05$) compared to ZM control defatted flour. Also, when comparing the defatted flours to the flours (Table 5), the OHC increased. The decrease in OHC remained constant with the results obtained in Table 5. This means that while the defatting of the flours slightly increases their OHC, the PEF treatments did have an impact ($p<0.05$), decreasing their OHC.

For FC, Fig. 3 (B) exhibits that T5-6.10 and T5.6-200 resulted in a decreased FC, compared to ZM control defatted flour. These treatments also yielded the highest lipid extraction (50.50 and 54.56%). It could be hypothesized that defatting the ZM flours exposed the impact of the PEF treatment on the defatted flours. Marco-Molés et al. (2011) also obtained a decreased FC on PEF-treated liquid whole egg (43.75% at 37 kV/cm) compared to the control (85.75%). The voltage, frequency, and pulse width could explain the alteration of the FC of the PEF-treated defatted flours (Han et al., 2018). The effect of PEF could be either by the proteins (such as globular proteins) not being able to unfold and rearrange at the water-air interface (Jeong et al., 2021; Villaseñor et al., 2022; Zielińska et al., 2018), or the disintegration of proteins, which play a crucial part in the stabilization of the water-air interface (Purschke et al., 2018). Furthermore, in contrast with the flours (Table 5), FC increased in all defatted flours. The presence of fat has reduced the FC of full-fatted *G. sigillatus*, *T. molitor*, and *Locusta migratoria* L., compared to their defatted flours (Dion-Poulin et al., 2020; Ndiritu et al., 2017). Therefore, the increase in the WHC, OHC, and FC of the defatted flours in comparison to the flours (Table 5) was related to the defatting of the flours.

Table 6 shows the EAI results of the ZM control defatted flour and the PEF-treated defatted-flours at 0 and 60 min. At t = 0 the highest EAI was shown by T3-200-defatted flour and ZM control defatted flour (0.183 and 0.177 m^2/g). While T5.6–10 and T5.6-200 resulted in a decreased EAI. The decrease in EAI observed in this work was consistent with the results obtained in WHC and OHC. The accumulation of hydrophilic pores reduces the EAI, in contrast to the control defatted flour (Jeyamkondan et al., 1999). Also, the EAI at t = 60 min showed no significant difference among the evaluated defatted flours ($p>0.05$).

Table 6
EAI of ZM control defatted flour and PEF-treated defatted flours at t = 0 min and t = 60 min.

PEF-treated defatted-flour	EAI (m^2/g) t = 0 min	EAI (m^2/g) t = 60 min
ZM control	0.177 ± 0.007 ^{a}	0.032 ± 0.006 ^{a}
T3-200	0.183 ± 0.018 ^{a}	0.030 ± 0.006 ^{a}
T5.6-10	0.157 ± 0.012 ^{b}	0.027 ± 0.004 ^{a}
T5.6-200	0.159 ± 0.007 ^{b}	0.033 ± 0.006 ^{a}

EAI: Emulsion Activity Index.
Means not sharing a common superscript letter are significantly different ($p<0.05$) within each column.
Mean and standard deviation values of three replicates.

3.3.2. *Protein characterization*

Protein solubility determination is shown in Fig. 4 (A). The ZM control defatted flour had a protein solubility of 30.35%. This result was similar to the defatted *T. molitor* and *Gryllus bimaculatus* (»30% and »25%) at pH 6 (Buβler et al. (2016). The defatted flours solubility ranged between 28.08% and 30.35% at pH 5.8–6. As for the PEF-treated defatted flours, T5.6-200 had a significant reduction ($p<0.05$) in its protein solubility (28.08%) in contrast to the ZM control defatted flour (30.35%). Similarly, the solubility of PEF-treated pea, rice, and gluten concentrates at pH 6 was significantly decreased ($p<0.05$) (Melchior et al., 2020). In addition, since T5.6-200-defatted flour had the highest extracted lipid yield (54.56%), denaturation of proteins may have occurred since the extraction of lipids enhanced the contact of different protein regions, therefore unfolding and collapsing the protein's structure (Jeong et al., 2021).

Fig. 4 (B) presents the IVPD analysis, where soy protein was used for comparison. Soy protein was significantly higher ($p<0.05$) (88.99%) than the insect samples (78.68–79.86%). The difference in the IVPD of ZM in comparison to other matrices (such as vegetables) may be associated with the presence of chitin. A good protein solubility is desired for IVPD (Mishyna et al., 2021), but chitin is insoluble in water. In humans, it is not degraded or absorbed but can partially be digested by chitinolytic enzymes (chitinase and chitobiase) that turn chitin into chitosan (Lopez-Santamarina et al., 2020; Rodríguez-Rodríguez et al., 2022). Since these enzymes are not in the enzyme cocktail, the IVPD of ZM is limited to the action of the proteases, and the reduced IVPD may be related to three different reasons: 1) the presence of antinutritional factors (Rodríguez-Rodríguez et al., 2022); 2) chitin's structure is similar to that of cellulose, but it may be packed to chitin-binding proteins, thus the higher hydrogen bonds bound to these polymer chains provide more resistance, hindering the IVPD (Freccia et al., 2020; Refael et al., 2022).

The IVPD of the insect samples showed no significant difference ($p>0.05$) (78.68–79.86%). ZM control defatted flour had an IVPD of 79.86%. This result was slightly lower than the digestibility of ZM (82.05–84.05%) by Dragojlović et al. (2022). Also, the digestibility of

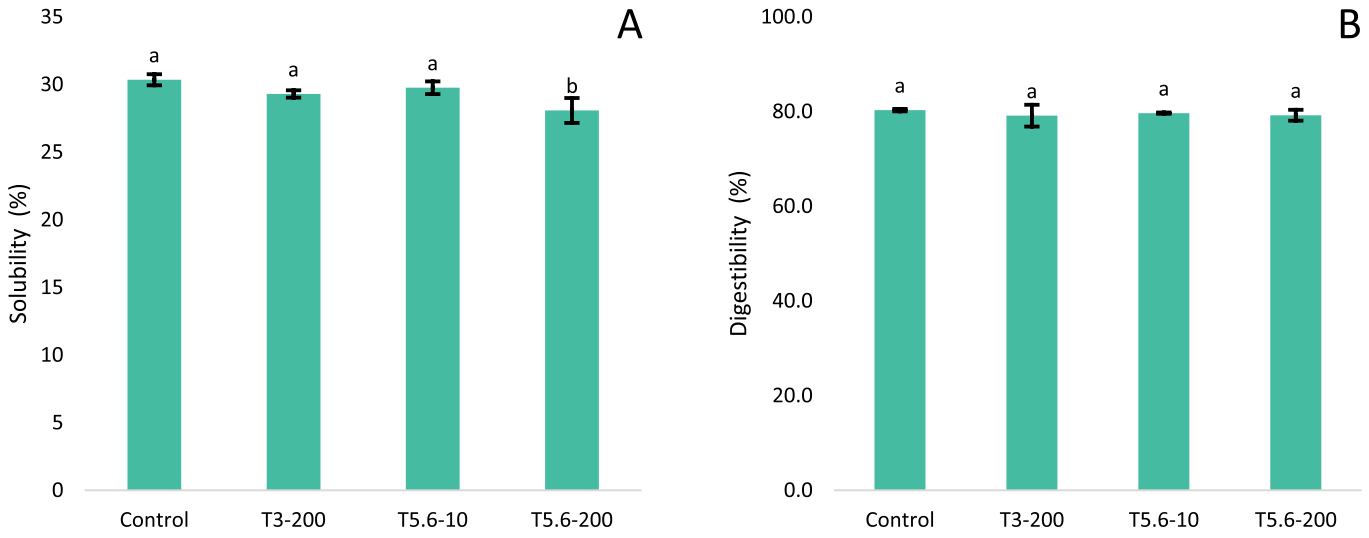

Fig. 4. Protein solubility and *in vitro* protein digestibility of ZM control defatted-flour, and PEF-treated defatted-flours
A) Protein solubility; **B)** *In vitro* protein digestibility (IVDP) Error bars represent the standard deviation of three replicates. Means not sharing a common superscript letter are significantly different ($p<0.05$) within the protein characteristic.

A. domesticus after defatting with hexane had no significant difference ($p>0.05$) (Ndiritu et al., 2017). Up to now, no literature on the effect of PEF on the IVPD of insects has been reported. However, Chian et al. (2019) reported that PEF treatment on beef muscle significantly increased ($p<0.05$) the digestibility by 18% compared to control (12.27%). Therefore, improving IVPD after PEF treatment may be attributed to the food matrix, but further investigation is needed.

The SDS-PAGE electrophoretic pattern of the proteins can be seen in Fig. 5. The protein sizes ranged from ~15–250 kDa. The reducing and non-reducing conditions showed 6 major bands. The bands from 15 to 30 kDa could be cuticle proteins with different molecular weights (Buβler et al., 2016; Cortazar-Moya et al., 2023). In this same range, the 25–27 kDa band might be chymotrypsin-like proteinase, present at 24 kDa (Buβler et al., 2016) on *T. molitor*. At 40–43 kDa arginine kinase could be present, a transferase enzyme in insects and crustaceans (identified as an allergen) (Brogan et al., 2021), or a melanization-inhibiting protein (Buβler et al., 2016). Trypsin-like proteinases and β-glycosidase may be present at 60 kDa. Whereas melanization-engaging types protein could be present at 80–90 kDa. Finally, the protein band at 245–250 kDa may be myosin, a muscle protein in insects (Brogan et al., 2021). Similar findings were reported on edible insects: *T. molitor*, *A. domesticus, Bombyx mori, Locusta migratoria*, and *Arsenura amida* (Brogan et al., 2021; Buβler et al., 2016; Cortazar-Moya et al., 2023). The main difference can be observed in the third lane of the non-reducing conditions (Fig. 5 A) (T5.6-200), where bands at the ~12, 19, and 23 kDa seemed less intense than the other lanes within the same gel. However, these same bands can be seen in the reducing gel (Fig. 5 B). Hence, the low intensity of these bands may have been related to the low exposure of the sample to the sample buffer. Regardless, the molecular size of the PEF-treated defatted flours proteins

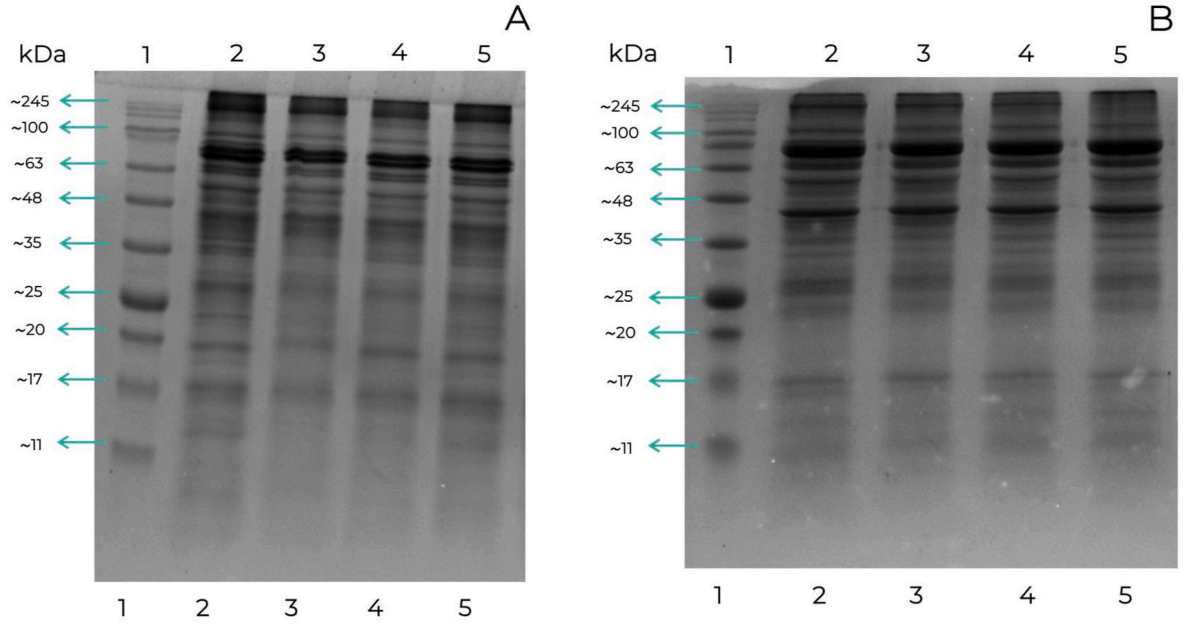

Fig. 5. SDS-PAGE of ZM control defatted-flour and PEF-treated defatted-flours
A) Non-reducing conditions **B)** Reducing conditions Lane 1: Molecular weight marker; lane 2: ZM control defatted-flour; lane 3: T5.6-200; lane 4: T3-200; lane 5: T5.6-10.

was not altered compared to the ZM control defatted flour. Melchior et al. (2020) also found no differences in the protein bands of pea, rice, and gluten concentrates after PEF treatment. The lack of differences in the protein's molecular weight of the PEF-treated defatted flours could be attributed to the low intensity PEF conditions used in the present study. Further research on the effect of the PEF on the molecular weight of the ZM proteins with different PEF parameters is needed.

4. Conclusions

The present research provided the characterization of ZM flour comprising its proximal composition, AA and FA profiles, techno-functional properties, protein characterization, and the effect of PEF on ZM. It has been shown that ZM is rich in lipid and protein content, its limiting AA is methionine + cysteine, and it is rich in MUFA. Hence, its consumption may be beneficial due to its nutritious profile. The use of PEF enhanced the lipid extraction at 5.6 kV/cm (200 and 10 pulses), and 3 kV/cm (200 pulses), leading to a 77.60, 64.38, and 64.48% increase. Additionally, the WHC was increased in virtually all the samples, at 3 kV/cm (10 pulses) it was increased up to 19%, and at 5.6 kV/cm (10 pulses) the OHC decreased (by 11%). PEF, in addition to the defatting process, modified the techno-functional values of WHC, OHC, and FC by increasing them. The protein characteristics of ZM had no major alterations after PEF, while ZM exhibited high IVPD (78.68–79.86%).

The tested PEF parameters improved the lipid extraction on ZM, and the extracted lipids could be used for other purposes, such as frying, cooking, emulsifying, flavor improvement, among others. Using PEF on ZM could be proposed as an alternative to increasing the WHC and decreasing the OHC while maintaining its protein solubility and high IVPD. Also, PEF and a defatting process can be used to increase the techno-functionality of ZM and its utilization as a food ingredient while maintaining a sustainable process.

Some limitations of this current study might include the effect of PEF on sensory attributes (color, flavor, odor), which are critical for consumer acceptance. Further investigation shall include the long-term stability of the PEF-treated flours and their extracted lipids, and the effect of PEF on bioactive compounds. Also, the application of more intense PEF parameters on the same matrix or other edible insects, and to evaluate their effect on the techno-functionality and protein characteristics, to use the resulting PEF-treated flour in food formulations. Finally, due to the high EC and WHC of ZM, the resulting materials could be used as a substitute in food formulations as baked goods or meats, as a way to increase their protein content, along with consumer acceptance tests to validate the flavor and texture profile, and the acceptance of the addition of insects to food and their inclusion in their diets.

CRediT authorship contribution statement

Michelle Fernández-Salas: Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Johanan Espinosa-Ramírez:** Writing – review & editing, Supervision, Resources, Methodology, Formal analysis. **Mariana Morales-de la Peña:** Methodology. **Luis Eduardo Garcia-Amezquita:** Resources, Methodology. **Sandra Teresita Martín-del-Campo:** Writing – review & editing, Methodology. **Viridiana Tejada-Ortigoza:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgments

Funding: Bio-Foods Research Lab from Tecnólogico de Monterrey funded the present research. The first author (Michelle Fernández-Salas) expresses her gratitude to Tecnólogico de Monterrey, and CONAHCYT (now SECIHTI) for their Scholarship programs (CVU: 1186000), and Zofo® for the *Zophobas morio* donation.

Data availability

Data will be made available on request.

References

Aguanta, L. M., Sabado, E. M., & Consolacion, E. M. (2023). *Consumer acceptance of Zophobas morio (Superworm) based food*.

Aguilar, J. G., & dos, S. (2021). An overview of lipids from insects. In *Biocatalysis and agricultural biotechnology* (Vol. 33). Elsevier Ltd. https://doi.org/10.1016/j.bcab.2021.101967

Ahmmed, M. K., Carne, A., Tian, H., Sabrina, & Bekhit, A. E. D. A. (2022). The effect of pulsed electric fields on the extracted total lipid yield and the lipidomic profile of hoki roe. *Food Chemistry, 384*. https://doi.org/10.1016/j.foodchem.2022.132476

AOAC International. (2022). *Official methods of analysis* (21st ed.).

Bakhshabadi, H., Mirzaei, H. O., Ghodsvali, A., Jafari, S. M., & Ziaiifar, A. M. (2018). The influence of pulsed electric fields and microwave pretreatments on some selected physicochemical properties of oil extracted from black cumin seed. *Food Science and Nutrition, 6*(1), 111–118. https://doi.org/10.1002/fsn3.535

Brogan, E. N., Park, Y.-L., Matak, K. E., & Jaczynski, J. (2021). Characterization of protein in cricket (Acheta domesticus), locust (Locusta migratoria), and silk worm pupae (Bombyx mori) insect powders. *Lebensmittel-Wissenschaft & Technologie, 152*, Article 112314. https://doi.org/10.1016/j.lwt.2021.112314

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), Article e00218. https://doi.org/10.1016/j.heliyon.2016.e00218

Caparros Megido, R., Gierts, C., Blecker, C., Brostaux, Y., Haubruge, É., Alabi, T., & Francis, F. (2016). Consumer acceptance of insect-based alternative meat products in Western countries. *Food Quality and Preference, 52*, 237–243. https://doi.org/10.1016/j.foodqual.2016.05.004

Chian, F. M., Kaur, L., Oey, I., Astruc, T., Hodgkinson, S., & Boland, M. (2019). Effect of Pulsed Electric Fields (PEF) on the ultrastructure and in vitro protein digestibility of bovine longissimus thoracis. *Lebensmittel-Wissenschaft & Technologie, 103*, 253–259. https://doi.org/10.1016/j.lwt.2019.01.005

Cortazar-Moya, S., Mejía-Garibay, B., López-Malo, A., & Morales-Camacho, J. I. (2023). Nutritional composition and techno-functionality of non-defatted and defatted flour of edible insect Arsenura armida. *Food Research International, 173*. https://doi.org/10.1016/j.foodres.2023.113445

Corzo-Ríos, L. J., Sánchez-Chino, X. M., Martin del Campo, S. T., Jiménez Martínez, C., Garduño Siciliano, L., & Martínez Herrera, J. (2022). Biological and toxicological evaluation of edible Jatropha curcas L. oil. *Food Science and Technology, 42*. https://doi.org/10.1590/fst.66722

de Carvalho, N. M., Madureira, A. R., & Pintado, M. E. (2020). The potential of insects as food sources–a review. In *Critical reviews in food science and nutrition* (Vol. 60, pp. 3642–3652). Bellwether Publishing, Ltd. https://doi.org/10.1080/10408398.2019.1703170, 21.

Dion-Poulin, A., Laroche, M., Doyen, A., & Turgeon, S. L. (2020). Functionality of cricket and mealworm hydrolysates generated after pretreatment of meals with high hydrostatic pressures. *Molecules, 25*(22). https://doi.org/10.3390/molecules25225366

Dragojlović, D., Đuragić, O., Pezo, L., Popović, L., Rakita, S., Tomičić, Z., & Spasevski, N. (2022). Comparison of nutritional profiles of super worm (zophobas morio) and yellow mealworm (Tenebrio molitor) as alternative feeds used in animal husbandry: Is super worm superior? *Animals, 12*(10). https://doi.org/10.3390/ani12101277

Espinosa-Ramírez, J., & Serna-Saldívar, S. O. (2016). Functionality and characterization of kafirin-rich protein extracts from different whole and decorticated sorghum genotypes. *Journal of Cereal Science, 70*, 57–65. https://doi.org/10.1016/j.jcs.2016.05.023

Finke, M. D. (2015). Complete nutrient content of four species of commercially available feeder insects fed enhanced diets during growth. *Zoo Biology, 34*(6), 554–564. https://doi.org/10.1002/zoo.21246

Freccia, A., Sergio Bee Tubin, J., Nishioka Rombenso, A., & Gustavo Coelho Emerenciano, M. (2020). Insects in aquaculture nutrition: An emerging eco-friendly approach or commercial reality?. In *Emerging technologies, environment and research for sustainable aquaculture*. IntechOpen. https://doi.org/10.5772/intechopen.90489.

Han, Z., Cai, M. jie, Cheng, J. H., & Sun, D. W. (2018). Effects of electric fields and electromagnetic wave on food protein structure and functionality: A review. In *Trends in food science and technology* (Vol. 75, pp. 1–9). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2018.02.017

Hsu, H. W., Vavak, D. L., Satterlee, L. D., & Miller, G. A. (1977). A multienzyme technique for estimating protein digestibility. *Journal of Food Science, 42*(5), 1269–1273. https://doi.org/10.1111/j.1365-2621.1977.tb14476.x

Janssen, R. H., Vincken, J.-P., van den Broek, L. A. M., Fogliano, V., & Lakemond, C. M. M. (2017). Nitrogen-to-crotein conversion factors for three edible insects: Tenebrio molitor, Alphitobius diaperinus, and hermetia illucens. *Journal of Agricultural and Food Chemistry, 65*(11), 2275–2278. https://doi.org/10.1021/acs.jafc.7b00471

Jeong, M. S., Lee, S. D., & Cho, S. J. (2021). Effect of three defatting solvents on the techno-functional properties of an edible insect (Gryllus bimaculatus) protein concentrate. *Molecules, 26*(17). https://doi.org/10.3390/molecules26175307

Jeyamkondan, S., Jayas, D. S., & Holley, R. A. (1999). Pulsed electric field processing of foods: A review. *Journal of Food Protection, 62*(Issue 9).

Joint FAO/WHO/UNU Expert Consultation on Protein. (2007). *Protein and amino acid requirements in human nutrition : Report of a joint FAO/WHO/UNU expert consultation* (p. 265). World Health Organization.

Kumari, B., Tiwari, B. K., Hossain, M. B., Brunton, N. P., & Rai, D. K. (2018). Recent advances on application of ultrasound and pulsed electric field technologies in the extraction of bioactives from agro-industrial by-products. In *Food and bioprocess technology* (Vol. 11, pp. 223–241). New York: Springer. https://doi.org/10.1007/s11947-017-1961-9. LLC.

Li, Y., Chen, Z., & Mo, H. (2007). Effects of pulsed electric fields on physicochemical properties of soybean protein isolates. *LWT - Food Science and Technology, 40*(7), 1167–1175. https://doi.org/10.1016/j.lwt.2006.08.015

Liceaga, A. M. (2021). Processing insects for use in the food and feed industry. In *Current opinion in insect science* (Vol. 48, pp. 32–36). Elsevier Inc. https://doi.org/10.1016/j.cois.2021.08.002

Lopez-Santamarina, A., Mondragon, A., del, C., Lamas, A., Miranda, J. M., Franco, C. M., & Cepeda, A. (2020). Animal-origin prebiotics based on chitin: An alternative for the future? A critical review. In *Foods* (Vol. 9). MDPI Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/foods9060782, 6.

Marco-Molés, R., Rojas-Graü, M. A., Hernando, I., Pérez-Munuera, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2011). Physical and structural changes in liquid whole egg treated with high-intensity pulsed electric fields. *Journal of Food Science, 76*(2). https://doi.org/10.1111/j.1750-3841.2010.02016.x

Melchior, S., Calligaris, S., Bisson, G., & Manzocco, L. (2020). Understanding the impact of moderate-intensity pulsed electric fields (MIPEF) on structural and functional characteristics of pea, rice and gluten concentrates. *Food and Bioprocess Technology, 13*(12), 2145–2155. https://doi.org/10.1007/s11947-020-02554-2

Mishyna, M., Keppler, J. K., & Chen, J. (2021). Techno-functional properties of edible insect proteins and effects of processing. In *Current opinion in colloid and interface science* (Vol. 56). Elsevier Ltd. https://doi.org/10.1016/j.cocis.2021.101508

Moron, C., Zacarias, I., & Pablo, S. de (1997). Producción y manejo de datos de composición química de alimentos en nutrición. In *Producccion y manejo de datos de composicion quimica de alimentos en nutricion* (p. 356).

Naliyadhara, N., Kumar, A., Girisa, S., Daimary, U. D., Hegde, M., & Kunnumakkara, A. B. (2022). Pulsed electric field (PEF): Avant-garde extraction escalation technology in food industry. In *Trends in food science and technology* (Vol. 122, pp. 238–255). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2022.02.019

Nascimento, R. Q., Di Mambro Ribeiro, C. V., Colauto, N. B., da Silva, L., Lemos, P. V. F., de Souza Ferreira, E., Linde, G. A., Machado, B. A. S., Tavares, P. P. L. G., Biasoto, A. C. T., Umsza Guez, M. A., Carvalho, N., de Jesus Assis, D., da Silva, J. B. A., & de Souza, C. O. (2022). Utilization of agro-industrial residues in the rearing and nutritional enrichment of zophobas atratus larvae: New food raw materials. *Molecules, 27*(20). https://doi.org/10.3390/molecules27206963

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. https://doi.org/10.1007/s11694-017-9584-4

Oibiokpa, F. I., Akanya, H. O., Jigam, A. A., Saidu, A. N., & Egwim, E. C. (2018). Protein quality of four indigenous edible insect species in Nigeria. *Food Science and Human Wellness, 7*(2), 175–183. https://doi.org/10.1016/j.fshw.2018.05.003

Oonincx, D. G. A. B., & de Boer, I. J. M. (2012). Environmental impact of the production of mealworms as a protein source for humans - a life cycle assessment. *PLoS One, 7*(12). https://doi.org/10.1371/journal.pone.0051145

Pearce, K. N., & Kinsella, J. E. (1978). Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *Journal of Agricultural and Food Chemistry, 26*(3), 716–723.

Psarianos, M., Dimopoulos, G., Ojha, S., Cavini, A. C. M., Bußler, S., Taoukis, P., & Schlüter, O. K. (2022). Effect of pulsed electric fields on cricket (Acheta domesticus) flour: Extraction yield (protein, fat and chitin) and techno-functional properties. *Innovative Food Science and Emerging Technologies, 76*. https://doi.org/10.1016/j.ifset.2021.102908

Purschke, B., Meinlschmidt, P., Horn, C., Rieder, O., & Jäger, H. (2018). Improvement of techno-functional properties of edible insect protein from migratory locust by enzymatic hydrolysis. *European Food Research and Technology, 244*(6), 999–1013. https://doi.org/10.1007/s00217-017-3017-9

Ramos-Elorduy, J. (2009). Anthropo-entomophagy: Cultures, evolution and sustainability. *Entomological Research, 39*(5), 271–288. https://doi.org/10.1111/j.1748-5967.2009.00238.x

Refael, G., Riess, H. T., Levi, C. S., Magzal, F., Tamir, S., Koren, O., & Lesmes, U. (2022). Responses of the human gut microbiota to physiologically digested insect powders or isolated chitin thereof. *Future Foods, 6*. https://doi.org/10.1016/j.fufo.2022.100197

Rodríguez-Rodríguez, M., Barroso, F. G., Fabrikov, D., & Sánchez-Muros, M. J. (2022). In vitro crude protein digestibility of insects: A review. *Insects, 13*(Issue 8). https://doi.org/10.3390/insects13080682. MDPI.

Ruiz Haddad, L., Tejada-Ortigoza, V., Martín-del-Campo, S. T., Balderas-León, I., Morales-de la Peña, M., Garcia-Amezquita, L. E., & Welti-Chanes, J. (2022). Evaluation of nutritional composition and technological functionality of whole American Bullfrog (Lithobates catesbeianus), its skin, and its legs as potential food ingredients. *Food Chemistry, 372*. https://doi.org/10.1016/j.foodchem.2021.131232

Rumbos, C. I., & Athanassiou, C. G. (2021). The superworm, zophobas morio (Coleoptera:Tenebrionidae): A 'sleeping giant' in nutrient sources. *Journal of Insect Science, 21*(2). https://doi.org/10.1093/jisesa/ieab014

Scholliers, J., Steen, L., & Fraeye, I. (2020). Partial replacement of meat by superworm (Zophobas morio larvae) in cooked sausages: Effect of heating temperature and insect:Meat ratio on structure and physical stability. *Innovative Food Science & Emerging Technologies, 66*, Article 102535. https://doi.org/10.1016/j.ifset.2020.102535

Sharma, V. N.&S. (2021). *Handbook of research on food processing and preservation technologies, ume 5*. NEW INDIA PUBLISHING AGENCY. NIPA.

Silve, A., Papachristou, I., Wüstner, R., Sträßner, R., Schirmer, M., Leber, K., Guo, B., Interrante, L., Posten, C., & Frey, W. (2018). Extraction of lipids from wet microalga Auxenochlorella protothecoides using pulsed electric field treatment and ethanol-hexane blends. *Algal Research, 29*, 212–222. https://doi.org/10.1016/j.algal.2017.11.016

Smetana, S., Mhemdi, H., Mezdour, S., & Heinz, V. (2020). Pulsed electric field-treated insects and algae as future food ingredients. In *Pulsed electric fields to obtain healthier and sustainable food for tomorrow* (pp. 247–266). Elsevier. https://doi.org/10.1016/B978-0-12-816402-0.00011-2.

Soares Araújo, R. R., dos Santos Benfica, T. A. R., Ferraz, V. P., & Moreira Santos, E. (2019). Nutritional composition of insects Gryllus assimilis and Zophobas morio: Potential foods harvested in Brazil. *Journal of Food Composition and Analysis, 76*, 22–26. https://doi.org/10.1016/j.jfca.2018.11.005

Sriprablom, J., Kitthawee, S., & Suphantharika, M. (2022). Functional and physicochemical properties of cookies enriched with edible insect (Tenebrio molitor and Zophobas atratus) powders. *Journal of Food Measurement and Characterization, 16*(3), 2181–2190. https://doi.org/10.1007/s11694-022-01324-2

Stone, A. K., Tanaka, T., & Nickerson, M. T. (2019). Protein quality and physicochemical properties of commercial cricket and mealworm powders. *Journal of Food Science and Technology, 56*(7), 3355–3363. https://doi.org/10.1007/s13197-019-03818-2

Taha, A., Casanova, F., Šimonis, P., Stankevič, V., Gomaa, M. A. E., & Stirkė, A. (2022). Pulsed electric field: Fundamentals and effects on the structural and techno-functional properties of dairy and plant proteins. *Foods, 11*(11). https://doi.org/10.3390/foods11111556

Villaseñor, V. M., Enriquez-Vara, J. N., Urías-Silva, J. E., & Mojica, L. (2022). Edible insects: Techno-functional properties food and feed applications and biological potential. In *Food reviews international* (Vol. 38, pp. 866–892). Taylor and Francis Ltd. https://doi.org/10.1080/87559129.2021.1890116. S1.

Yi, L., Lakemond, C. M. M., Sagis, L. M. C., Eisner-Schadler, V., Huis, A. Van, & Boekel, M. A. J. S. V. (2013). Extraction and characterisation of protein fractions from five insect species. *Food Chemistry, 141*(4), 3341–3348. https://doi.org/10.1016/j.foodchem.2013.05.115

Zhang, X., Tang, H., Chen, G., Qiao, L., Li, J., Liu, B., Liu, Z., Li, M., & Liu, X. (2019). Growth performance and nutritional profile of mealworms reared on corn stover, soybean meal, and distillers' grains. *European Food Research and Technology, 245*(12), 2631–2640. https://doi.org/10.1007/s00217-019-03336-7

Zhang, L., Wang, L.-J., Jiang, W., & Qian, J.-Y. (2017). Effect of pulsed electric field on functional and structural properties of canola protein by pretreating seeds to elevate oil yield. *Lebensmittel-Wissenschaft & Technologie, 84*, 73–81. https://doi.org/10.1016/j.lwt.2017.05.048

Zielińska, E., Karaś, M., & Baraniak, B. (2018). Comparison of functional properties of edible insects and protein preparations thereof. *Lebensmittel-Wissenschaft & Technologie, 91*, 168–174. https://doi.org/10.1016/j.lwt.2018.01.058