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Nutritional Value and Microbiological Aspects of Dried Yellow Mealworm (*Tenebrio molitor* L.) Larvae Pretreated with a Pulsed Electric Field

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Abstract: Complete protein, which includes all the essential amino acids, and bioactive compounds needed in human nutrition, can be found in edible insects. Bioactive compounds play a crucial role in protecting cells from the damage caused by free radicals. Therefore, in this study, fat extraction yield, protein content, amino acid profile, total polyphenol content, antioxidant properties, crustacean and mollusk content, and microbiological quality have been assessed to evaluate the influence of the drying method and pulsed electric field (PEF) pretreatment. To this end, the sample was processed by the PEF at varied specific energy intakes (5, 20, and 40 kJ/kg) and dried by means of two methods: convective (CD) and infrared-convective (IR-CD). A comparable protein content (47.5–48.7 g/100 g d.m.) was determined for most of the samples tested. A significantly higher lysine and methionine content was detected in the CD insects, especially for samples treated by the PEF at 40 kJ/kg. The IR-CD samples exhibited a significantly higher content of polyphenols as compared to those obtained by means of the CD method, whereas the PEF apparently had a significant impact to the extent of increasing their content. Taking into account allergenicity, the crustacean content was approximately 10 times higher than the mollusk content. The study has shown that the PEF treatment prior to infrared-convective drying resulted in the assurance of the microbiological quality of dried insects for food use. Furthermore, a dose of the PEF at 20 and 40 kJ/kg demonstrated the antimicrobial effect. The results have proven that, in this case, a high temperature during the CD method did not cause the undesirable changes that had been expected. Therefore, PEF-assisted convective drying may conceivably be considered to obtain highly nutritionally valuable insects; however, it is crucial to utilize appropriate parameters in the course of the PEF processing.

Keywords: edible insects; pulsed electric field treatment; drying; protein content; amino acid profile; bioactive compounds; allergens

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1. Introduction

Given a growing world population, increasing costs of animal production, increasing numbers of emerging diseases in livestock, dwindling natural resources, climate change, increasing food prices, and resource disparities, there is a requirement to search for alternative and sustainable food [1–3]. Edible insects have emerged as a promising and sustainable source of nutrition in response to global challenges related to food security and environmental sustainability [3,4].

Owing to their high nutritional composition, edible insects are becoming more and more popular as an alternative food source. They constitute a valuable source of protein, lipids, vitamins, and micronutrients for human life. They additionally differ in the quantity of polyunsaturated fatty acids and essential amino acids [5–7]. Alternatively, the nutritional compositions of edible insects vary significantly between and within species, depending on the metamorphosis stage of an insect, the origin of the insect, and farming conditions [6,8]. Diverse processing techniques may also influence the nutritional and functional properties of edible insects [9]. For example, varied drying pretreatments have been investigated to enhance the final quality of the edible insects, extend their shelf life, and reduce overall energy consumption [10,11].

The majority of studies have been focused on yellow mealworms (*Tenebrio molitor* L.). In Europe, this insect species is currently approved for human consumption and holds great potential for industrial adoption and large-scale commercial production [12]. It has been stated that yellow mealworm larvae encompass a high amount of mono- and polyunsaturated fatty acids and almost all types of amino acids, especially those essential ones (199 g/kg d.m.), as well as fat (18–40% d.m.) and protein (18–64% d.m.) [2,12,13]. Scientific studies have emphasized the critical impact of thermal processing of yellow mealworms intended for human consumption. The industrial production of yellow mealworms requires optimized processing techniques, with drying being the first postharvest step that is crucial to the end product's quality [14]. Various drying technologies were used for investigating their effects on nutrient stability and possible lipid oxidation in yellow mealworms (*Tenebrio molitor* L.). Selaledi and Mabelebele [15] reported that sun-drying yellow mealworms resulted in the same nutritional quality as drying in the oven and by means of freeze-drying methods. Moreover, the process of freezing yellow mealworms followed by 20 s of blanching reduced microbial counts and inactivated degradative enzymes responsible for food poisoning and spoilage. Microwave drying may also be an appropriate alternative to freeze-drying. There were slight differences in the approximate composition of yellow mealworms processed by means of the compared techniques, and microwave drying resulted in a greater decrease in vitamin B_{12} content. Lipid oxidation was also lower in microwave-dried than in freeze-dried yellow mealworms [13]. Kröncke et al. [9] showed that drying in a vacuum oven and microwave drying could be an alternative to conventional freeze-drying for yellow mealworms. However, more in-depth knowledge of the changes in the quality of yellow mealworms in the course of processing is required to develop improved drying techniques that maintain the desired properties and reduce or eliminate undesirable ones.

Some studies have shown that the consumption of insects may be potentially risky because they may contain potential microbiological hazards (pathogens) or antinutrients and allergenic substances [2,7,8]. Safety aspects of edible insects may be guaranteed by heat treatments such as cooking, boiling, or processing. However, non-thermal technologies such as pulsed electric fields as well as additional hygiene practices should be considered to ensure the microbiological safety of edible insects during on-farm production, processing steps, and handling [5,16].

One of the problems is that edible insects may become contaminated by microorganisms such as bacteria and fungi, especially when present in their digestive tract. The genera of bacteria including *Salmonella*, *Escherichia*, *Enterococcus*, *Listeria*, *Staphylococcus*, *Bacillus*, or *Clostridium* [8,17–19] may be recognized in dried yellow mealworm larvae. Therefore, it is required to process insects before they are intended for human consumption. The most well-known traditional processing method is blanching. This method is related to treatment with hot water or steam, which allows for a sufficient reduction in the number of microorganisms. Previous studies have shown that the use of blanching may greatly reduce the microbial load of yellow mealworm larvae, even by 6.4 log CFU/g [17,20]. Furthermore, the study demonstrated by Cacchiarelli et al. [21] provided information about the reduction of microorganisms to a value below the detection limit. Pulsed electric fields (PEF), as an example of non-thermal technologies, may also be applied to decrease the number of

microorganisms liable for foodborne illnesses. The utilization of the PEF, which is based on the use of high-voltage electric pulses, may disturb the integrity of the cell walls as well as membranes of microorganisms, and as a result cause their death [22,23].

Therefore, the objective of this research has been to evaluate how PEF pretreatment at varied specific energy intake levels and drying methods (convective or infrared-convective) affect the selected nutritional properties and microbiological quality of yellow mealworm (*Tenebrio molitor* L.) larvae. The studies have aimed at assessing the effect of the PEF treatment and the drying methods on protein content, amino acid profile, total polyphenol content, antioxidant activity, and microbial load.

2. Materials and Methods

2.1. Material

The yellow mealworm (*Tenebrio molitor* L.) larvae were provided by a local producer (Cirwins, Kamień Duży, Poland). The material was stored in a tray filled with wheat bran at a temperature of 4 ± 1 °C and humidity of 80–90% until the experiment. Before proceeding, larvae were separated from wheat bran, rinsed under tap water, and then tapped with blotting paper.

2.2. Technological Treatments

2.2.1. Pulsed Electric Field (PEF) Treatment

For PEF treatment, the PEF Pilot™ Dual system (Elea GmbH, Quakenbrück, Germany), which generates peak voltage up to 30 kV and monopolar, near-rectangular pulses (frequency 2 Hz, pulse width 7 µs, pulse duration 40 µs) was used. In each experiment, about 350 ± 5 g of larvae were placed inside the treatment chamber, which was filled with tap water (σ = 220 µS/cm, t = 22 ± 1 °C) until the total mass of 1000 g was reached. The trials were carried out using an electric field strength of 1 kV/cm, and a specific energy intake (W_{spec}) at a level of 5, 20, and 40 kJ/kg was adjusted by applying electric pulses [16]. After treatment, the larvae were separated from the water, gently tapped with blotting paper, and then weighed. The treatment was carried out in at least three repetitions.

2.2.2. Convective Drying (CD)

The untreated and PEF-treated larvae were dried using the prototype laboratory dryer (Warsaw, Poland). The larvae, in amounts of 700 g, were placed as a layer on a sieve of 43 × 30 cm, receiving a load of 5.4 kg/m^2. The material was dried at a temperature of 90 °C and air velocity of 2 m/s until the constant weight of the material was reached. The dried material was gently taken and packed in air and light barrier PET12/AL8/PE100 bags (Pakmar, Warsaw, Poland). For each sample, the drying process was undertaken two times.

2.2.3. Infrared-Convective Drying (IR-CD)

The untreated and PEF-treated larvae were dried using the prototype laboratory dryer (Warsaw, Poland) equipped with nine lamps positioned 0.25 m from the surface of the material and emitting radiation at 7.9 kW/m^2. The larvae, in amounts of 700 g, were placed as a layer on a sieve of 43 × 30 cm, receiving a load of 5.4 kg/m^2. The material was dried at a temperature of 40 °C and air velocity of 0.8 m/s until the constant weight of the material was reached. The dried material was gently taken and packed in air and light barrier PET12/AL8/PE100 bags (Pakmar, Warsaw, Poland). For each sample, the drying process was carried out two times.

2.3. Fat Extraction Yield

For oil extraction yield determination, the Soxhlet method and a Behrotest ET2 Control Unit (Behr Labor-Technik GmbH, Düsseldorf, Germany) were used. Petroleum ether (Chempur, Piekary Śląskie, Poland) was used as a solvent and an extraction process was carried out for 6 h. The analysis was performed in duplicate for each sample [24].

2.4. Protein Content

For protein content determination, the Kjeldahl method and a KjelFlex K-360 apparatus (Büchi, Flawil, Switzerland) integrated with a TitroLine 5000 automatic titrator (SIAnalytics, Weilheim, Germany) were used. Protein content was calculated by multiplying a nitrogen concentration by a conversion factor of 4.76 [25]. The analysis was performed in duplicate for each sample.

2.5. Amino Acid Profile

The amino acid profile was analyzed following the method described previously by Smarzyński et al. [26], using an LC Agilent Technologies 1200 Rapid Resolution system equipped with a UV-Vis detector DAD 1260 and a reversed-phase Zorbax Eclipse Plus C18 column (4.6 × 150 mm, 5 µm) from Agilent Technologies (Santa Clara, CA, USA). Firstly, acidic hydrolysis was performed in a 6 M HCl solution (Honeywell, Germany) at 110 °C for 24 h. The amino acid content was determined by converting into derivatives of phenylisothiocyanate (PITC) with the addition of norleucine (500 nM) as an internal standard.

2.6. Chemical Analyses

2.6.1. Extract Preparation

For extraction preparation, 2 g of previously grounded sample (IKA A11 basic; IKA-Werke GmbH, Staufen, Germany) was mixed with 10 mL of 80% ethyl alcohol (Chempur, Piekary Śląskie, Poland) in the tube [27]. The extraction was performed in the dark for 12 h at a temperature of 20 °C using a shaker (Multi Reax, Heidolph Instruments, Schwabac, Germany). After that, the solution was centrifugated at 4350 rpm for 2 min using a laboratory centrifuge (MegaStar 600, VWR, Leuven, Belgium). For each sample, two independent extraction processes were carried out.

2.6.2. Total Polyphenol Content (TPC)

For total polyphenol content, the Folin–Ciocalteu spectrophotometric method was used. A sample extract of 10 µL, 10 µL of distilled water, and 5-fold diluted Folin–Ciocalteu reagent (Sigma Aldrich, Saint Louis, MO, USA) in amount of 40 µLs were mixed in the 96-well plates. Then, 250 µL of supersaturated sodium carbonate (Sigma Aldrich, Saint Louis, MO, USA) was added after 3 min to the wells, and the mixtures were further incubated for 60 min at 25 °C [28]. The absorbance of the reaction mixture was measured against the blank without the sample extract at 750 nm using a Multiskan Sky plate reader (Thermo Electron Co., Waltham, MA, USA). The calibration curve was prepared for chlorogenic acid (0–100 µg/mL, Sigma-Aldrich, Saint Louis, MO, USA). The results were given in mg of chlorogenic acid per 100 g d.m. The analysis was performed in two repetitions for each sample.

2.6.3. Antioxidant Activity

ABTS Assay

For antioxidant activity, the ABTS radical solution was made by dissolving ABTS (abcr GmbH, Karlsruhe, Germany) and potassium persulfate (Sigma Aldrich, Saint Louis, MO, USA) in distilled water and keeping for 16 h at 4 °C. Before analysis, the ABTS radical solution was diluted in 80% ethyl alcohol (Chempur, Piekary Śląskie, Poland) to achieve an absorbance value ranged from 0.680 to 0.720, measured using absorbance at 734 nm. Sample extracts of 10 µL and 250 µL of the radical working solution were mixed in the 96-well plates, and the mixtures were further incubated for 6 min at 25 °C [27]. The absorbance of the reaction mixture was measured using a Multiskan Sky plate reader (Thermo Electron Co., St. Louis, MO, USA) against the blank (without the sample extract). The antioxidant activity (the degree of radical scavenging in the presence of the antioxidant) was performed in duplicate for each sample. The results were given in mg of Trolox per 1 g d.m.

DPPH Assay

For antioxidant activity, the DPPH radical solution was made by dissolving DPPH (abcr GmbH, Karlsruhe, Germany) in methyl alcohol (Chempur, Piekary Śląskie, Poland) and storing in darkness for 24 h at 4 °C. Before analysis, the DPPH radical solution was diluted in 80% ethyl alcohol (Chempur, Piekary Śląskie, Poland) to achieve an absorbance value ranging from 0.680 to 0.720, measured using absorbance at 515 nm. Sample extracts of 10 µL and 250 µL of the radical working solution were mixed in the 96-well plates, and the mixtures were further incubated for 30 min at 25 °C [28]. The absorbance of the reaction mixture was measured using a Multiskan Sky plate reader (Thermo Electron Co., St. Louis, MO, USA) against the blank (without the sample extract). The antioxidant activity (the degree of radical scavenging in the presence of the antioxidant) was assessed in duplicate for each sample. The results were given in mg of Trolox per 1 g d.m.

2.7. Allergen Content

For allergen content, an ELISA assay [29] and crustacean and mollusk quantitative tests (Demeditec Diagnostics GmbH, Kiel, Germany) were used. The procedure involved extraction with a buffer, incubation in a water bath (15 min, 40 °C), centrifugation (10 min, 2000× *g*), filtration, and a quantity analysis of allergen content, as stated by the manufacturer's instructions. After analysis, the measurement of the reaction mixture absorbance was carried out using a plate reader (BioTek™ 800TS, Winooski, VT, USA) at a wavelength of 450 nm (reference wavelength 620 nm).

2.8. FTIR Analysis

For FTIR spectra measurement, a spectrometer model Cary 630 (Agilent Technologies Inc., Santa Clara, CA, USA) coupled with an ATR single diamond was used. The FTIR spectra determination was conducted with the following settings: wavelength 650–4000 cm^{-1}, resolution 4 cm^{-1}, 32 scan per sample. The collected spectra were processed by using MicroLab PC 5.7 software (Agilent Technologies Inc., Santa Clara, CA, USA).

2.9. Microbiological Analysis

Microbial analysis focused on total viable count (TVC), total yeast and mold count (TYMC), *Listeria monocytogenes* count, *Escherichia coli* count, *Staphylococcus aureus* count, and aerobic and anaerobic spore-forming bacteria count. Ten grams of sample (in triplicates) was weighed and homogenized in 90 mL 0.85% NaCl (Stomacher 400 Circulator, UK) for 5 min. TVC were enumerated on plate count agar (PCA, Biomaxima, Lublin, Poland) incubated at 30 °C for 72 h. TYMC were counted on Dichloran Rose Bengal Chloramphenicol (DRBC) agar for fresh insects or Dichloran Glicerol DG 18 agar for dried insects after incubation at 25 °C (Binder, Holzgerlingen, Germany) for 5 days. *E. coli*, *L. monocytogenes*, and *S. aureus* were counted on chromogenic agar (accordingly TBX, ALOA, Chapman; Biomaxima, Lublin, Poland), after incubation at 37 °C for 24 h. Bacterial endospores were enumerated on PCA medium or Wilson Blair (accordingly for aerobic or anaerobic spore-forming bacteria). Heat shocking sample dilution was performed for 20 min at 80 °C in a sterile tube. Plate with medium and samples were incubated at 30 °C for 48 h [30,31]. The number of microorganisms was counted (ProtoCOL 3, Synbiosis, Frederick, MD, USA) and determined in log CFU/g, while the limit of detection was 2.0 log CFU/g.

2.10. Statistical Analysis

For statistical analyses, STATISTICA 13.1 software (TIBCO Software, Palo Alto, CA, USA) was used. One-way analysis of variance (ANOVA) followed by Tukey's test with $\alpha = 0.05$ was performed to determine homogeneous groups. Two-way analysis of variance (ANOVA) was performed to indicate the significance of the type of treatment (drying method or specific energy intake of PEF). The average values with standard deviations (±SD) presented on the graphs were established using MS 356 Excel software (Microsoft, Redmond, WA, USA).

3. Results and Discussion

3.1. The Impact of PEF Pretreatment and Drying Methods on Fat Extraction Yield and Protein Content

The fat extraction yield of fresh larvae equaled 1.67 ± 0.06 g/100 g wet mass (w.m.), while dried yellow mealworm equaled 22.09 ± 0.07 and 16.01 ± 0.23 g/100 g d.m. for convective and infrared-convective samples, respectively. The two-factor ANOVA showed that the drying method (η^2 = 0.963), as well as the dose of the PEF energy intake (η^2 = 0.959), and the interaction between the dose of PEF energy intake and the drying method (η^2 = 0.970), affected the fat extraction yield. The fat extraction yield was in the range of 17.7–24.7 g/100 g d.m. (Figure 1a). The samples subjected to the PEF treatment resulted in a greater fat extraction yield for most of the analyzed samples. Higher extraction yields for the PEF-treated olives [32], sunflower seeds [33], and also insects [34] were provided in the related literature. What is more, for almost all of the convective-dried samples, a significantly higher fat extraction yield was recorded. The exception was a PEF-treated sample at 5 kJ/kg. The differences in the results obtained may be related to the varied composition of the insects studied, as well as structural changes (due to the electroporation phenomenon) and shrinkage in the course of drying. This may have caused the migration of fat globules to the tissue surface and subsequent loss [35,36]. This may be related to the varied distribution of fat in tissue or its binding to other components, which requires further research using nuclear magnetic resonance (NMR), scanning electron microscopy (SEM) or laser confocal scanning microscopy (LCSM).

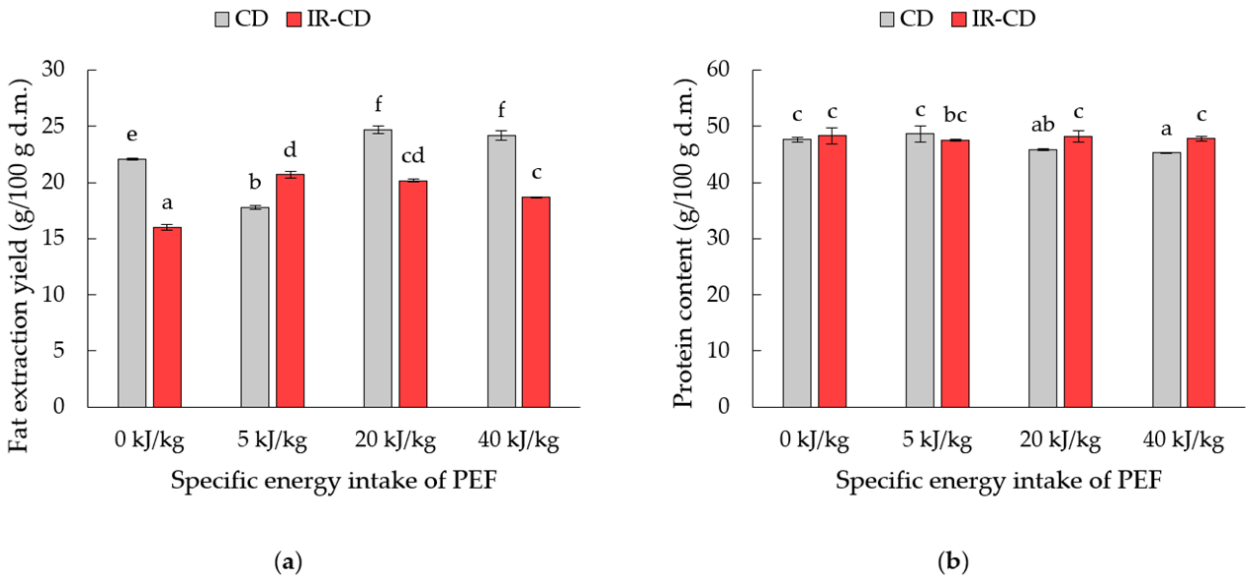

Figure 1. Effect of the PEF pretreatment and drying methods (CD—convective drying, IR-CD—infrared-convective drying) on (**a**) fat extraction yield and (**b**) protein content of yellow mealworm larvae. The different letters above columns denote statistical differences between samples ($p < 0.05$).

Protein is one of the most important macronutrients found in insects. The protein content assayed in fresh yellow mealworm equaled 15.45 ± 0.09 g/100 g w.m., while in dried yellow mealworm larvae content was comparable and equaled 47.5–48.7 g/100 g d.m. (Figure 1b). The two-factor ANOVA illustrated that the dose of the PEF energy intake (η^2 = 0.635), the drying method (η^2 = 0.569), and the interaction between the dose of the PEF energy intake and the drying method (η^2 = 0.699) affected protein content. The significant impact was noticed when the sample was PEF-treated at 20 and 40 kJ/kg before drying by means of the CD method. This significant decline in protein content may be connected to some structural changes, proteolysis, and reduced extractability due to the electroporation phenomenon and high temperature in the course of drying [9,34]. The protein content in insects after the PEF treatment was also elaborated upon in the related

literature. For instance, the study performed on yellow mealworm larvae showed that the PEF application at 20 kJ/kg prior to freeze-drying significantly decreased protein content by 5.5% as compared to an untreated sample [16]. Psarianos et al. [37] illustrated that the PEF-assisted extraction process of house cricket (*Acheta domesticus*) flour allowed the protein yield to increase, especially after PEF at 4.9 kJ/kg and an extraction time of 60 min.

3.2. The Impact of PEF Pretreatment and Drying Methods on Amino Acid Profile

The high protein content in insects is often indicated in the scientific literature. A nutritional perspective emphasizes the significance of both the protein content and the amino acid profile [38]. Significant differences in amino acid content levels were observed depending on the PEF energy and the drying method (Table 1). It was noticed that the use of the IR-CD method brought a lower content of individual amino acids, especially exogenously, than for samples obtained using the CD method, given the comparison of samples with the same PFE energy. Among the analyzed exogenous amino acids, the isoleucine content was the highest, followed by valine and leucine, which corresponds to the literature data on the amino acid profile of yellow mealworm larvae [39,40]. Other sources in the literature also indicate a high lysine content in yellow mealworm larvae [41], which was also observed in the samples analyzed in our research. Therefore, the obtained insects can be used for enriching food naturally low in the mentioned amino acids, e.g., cereal products that are low in lysine [42].

Table 1. Effect of the PEF pretreatment and drying methods (CD—convective drying, IR-CD—infrared-convective drying) on amino acids profile (mg/g of protein) of yellow mealworm larvae.

Amino Acid	PEF0_CD	PEF5_CD	PEF20_CD	PEF40_CD	PEF0_IR-CD	PEF5_IR-CD	PEF20_IR-CD	PEF40_IR-CD
Essential amino acids (EAAs)								
Phe *	18.80 ± 0.21 e [1]	14.72 ± 0.08 a	15.08 ± 0.45 ab	18.71 ± 0.29 e	17.24 ± 0.72 cde	16.40 ± 0.44 bc	17.00 ± 0.19 cd	18.21 ± 1.19 de
His	16.86 ± 0.03 d	13.93 ± 0.13 b	14.96 ± 0.49 c	16.72 ± 0.21 d	13.49 ± 0.07 b	12.48 ± 0.05 a	13.40 ± 0.16 b	14.18 ± 0.57 bc
Ile	36.99 ± 0.12 cd	23.76 ± 3.55 a	26.73 ± 0.12 abcd	40.13 ± 0.10 d	24.17 ± 0.16 abc	23.62 ± 0.49 abc	22.49 ± 1.59 ab	30.10 ± 0.04 bcd
Leu	30.22 ± 0.10 c	23.37 ± 1.32 ab	22.48 ± 1.89 ab	31.50 ± 0.44 c	21.82 ± 0.45 ab	20.07 ± 0.09 a	19.96 ± 2.39 a	25.13 ± 0.82 b
Lys	26.71 ± 0.01 cd	26.22 ± 0.07 c	24.99 ± 0.08 b	27.66 ± 0.24 d	24.66 ± 0.14 ab	23.81 ± 0.70 a	24.44 ± 0.65 ab	25.08 ± 0.08 b
Val	33.97 ± 0.13 d	21.85 ± 0.21 b	21.89 ± 0.28 b	34.66 ± 0.26 d	19.97 ± 0.34 a	19.68 ± 0.47 a	19.65 ± 1.19 a	25.45 ± 0.34 c
Met	6.38 ± 0.05 c	4.16 ± 0.12 ab	4.39 ± 0.04 ab	6.78 ± 0.11 c	3.78 ± 0.18 a	3.81 ± 0.14 ab	3.96 ± 0.25 ab	4.47 ± 0.58 b
Thr	21.64 ± 0.07 cd	20.43 ± 0.35 bc	23.46 ± 1.22 e	22.32 ± 0.30 de	18.97 ± 0.01 ab	18.15 ± 0.73 a	20.09 ± 0.42 bc	20.46 ± 0.12 bc
Non-essential amino acids (NEAAs)								
Asp	42.32 ± 0.04 b	36.31 ± 0.49 a	41.33 ± 2.16 b	43.00 ± 0.79 b	34.73 ± 0.41 a	34.21 ± 0.95 a	36.36 ± 1.03 a	35.40 ± 1.19 a
Ser	23.80 ± 0.12 cd	22.35 ± 0.77 bc	25.10 ± 1.14 de	25.47 ± 0.35 e	20.54 ± 0.42 a	20.51 ± 0.40 a	21.81 ± 0.46 ab	23.13 ± 0.31 bc
Glu	53.33 ± 0.19 bcd	40.44 ± 0.53 a	49.21 ± 4.14 b	56.86 ± 0.07 de	58.53 ± 0.27 e	54.74 ± 0.50 cde	51.82 ± 1.51 bc	53.29 ± 0.61 bcd
Pro	26.34 ± 0.16 b	32.41 ± 2.29 cd	27.61 ± 2.35 b	20.00 ± 0.14 a	35.52 ± 0.41 d	30.16 ± 0.57 bc	32.68 ± 2.44 cd	32.93 ± 1.21 cd
Gly	25.53 ± 0.09 d	21.15 ± 0.25 bc	22.63 ± 0.51 c	25.55 ± 0.41 d	20.43 ± 0.28 ab	18.87 ± 0.25 a	20.05 ± 0.95 ab	22.24 ± 1.14 c
Ala	32.65 ± 0.05 bc	27.81 ± 1.19 a	27.31 ± 0.93 a	35.78 ± 0.75 c	26.78 ± 0.15 a	27.11 ± 0.72 a	27.50 ± 1.05 a	31.70 ± 2.86 b
Cys	0.27 ± 0.00 d	0.16 ± 0.04 bc	0.21 ± 0.02 c	0.72 ± 0.01 e	0.08 ± 0.01 a	0.15 ± 0.01 b	0.05 ± 0.03 a	0.18 ± 0.01 bc
Tyr	30.90 ± 0.19 e	20.32 ± 0.04 ab	21.16 ± 0.49 bc	30.55 ± 0.12 e	21.30 ± 0.26 bc	19.47 ± 0.22 a	21.48 ± 0.31 c	23.84 ± 0.78 d
Arg	29.86 ± 0.14 e	22.58 ± 0.34 c	21.85 ± 40 bc	27.31 ± 0.15 d	22.14 ± 0.01 bc	20.11 ± 0.04 a	21.02 ± 0.20 ab	23.13 ± 1.27 c

* Essential amino acids (EAAs): Phenylalanine (Phe), Histidine (His), Isoleucine (Ile), Leucine (Leu), Lysine (Lys), Valine (Val), Methionine (Met), Threonine (Thr); Non-essential amino acids (NEAAs): Asparagine (Asp), Serine (Ser), Glutamic acid (Glu), Proline (Pro), Glycine (Gly), Alanine (Ala), Cysteine (Cys), Tyrosine (Tyr), Arginine (Arg). [1] The different letters in rows denote statistical differences between samples ($p < 0.05$).

3.3. The Impact of PEF Pretreatment and Drying Methods on Total Polyphenol Content (TPC) and Antioxidant Activity (AA)

Bioactive compounds such as polyphenols are important for human health. These compounds reduce oxidative stress and protect cells' exposure to reactive oxygen species (ROS) against damage or death [43]. In terms of food, bioactive compounds exhibit some of the most crucial properties: antioxidant and antimicrobial [43,44]. The raw material was characterized by a higher total polyphenol content (124.3 ± 0.4 mg chlorog. acid/100 g d.m.) than dried samples. The two-factor ANOVA showed that the dose of the PEF energy intake (η^2 = 0.957), the drying method (η^2 = 0.818), and the interaction between the dose of the PEF energy intake and the drying method (η^2 = 0.570) affected the total polyphenol content. There was no statistical difference in the TPC between the untreated samples dried by means of the CD and IR-CD methods (Figure 2). For PEF-treated samples, the noticed TPC for samples dried by means of the IR-CD method was significantly higher, regardless of the PEF energy intake. The insects subjected to the PEF at 40 kJ/kg were observed to present the highest TPC among all of the samples tested. Bioactive compounds are sensitive to high temperature [28,45], and therefore CD-dried insects exhibited a lower TPC, contrary to insects dried by means of the IR-CD method. Similar observations were found in the study performed on yellow mealworm larvae dried by means of convective and freeze-drying methods, in which a higher TPC was found in the freeze-dried insects [46]. Furthermore, the impact of the PEF pretreatment on dried yellow mealworm larvae was observed. The differences for those samples might be referred to the electroporation phenomenon and structural damage, resulting in greater solvent penetration and the availability of bioactive compounds.

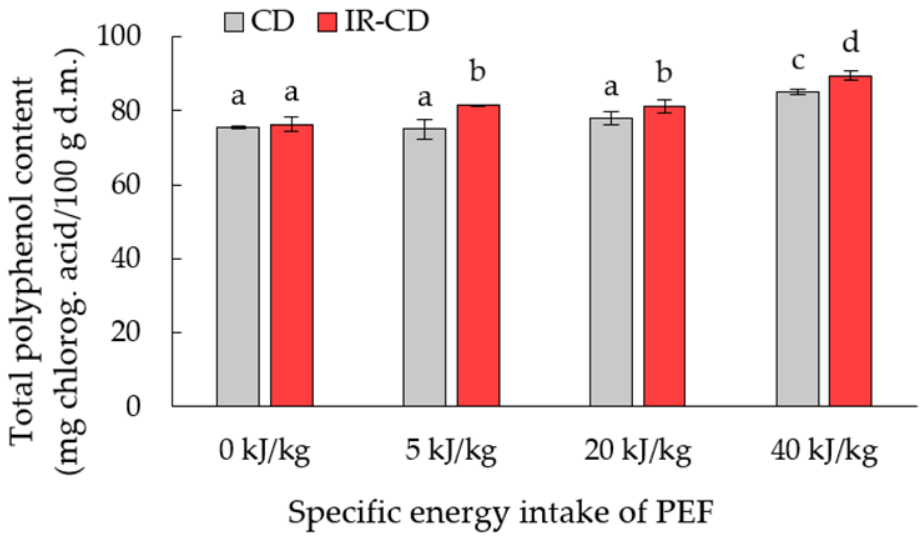

Figure 2. Effect of the PEF pretreatment and drying methods (CD—convective drying, IR-CD—infrared-convective drying) on total polyphenol content of yellow mealworm larvae. The different letters above columns denote statistical differences between samples ($p < 0.05$).

The antioxidant properties of edible insects are important in terms of incorporating them into foodstuff. In general, the outcomes obtained by the ABTS and DPPH assay method resulted in different antioxidant activity, and there the ABTS values were higher than the DPPH values (Figure 3). This was true both for the fresh and the dried materials. The antioxidant properties assayed in the fresh larvae equaled 9.0 ± 0.3 and 3.1 ± 0.3 mg Trolox/g d.m. for ABTS and DPPH, respectively, and were characterized by higher antioxidant properties compared to dried insects. However, the two-factor analysis showed that the specific energy intake of the PEF (ABTS: η^2 = 0.949, DPPH: η^2 = 0.984), as well as the drying method (ABTS: η^2 = 0.978, DPPH: η^2 = 0.828) and the interaction between the specific energy intake of the PEF and the drying method (ABTS: η^2 = 0.987,

DPPH: $\eta^2 = 0.977$), affected the antioxidant activity. Furthermore, the CD samples presented a higher antioxidant capacity as compared to the IR-CD samples, except for the sample treated with the PEF at 40 kJ/kg. Based on the results, it was noted that for the PEF-treated and CD samples, the ABTS values were significantly lower than those for the untreated sample and exhibited a decreasing trend with increasing PEF energy intake (Figure 3a). In turn, the DPPH test showed a significant increase for PEF-treated insects at 20 and 40 kJ/kg (Figure 3b). For the IR-CD samples, the ABTS results depended on the dose of the PEF energy intake. The significantly higher antioxidant activity values were found for the PEF-treated sample at 5 and 40 kJ/kg. The antioxidant activity was higher for CD insects than for the IR-CD insects. This is likely connected to a lower drying time and exposure to oxygen atmosphere and temperature or due to thermal treatment, which may cause the antioxidant activity to increase [44]. In addition, the use of the PEF and the initial high drying temperature may bring about the partial inactivation of exogenous enzymes needed for different chemical reactions, and therefore the demand for antioxidant compounds throughout the drying process, which results in higher antioxidant capacity as far as the dried matter is concerned [47]. Another explanation could be associated with protein content. Lower protein content in PEF-treated and CD-dried insects may result in a poorer antioxidant capacity. This is because some low molecular weight peptides may exhibit properties similar to those of antioxidant compounds [48,49].

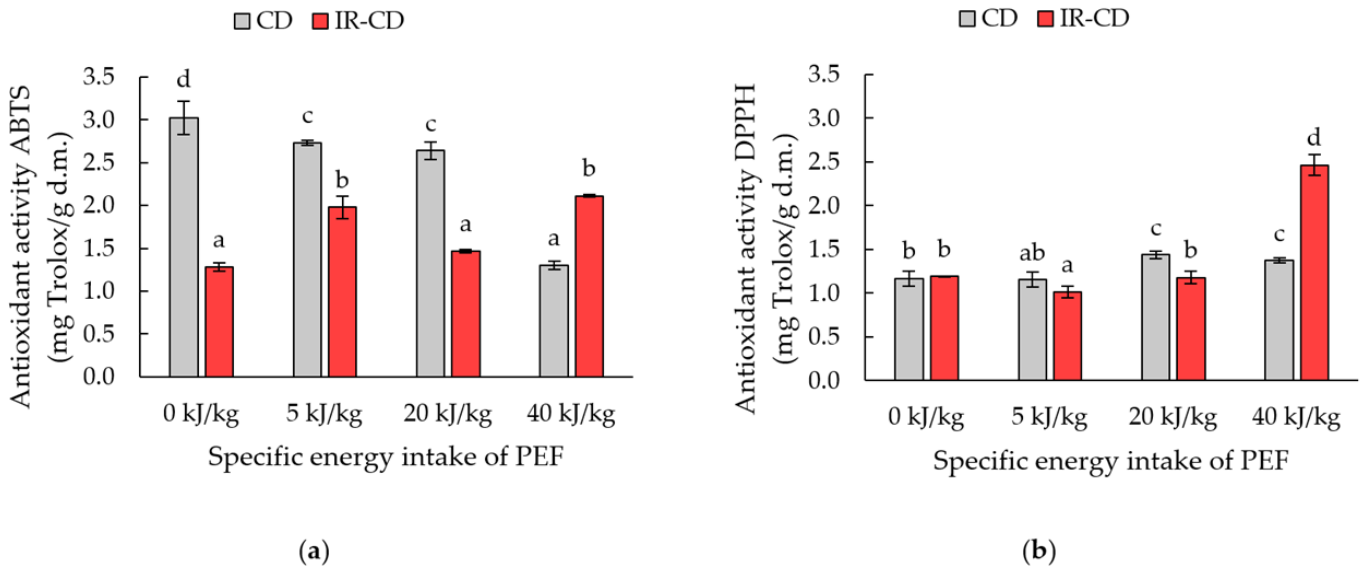

Figure 3. Effect of the PEF pretreatment and drying methods (CD—convective drying, IR-CD—infrared-convective drying) on antioxidant activity of yellow mealworm larvae against (**a**) ABTS radical, (**b**) DPPH radical. The different letters above columns denote statistical differences between samples ($p < 0.05$).

3.4. The Impact of PEF Pretreatment and Drying Methods on Allergen Content

Allergens are proteins that are not desirable in human nutrition because they can cause food allergies and various related symptoms [2]. Therefore, the presence of crustaceans and mollusks, possible allergens that may occur, was detected in dried yellow mealworm larvae prior to incorporating them into foodstuffs. Based on the results presented in Figure 4, it is plausible to conclude that the content of crustaceans was approximately 10 times higher than that of mollusks. This is probably due to documented cross-relationships that exist between crustaceans and edible insects [50,51].

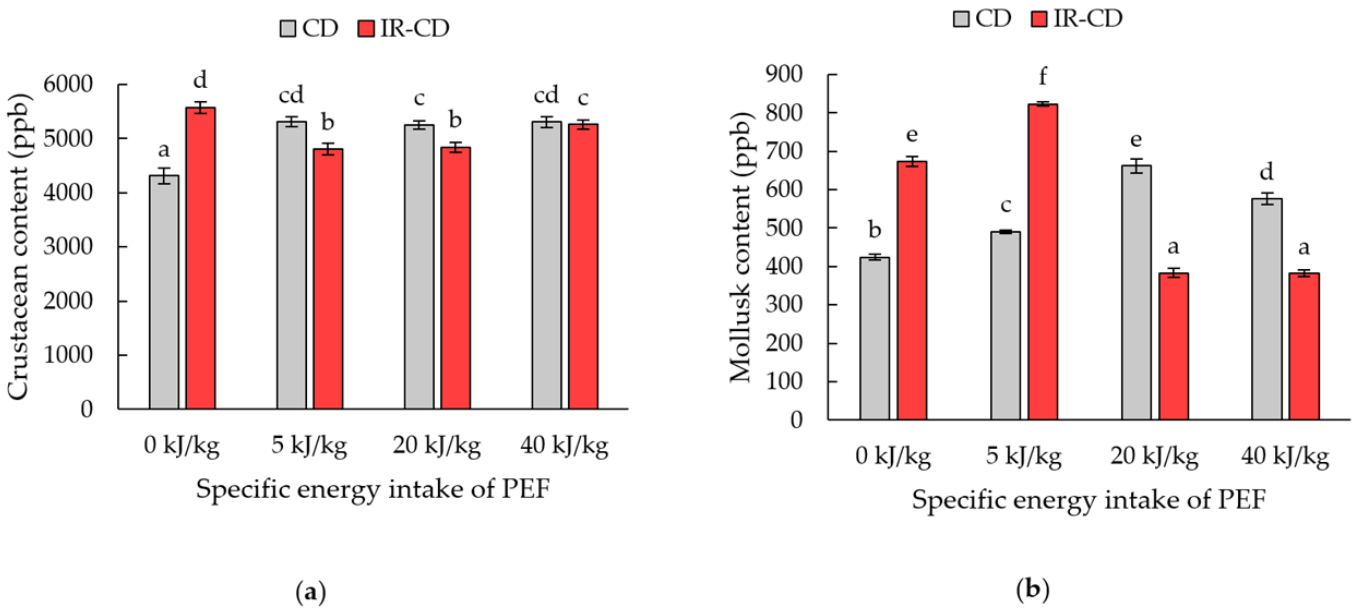

Figure 4. Effect of the PEF pretreatment and drying methods (CD—convective drying, IR-CD—infrared–convective drying) on (**a**) crustacean and (**b**) mollusk content of yellow mealworm larvae. The different letters above columns denote statistical differences between samples ($p < 0.05$).

The application of the PEF followed by convective drying caused a significant increment in crustacean content as compared to an untreated sample. The two-factor ANOVA also illustrated that the dose of the PEF energy intake (crustacean: $\eta^2 = 0.682$, mollusk: $\eta^2 = 0.981$) had a significant impact on both studied allergens' content. Samples subjected to the PEF treatment before the IR-CD drying method exhibited a significantly lower crustacean content as compared to untreated insects. This tendency was also similar in the case of mollusk content. The lower allergen content for PEF-treated and IR-CD dried insects was probably associated with destructive alterations in the secondary and tertiary structures of the molecules [52] that are related to the protein quality [53]. According the two-factor ANOVA, the drying method (mollusk: $\eta^2 = 0.688$), and the interaction between the dose of the PEF energy intake and the drying method (crustacean: $\eta^2 = 0.946$, mollusk: $\eta^2 = 0.995$) also affected the particular allergen content. By and large, studies on allergen content in insects are limited, which confirms the need for further research in this area.

3.5. The Impact of PEF Pretreatment and Drying Methods on FTIR Spectra

The FTIR spectra analysis was performed to assess the changes in the chemical structure of untreated and PEF-treated yellow mealworm larvae dried by means of diverse methods (Figure 5).

In the region of 3200–3300 cm^{-1}, weak stretching vibrations of the O–H bond related to water molecules, phenolic compounds, or amino acids [27,54] are visible, as well as weak stretching vibrations of the N–H bond of the amide A group [55]. Strong stretching vibrations of the C–H bond of the $–CH_2$ and $–CH_3$ alkyl groups are seen at around 2920 cm^{-1} and 2850 cm^{-1}, respectively. Moreover, a strong band at 2920 cm^{-1} may also indicate stretching vibrations of the C–H bond of the amide B group [54,56]. A medium band around 1730–1760 cm^{-1} is related to amide I [57], while strong bands at 1400–1560 cm^{-1} and 1250–1300 cm^{-1} may be identified as amide II and amide III, respectively [54,56]. Bioactive compounds such as polyphenols may be related to a strong peak around 1630 cm^{-1} [28]. Protein vitality (quality) and structure is determined mainly by amide I and amide II owning to the presence of a sensitive C=O bond [57,58]. Greater protein quality (demonstrated by a higher absorbance) was noticed for the sample dried by means of the CD method compared to the one dried by means of the IR-CD method. The PEF pretreatment affected the quality of dried yellow mealworm larvae. For the CD

method, it was found that the PEF-treated sample at 5 kJ/kg indicated the most damaged structure. The PEF-treated samples at 20 and 40 kJ/kg showed better quality, although nonetheless lower than the sample without PEF treatment (based on the absorbance of amide I C=O bond). For the IR-CD method, similar FTIR spectra were obtained when a dose of the PEF energy at 20 and 40 kJ/kg was applied, while the PEF-treated sample at 5 kJ/kg showed reduced absorbance. The untreated sample represented the highest structural damage (the lowest absorbance). Furthermore, similar FTIR spectra were obtained for samples marked as PEF20_CD, PEF40_CD, and PEF5_IR-CD. Thus, it may be concluded that the PEF pretreatment had a major impact on the protein structure and infrared light absorption.

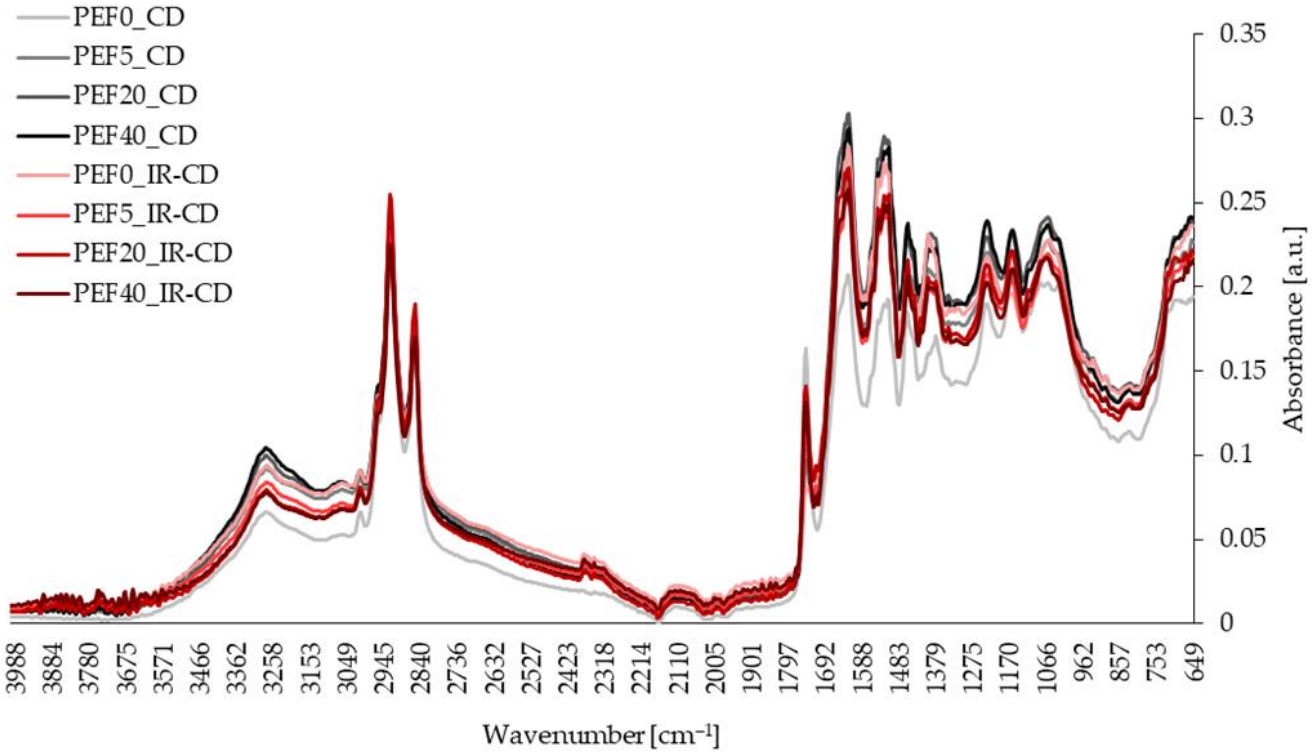

Figure 5. FTIR spectra of untreated and PEF-treated yellow mealworm larvae dried with convective (CD) and infrared-convective (IR-CD) methods.

3.6. The Impact of PEF Pretreatment and Drying Methods on Microbiological Quality

The microbiological quality of a product is one of the decisive factors determining whether a product is approved for consumption. The levels of permitted microbial contamination of yellow mealworm larvae are included in the European Union Regulation [59]. In conformity with this Regulation, the level of selected groups of microorganisms in dried larvae has been checked in our study. Table 2 shows the level of microbial contamination of dried yellow mealworm larvae as compared with larvae not subjected to heat treatment and PEF pretreatment.

Fresh larvae were characterized by high levels of total viable count (TVC) and total yeast and mold count (TYMC) as well as aerobic spore-forming bacteria, which exceeded the levels of microorganisms allowed in the regulation (5 log CFU/g for TVC, 2 log CFU/g for TYMC and aerobic spore-forming bacteria) [59]. The samples did not contain any contamination with waterborne and foodborne pathogenic bacteria, e.g., *L. monocytogenes*, *E. coli*, and *S. aureus*, as well as anaerobic spore-forming bacteria.

Due to the PEF pre-treatment and the drying process, the reduction in the count of microorganisms is noticeable. However, the method of drying has an impact on microorganism contamination, too. The infrared-convective dried samples were characterized by a lower microbial contamination than those dried only by means of the convective method.

The use of infrared-convective drying allowed for the reduction in the count of yeasts and molds below the detection level, and the combination of this technique with the PEF pretreatment in higher doses, e.g., 20 and 40 kJ/kg, reduced the TVC above three log cycles (antimicrobial effect was demonstrated), as well as reducing aerobic spore-forming bacteria below the detection level. Convective drying did not result in such a high reduction in the total viable count (reduction by 1.6 log cycles was noticed for the highest dose of the PEF pretreatment). To sum up, the use of the PEF pretreatment followed by IR-CD drying can alleviate the microorganism count, ensuring yellow mealworm safety.

Table 2. The changes in the microbial quality (log CFU/g) of fresh yellow mealworm larvae and PEF-treated and dried with convective (CD) and infrared-convective (IR-CD) methods.

Sample	Total Viable Count (TVC)	Total Yeast and Mold Count (TYMC)	*Listeria monocytogenes*	*Escherichia coli*	*Staphylococcus aureus*	Aerobic Spore-Forming Bacteria	Anaerobic Spore-Forming Bacteria
			Microbial count (log CFU/g)				
Fresh	6.9 ± 0.2	4.3 ± 0.2	<2.0	<2.0	<2.0	3.9 ± 0.1	<2.0
PEF0_CD	6.5 ± 0.1	4.0 ± 0.1	<2.0	<2.0	<2.0	3.5 ± 0.2	<2.0
PEF5_CD	6.3 ± 0.2	3.7 ± 0.0	<2.0	<2.0	<2.0	3.3 ± 0.1	<2.0
PEF20_CD	5.8 ± 0.1	2.9 ± 0.2	<2.0	<2.0	<2.0	2.5 ± 0.3	<2.0
PEF40_CD	5.3 ± 0.4	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
PEF0_IR-CD	5.2 ± 0.2	<2.0	<2.0	<2.0	<2.0	3.1 ± 0.2	<2.0
PEF5_IR-CD	4.8 ± 0.1	<2.0	<2.0	<2.0	<2.0	2.2 ± 0.3	<2.0
PEF20_IR-CD	3.8 ± 0.3	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
PEF40_IR-CD	3.6 ± 0.2	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0

Research on the microbiological safety of edible insect larvae is a frequent supplement to physical and chemical tests of those raw materials. It is stated that the microbiological quality of edible insects very often depends on the breeding conditions, so improving those conditions may significantly reduce the count of microorganisms in the final product. It has been shown that even slight contamination with *L. innocua* causes the bacteria to enter the larvae's intestines, where they can multiply and pose a health risk to consumers that cannot be removed by washing, and it is necessary to look for other methods of treating edible insect larvae [60,61]. In turn, contamination of feed with *Salmonella* below two log cycles does not cause those bacteria to enter the intestines of the larvae. This indicates a lower risk of transmission of such type of bacteria by insect larvae, which may be due to the natural microflora of yellow mealworm larvae and the ability to compete with pathogenic bacteria [19]. Other studies did not show the presence of *Salmonella* and *Listeria* as pathogens of high risk to consumers in live insect larvae [62]. The research conducted by Costa et al. [61] showed a total viable count of 7.7 log CFU/g, which indicated a slightly higher insect contamination than in our study. Furthermore, the authors did not detect the presence of *L. monocytogenes*, *E. coli*, and *S. aureus*, which was also found in our study. Similar results were obtained by Stoops et al. [63], in which the TVC was recorded at the level of 7.7–8.3 log CFU/g, and fungi at 5.2–5.7 log CFU/g. Contamination with spore-forming bacteria varied depending on the batch, which may have been related to the cultivation methods (e.g., access to soil may have increased the content of spore-forming bacteria). The high content of TVC, TYMC, and aerobic spore-forming bacteria indicates the need to use antimicrobial techniques, such as pre-treatment or drying. Previous studies have shown that a short heating step (1 min of boiling) could eliminate *Enterobacteriacea* from the larvae, reducing the TVC by more than three log cycles, while spore-forming bacteria remain in the product, unable to germinate [64]. Vandeweyer et al. [17] also obtained a reduction in TVC, fungi, and *Enterobacteriacea* after blanching yellow mealworm larvae, while the microwave drying of blanched larvae did not cause a significant reduction in spore-forming bacteria, which was achieved in our study after the PEF pretreatment was applied before drying [17]. Th genetic analysis of aerobic spore-forming bacteria indicates the frequent presence of *B. cereus*, *B. cytotoxicus*, and *B. thuringiensis* in edible insect larvae,

which may pose a danger to consumers by producing toxins, so reducing the number of those microorganisms and controlling their level is a key parameter for the approval for consumption of yellow mealworm larvae [65].

4. Conclusions

The results of the conducted study show that the utilization of the PEF applied before convective (CD) and infrared-convective (IR-CD) drying allows dried yellow mealworm larvae to be obtained with a high content of valuable compounds. The utilization of the PEF treatment before drying accounts for a higher fat extraction yield and total polyphenol content, as well as reduced microbiological contamination as compared to an untreated sample.

The PEF-treated and convective dried yellow mealworms exhibited a higher fat extraction yield and antioxidant properties measured against ABTS radicals. For yellow mealworms PEF-treated and dried by means of the IR-CD method, a higher total polyphenol content was observed. Moreover, larvae subjected to the highest energy input of the PEF (40 kJ/kg) before IR-CD drying exhibited significantly higher antioxidant activity (both ABTS and DPPH assay) compared to those dried by means of the CD method.

The protein content across the tested samples was comparable; however, a significantly lower content for larvae subjected to higher PEF energy input (20 and 40 kJ/kg) and dried by means of the CD method was recorded.

This study has also shown that PEF-treated and IR-CD yellow mealworms complied with the microbiological quality requirements for insects intended for food production. In the case of allergens, the crustacean content was up to 10 times higher than the mollusk content. It was also observed that insects treated by means of a lower PEF energy (0 and 5 kJ/kg) and CD method exhibited lower mollusk content, whereas a higher PEF energy (20 and 40 kJ/kg) and the CD method resulted in a higher mollusk content as compared to the content detected in the IR-CD samples. Thus, in order to explain all the changes occurring in protein and bioactive compound molecules throughout the yellow mealworm larvae processing by means of the convective and infrared-convective drying methods, further studies in detail are needed.

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