The Pulsed Electric Field Treatment Effect on Drying Kinetics and Chosen Quality Aspects of Freeze-Dried Black Soldier Fly (Hermetia illucens) and Yellow Mealworm (Tenebrio molitor) Larvae

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Abstract: Freeze drying is employed as one of the most effective preservation techniques, allowing dried material to be obtained characterized by high-quality properties. However, it also stands out as being one of the most energy-intensive methods and, consequently, expensive processes. Therefore, the objective of this study was to examine how the application of pulsed electric field (PEF) at 5 and 20 kJ/kg impacts the drying kinetics and the final quality of freeze-dried insects, including chemical composition, physical properties, and microbiological quality. For PEF-treated samples, a comparable content of protein (35.7–37.4 for H. illucens, 45.4–48.0 for T. molitor) to the untreated sample (35.8 for H. illucens and 48.0 for T. molitor) was noted. There were no significant distinctions found in the rehydration and hygroscopic characteristics across most tested samples. However, microtomography of freeze-dried H. illucens and T. molitor larvae unveils notable alterations in their internal structures influenced by both their species and the pre-treatment applied. The PEF-treated and freeze-dried H. illucens larvae exhibited a notably darker color (34.7–34.9) compared to the untreated sample (42.1), while a relatively consistent lightness for T. molitor larvae was observed. The performed study outlines that PEF treatment did not enhance the freeze-drying process of insect biomass and did not exhibit suitable microbiological quality for food purposes. Only fungi exhibited greater susceptibility to the effects of PEF treatment in comparison to bacteria, resulting in a reduction of 1.9 to 2.6 log cycles. Furthermore, PEF treatment did not negatively affect valuable compounds such as protein or fat.

Keywords: edible insects; pulsed electric field; freeze drying; chemical composition; structure; color; microbiological evaluation

1. Introduction

The possibility of using edible insects as a novel food resource is gaining interest. Eating insects, typically termed as entomophagy, is not a new concept of food consumption. Insects have been part of the daily diet of approximately 2 billion people worldwide for many years. They are especially eaten in Asia, Africa, as well as South America, while their consumption is still in its infancy in Western countries [1–4]. Nevertheless, some insect species are studied in terms of feed and food uses. Among them, the most common are yellow mealworm (Tenebrio molitor) and black soldier fly (Hermetia illucens). It is also worth...
noting that yellow mealworm (*Tenebrio molitor*) has been recently approved and listed as a novel food in the European Union.

Interest in insects is related to their chemical composition, which makes them a valuable source of many important nutrients [5–8]. They are valued for their content of valuable compounds such as protein and fat. According to the Commission Implementing Regulation (EU) 2022/169 of 8 February 2022, the content of protein and fat for blanched and freeze-dried *T. molitor* larvae should be in the range of 54.0–60.0% and 27.0–30.0%, respectively. Comparable results were provided, for example, by Krzyżaniak et al. [6], obtaining 47.1–53.3% of protein and 29.5–38.4% of fat, as well as by Kröncke et al. [9], who obtained 53.2% of protein and 27.7% of fat. Despite these advantages, insects can also be a source of microbiological hazards, including viruses, bacteria, parasites, or fungi [10–12]. However, pathogenic microorganisms can also be found primarily in the digestive tract of insects [13,14]. This issue is very crucial due to their potential contribution to the transmission of numerous diseases [12,15]. To date, studies of yellow mealworm (*Tenebrio molitor*) and black soldier fly (*Hermetia illucens*) indicate the presence of Enterobacteriaceae [16–20], Vibrio [17,21], Listeria [19,21–23], Salmonella [16,19,21,24], Staphylococcus [16,18,25,26], and spore-forming bacteria [11,21–23]. Nevertheless, microbiological data for fresh and processed *H. illucens* are limited, indicating the need for further research. Microbiological hazards raise doubts about the possibility of the widespread use of insects for food purposes. Therefore, appropriate processing beforehand is needed [27,28], such as blanching before drying as the European Union Regulations indicate. This is not unexpected, since thermal processing effectively reduces microbial load [6,18,27].

Freeze drying is a method of drying based on the sublimation process, i.e., that removes water from frozen material under reduced pressure and low temperature. The complexity of the lyophilization process (freezing, ice sublimation, and desorption) results in its lasting a long time, and thus it is energy- and cost-intensive [29,30]. Thus, the freeze-drying process can be enhanced by disrupting cellular structure using pretreatment methods. A traditional thermal method is blanching, but some unfavorable changes in texture, sensory profile, and nutritional value may occur [29]. Therefore, non-thermal techniques, such as pulsed electric field (PEF), can also induce the disruption of the cellular structure. The PEF treatment involves the application of a high-voltage electric field to disrupt the integrity of cell membranes. As a consequence, cell membranes undergo an electroporation phenomenon, which is associated with the formation of new membrane pores and the growth of existing ones. Thereby, its permeability increases and intensifies the mass and/or heat transfer processes [31,32]. For example, an enhanced freeze-drying time was reported for apple [33,34], potato [35], or basil leaves [32]. Moreover, reduced drying time was also observed for insects during convective drying [36,37] and infrared-convective drying [38]. On the other hand, PEF also may affect various physical properties of dried products like color or hygroscopicity. Generally, the hygroscopicity of PEF-treated samples is higher [39,40], which is not beneficial, as it negatively affects the stability of the product during storage or may promote microbial growth.

Based on the literature, the suggested processing with PEF and freeze drying appears as a highly promising technology for obtaining an insect biomass with good quality properties, especially considering the positive effect on its final quality in terms of chemical composition. Therefore, the objective of this study was to examine how PEF pretreatment at different energy levels prior to freeze drying affects the drying curves of black soldier fly (*Hermetia illucens*) and yellow mealworm (*Tenebrio molitor*) larvae. The analysis included the effect of the PEF treatment on the dry matter content, water activity, chemical composition, rehydration and hygroscopic properties, structure, color changes, and microbial quality.

2. Materials and Methods

2.1. Material

The materials for this research were purchased from a local German producer (Ahaus, Germany). The black soldier fly (*Hermetia illucens*) and yellow mealworm (*Tenebrio molitor*)
larvae were stored at a temperature of 4 ± 1 °C until the experiment. Before both PEF treatment and freeze drying, larvae were washed with tap water to remove residual feces and then tapped with the blotting paper. The moisture content of fresh *H. illucens* and *T. molitor* larvae was 63.4 ± 1.3 and 67.0 ± 0.1%, respectively.

2.2. Pulsed Electric Field Treatment (PEF)

The PEF treatment was investigated using the PEF Pilot™ Dual system (Elea GmbH, Quakenbrück, Germany), equipped with a system-generated peak voltage up to 30 kV with monopolar, near-rectangular pulses (pulse width of 7 µs, pulse duration of 40 µs, frequency of 2 Hz). In each experiment, about 100 ± 5 g of larvae were placed inside the treatment chamber, and tap water (with a conductivity of \( \sigma = 220 \mu S/cm \) and a temperature of 22 ± 1 °C) was added, bringing the total mass up to 1000 g. The trials were carried out using electric field strength of 1 kV/cm and a specific energy intake (\( W_{\text{spec}} \)) of 5 and 20 kJ/kg, which was adjusted by applying a specific number of pulses (approx. 500 and 2000 pulses). Thus, the PEF treatment took ca. 2 and 5 min. After treatment, the larvae were separated from the water, gently dried using filter paper, and then weighed. The calculation of specific energy intake (\( W_{\text{spec}} \)) and electric field strength (\( E \)) was conducted using Equations (1) and (2) as presented below [41]:

\[
W_{\text{spec}} = \frac{U^2 C n}{2m} \frac{IU t n}{1000 m'},
\]

(1)

\[
E = \frac{U}{d},
\]

(2)

where \( n \) is the number of pulses (–); \( m \) is the mass of the treated samples (kg); \( U \) is the voltage (kV); \( C \) is the capacitance (\( \mu F \)); \( I \) is the current (A); \( t \) is the width of the pulse (s); \( d \) is the distance between electrodes (cm).

2.3. Freeze Drying (FD)

Before freeze drying, larvae were placed on a petri dish and frozen at a temperature of −40 °C (Shock Freezer HCM 51.20; Irinox, Treviso, Italy) for 5 h. Freeze drying was performed using a Gamma 1–16 LSC laboratory dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). The drying process was started immediately after putting the samples into the dryer chamber and with the following parameters: a shelf temperature of 40 °C, a condenser temperature of −55 °C, and a pressure of 63 Pa. During the freeze-drying process, mass changes and temperature of the samples were registered continuously each 5 min during the first 120 min and each 15 min until the end of the process. Throughout the freeze-drying process, alterations in mass and temperature of the samples were recorded at 30-min intervals. The drying apparatus was integrated with a system for recording mass and temperature (SWL0125; Mensor, Warsaw, Poland), positioned within the drying chamber and linked to a custom-designed scale situated outside the chamber. The drying procedure was executed and monitored until the material achieved a constant mass.

The relative moisture ratio (MR) of larvae during the freeze-drying process was determined using Equation (3) [38]:

\[
\text{MR} = \frac{u_T}{u_0},
\]

(3)

where \( u_T \)—moisture content at each moment of the process (kg water/kg d.m.); \( u_0 \)—initial moisture content (kg water/kg d.m.).
2.4. Physical Properties

2.4.1. Water Activity

Water activity measurement was performed using the calibrated instrument HygroLab C1 (Rotronic, Bassersdorf, Switzerland) at 25 ± 1 °C at least in triplicate.

2.4.2. Rehydration Properties

The rehydration properties were determined in a 100 cm$^3$ beaker filled with distilled water at 22 ± 1 °C [38] at least in triplicate. The dried insects were weighed and immersed for 3 h, after that separated from the water, blotted with the filter paper, and weighed again. In the rehydrated samples, the dry matter content was also measured. Based on obtained results, the rehydration rate (RR) and soluble solid loss (SSL) were calculated according to Equations (4) and (5):

\[
RR = \frac{m_r}{m_0},
\]

\[
SSL = \frac{m_rdm_r}{m_0dm_0},
\]

where $m_r$—mass of sample after 3 h of rehydration (g); $m_0$—initial mass of a dried sample (g); $dm_r$—dry matter content of a sample after 3 h of rehydration (%); and $dm_0$—dry matter content of dried sample before rehydration (%).

2.4.3. Hygroscopic Properties

The hygroscopicity was determined after 1, 2, 3, 6, 9, 12, 24, 48, and 72 h [38] at least in triplicate. The dried insects were weighed and placed in the desiccator over distilled water (water activity of 1.0, temperature of 22 ± 1 °C). Based on obtained results, the hygroscopic properties after each moisture adsorption time were calculated using Equation (6):

\[
H = \frac{m_r}{m_0},
\]

where $m_r$—mass of sample after moisture adsorption time (g); and $m_0$—initial mass of a dried sample (g).

2.4.4. Color

Color parameters were measured with a Konica Minolta CR-5 chromameter (Konica Minolta, Osaka, Japan) [42] using at least 15 repetitions. During the measurements, D65 standard illuminant light source, 8° angle of viewing, CIE 2° Standard Observer, and a 30 mm measuring diameter were set. Additionally, based on the obtained L*, a*, and b* color parameter, the total color difference ($\Delta E$) was calculated using Equation (7):

\[
\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2},
\]

where L*—lightness (0–100), a*—chromatic parameter (redness-greenness) and b*—chromatic parameter (yellowness-blueness); $\Delta L^*$, $\Delta a^*$, $\Delta b^*$—the differences between L*, a* and b* measured for freeze-dried and raw edible insects.

2.5. Chemical Composition

2.5.1. Moisture Content and Dry Matter Content

The moisture content was examined using the oven method [43] at least in triplicate. Approximately 1 g of ground sample was dried at a temperature of 105 °C in a laboratory dryer SUP 65 W/G (Wamed, Warsaw, Poland) for 17 h until a constant weight was achieved. Then, the samples were cooled within a desiccator and then weighed.
2.5.2. Protein Content

The protein content was examined using the Kjeldahl method provided by Lenaerts et al. [43] with small modifications. Approximately 50 mg of ground sample was mineralized in a medium of sulfuric acid with the addition of Kjeltabs CT/3.5 as a catalyst. After mineralization, distilled water and 33% sodium hydroxide solution were added, and the liberated ammonia was distilled into excess 4% boric acid solution using a KjelFlex K-360 (Büchi, Flawil, Switzerland) apparatus. The nitrogen content was determined by titration with 0.1 M hydrochloric acid solution using a TitroLine 5000 automatic titrator (SIAnalytics, Weilheim, Germany). To determine crude protein content, the measured nitrogen concentration was multiplied by a conversion factor of 4.76 [44].

2.5.3. Fat Content

The fat content was examined using the Soxhlet method described by Kröncke et al. [9] with some modifications. Approximately 10 g of ground sample was moved to extraction thimbles and covered with cotton wool. The extraction procedure was carried out using petroleum ether as a solvent for 6 h at a temperature of 90 °C in the Soxhlet apparatus unit (Behr Labor-Technik GmbH, Düsseldorf, Germany). After extraction, the residual solvent was evaporated at a temperature of 105 °C in a laboratory dryer SUP 65 W/G (Wamed, Warsaw, Poland). Then, the extracted fat was cooled within a desiccator and its weight was measured.

2.5.4. Ash Content

The ash content was examined using the muffle oven method [43] at least in triplicate. Approximately 1 g of ground sample was subjected to complete incineration in a muffle furnace (SX4-12-12; Chemland, Stargard, Poland) at a temperature of 550 °C until a constant weight was reached [33]. Once the incineration process was completed, the sample was cooled within a desiccator and weighed.

2.5.5. FTIR Analysis

The dried insects were subjected to FTIR analysis using the Cary 630 instrument (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a single reflection diamond attenuated total reflection (ATR) setup [45]. The examination was carried out over a wavelength range spanning from 650 to 4000 cm\(^{-1}\), employing a resolution of 4 cm\(^{-1}\) and collecting 32 scan readings for the spectral analysis. To achieve this, the dried sample was firmly pressed onto the crystal utilizing a pressure clamp. Each specimen underwent 5 separate scans. The analytical data were captured and managed through the MicroLab FTIR software (version 5.7).

2.6. Structure with \(\mu\)Tomography

Internal structural determinations were conducted on freeze-dried worm samples using the micro CT Skyscan 1272 system (Bruker, Kontich, Belgium) [41]. The system fulfills two main roles: data acquisition and image reconstruction. Throughout the analysis, the X-ray source voltage was configured to 40 kV alongside a current of 193 \(\mu\)A. Scans were executed using a rotation step of 0.3° and a resolution of 25 \(\mu\)m. For the creation of 3D images from the \(\mu\)CT projections, the NRecon software (version 2.0, Bruker, Kontich, Belgium) was utilized for image reconstruction. In the course of this process, adjustments were applied to rectify beam hardening and ring artifacts, thereby improving image quality.

2.7. Microbiological Assessment

Microbiological quality of fresh and freeze-dried larvae included total viable count (TVC) on plate count agar (PCA, Biomaxima, Poland) medium incubated at 30 ± 1 °C for 48 h, total fungi count on dichloran rose Bengal chloramphenicol agar ((DRBC); Biomaxima, Lublin, Poland) medium incubated at 28 ± 1 °C for 5 days, number of spore-forming bacteria (after heat shocking of ten-fold diluted sample for 20 min at 80 °C) on plate count
agiar (PCA; Biomaxima, Lublin, Poland) medium incubated at 30 ± 1 °C for 48 h. The number of microorganisms was counted and then expressed as log CFU/g [16,46].

2.8. Statistical Analysis

The one-way analysis of variance (ANOVA) followed by Tukey’s test with α = 0.05 to group into homogeneous groups using STATISTICA 13.1 software (TIBCO Software, Palo Alto, CA, USA). Average values with standard deviations (±SD) were performed using MS Excel software (version 2019, Microsoft, Redmond, WA, USA).

3. Results and Discussion

3.1. Freeze-Drying Process of Edible Insects Larvae

The drying kinetics of untreated and PEF pre-treated H. illucens and T. molitor larvae during freeze drying (FD) are presented in Figure 1. Regarding to the course of the drying curves, it may be stated that the applied PEF treatment influenced the kinetics of the drying process. The freeze drying of the untreated larvae to reach a relative moisture content of MR = 0.1 lasted 5160 and 1245 min for H. illucens and T. molitor, respectively (Table 1). Saucier et al. [19] have reported that H. illucens larvae punctured on the surface prior to freeze drying were dried faster. As the authors observed, the time of the freeze-drying process declined equal to 66.7% in comparison to the untreated sample. The effect of puncturing the surface can be compared to PEF pretreatment, with the formation of electrically induced pores on the disintegrated cell membrane. However, drying of H. illucens larvae exposed to PEF treatment at an energy intake of 5 kJ/kg took 7320 min, that is, 41.9% longer than the untreated sample. A possible explanation could be related to the greater leakage of water during PEF treatment and then its low content in the material undergoing drying. Furthermore, some water could be strongly bound by other compounds (e.g., protein). This is evidenced by the mild drying curve at the initial stage (Figure 1) and also the final moisture content, as the typical course of the drying curve at the initial stage is sharp, indicating a high moisture content [47].

![Freeze-drying kinetics of untreated (0 kJ/kg) and PEF-treated (5 and 20 kJ/kg) H. illucens and T. molitor larvae.](image)

**Figure 1.** Freeze-drying kinetics of untreated (0 kJ/kg) and PEF-treated (5 and 20 kJ/kg) H. illucens and T. molitor larvae.
Table 1. Drying time, dry matter content, and water activity of freeze-dried untreated (0 kJ/kg) and PEF-treated (5 and 20 kJ/kg) H. illucens and T. molitor larvae.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Drying Time to MR = 0.1 (min)</th>
<th>Dry Matter Content (%)</th>
<th>Water Activity (−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. illucens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kJ/kg</td>
<td>5160 ± 467 a, *</td>
<td>83.2 ± 2.3 a</td>
<td>0.165 ± 0.008 a</td>
</tr>
<tr>
<td>5 kJ/kg</td>
<td>7320 ± 85 b</td>
<td>87.5 ± 0.5 b</td>
<td>0.232 ± 0.008 b</td>
</tr>
<tr>
<td>20 kJ/kg</td>
<td>5175 ± 191 a</td>
<td>83.2 ± 2.0 a</td>
<td>0.405 ± 0.001 c</td>
</tr>
<tr>
<td>T. molitor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kJ/kg</td>
<td>1245 ± 21 B</td>
<td>93.3 ± 0.2 A</td>
<td>0.145 ± 0.001 B</td>
</tr>
<tr>
<td>5 kJ/kg</td>
<td>1245 ± 21 B</td>
<td>95.8 ± 1.0 B</td>
<td>0.023 ± 0.002 A</td>
</tr>
<tr>
<td>20 kJ/kg</td>
<td>1200 ± 0 A</td>
<td>95.8 ± 0.1 B</td>
<td>0.023 ± 0.002 A</td>
</tr>
</tbody>
</table>

* The same letters in columns (lowercase for H. illucens larvae, uppercase for T. molitor larvae) denote the same homogeneous groups, which do not differ statistically (p < 0.05).

For T. molitor larvae, the results showed that only the samples subjected to the PEF with energy intake of 20 kJ/kg demonstrated a reduction in drying time equal to 45 min in comparison to the other ones. The decrease in the drying time of samples treated with PEF can be attributed to the phenomenon of electroporation affecting the cell membranes within the insect’s body; therefore, undergoing a loss of their partial permeability, thereby aiding in the release of water through the acceleration of its diffusion. During PEF treatment, the cell membranes are losing their semi-permeability, which leads to an enhancement of mass and heat transfer [38,41]. Following the permeabilization of the insect body, water molecules exhibit increased mobility through the pores, leading to heightened material porosity. This, in turn, reduces the time required for water molecules to reach the tissue surface via capillaries, resulting in an accelerated drying process [48]. It is worth emphasizing that drying time is not always reduced, especially when the specific energy intake is too high and the conductivity does not increase, which means the samples are oversaturated with electroporation, and thus did not result in shorter drying time. Furthermore, during the drying process, the tissue collapses due to larger PEF-induced disintegration of the cell membrane [36]. Based on the findings from this study, it can be concluded that the decrease in drying time was significantly influenced by both the specific energy intake of PEF pretreatment and the type of insect species.

3.2. Physical Properties of Freeze-Dried Edible Insects Larvae

3.2.1. Dry Matter Content and Water Activity of Freeze-Dried Edible Insects Larvae

The dry matter content in the dried larvae, was in the range of 83.2 to 95.8% (Table 1). The use of PEF treatment before freeze drying of H. illucens and T. molitor larvae caused significant changes in the content of dry matter. The samples subjected to PEF were characterized by higher dry matter content. Only H. illucens larvae treated with PEF at an energy intake of 20 kJ/kg had the same dry matter content as an untreated one. Changes in the dry matter content may be related to dry matter components leakage into the environment [49]. Also, such behavior can be attributed to the phenomenon that PEF treatment is capable of inducing irreversible electroporation. This, in turn, results in an increase in material porosity and facilitates the process of leaching, ultimately promoting the diffusion of water and dry matter compounds from the tissue into the surrounding environment [50].

The water activity of the freeze-dried insect larvae was in the range of 0.023 to 0.405 (Table 1). The increase in specific energy intake during H. illucens larvae treatment caused an increase in the water activity of the samples, while for T. molitor the reversed course was observed. The PEF pre-treated samples of T. molitor larvae showed a water activity below 0.1. It was also noted that the higher dry matter content in the dried larvae resulted in lower water activity. The elevated dry matter content and correspondingly low water activity in the dried products contribute to their favorable storage characteristics [51], because it
serves as a factor influencing numerous biochemical processes in food, particularly those instigated by the microbial growth of microorganisms. By maintaining the water activity below 0.6, the growth of microorganisms is inhibited, ensuring the physical and chemical stability of the product during storage [43,49]. Freeze-dried samples in this study obtained water activity below the value of 0.6, which indicates product stability.

3.2.2. Rehydration Rate and Hygroscopic Properties of Freeze-Dried Edible Insects Larvae

The rehydration attributes of freeze-dried insects are summarized in Table 2. Rehydration is a process opposite to the drying process, and it is an important indicator of the quality of the dried products. The primary goal of the rehydration is to obtain a product similar to those before the drying process. During rehydration, water permeates the dried tissue, while soluble solid compounds migrate into the water, leading to an expansion in the weight and volume of the rehydrated tissue. The structural transformations that occur in tissue during both drying and pretreatment exert an influence on its capacity to bind water, consequently affecting the restoration of the original material volume [42,52]. Despite the lack of significant differences, the use of PEF pretreatment slightly increased the rehydration rate of the dried product samples (Table 2), with the exception of T. molitor larvae which were treated at an energy intake of 5 kJ/kg. PEF treatment can cause various tissue changes, e.g., an increase in the number of pores, and thus allow better solvent penetration or a modified chitin fraction and protein structure forming the exoskeleton of insects [53]. As Khatun et al. [54] explains, at very low water activity, the lipid oxidation process can increase, which primarily affects protein oxidation and thus decrease its solubility, which in turn enhances its ability to bind water [9,55]. Connected with rehydration is also the loss of the water-soluble (SSL) compounds of the dry matter. The results for SSL loss after 3 h provided that the influence of PEF pretreatment, both for H. illucens and T. molitor larvae, was not significant (Table 2).

<table>
<thead>
<tr>
<th>Sample</th>
<th>RR [-]</th>
<th>SSL [-]</th>
<th>H [-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. illucens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kJ/kg</td>
<td>1.26 ± 0.04</td>
<td>1.13 ± 0.01</td>
<td>1.31 ± 0.07</td>
</tr>
<tr>
<td>5 kJ/kg</td>
<td>1.25 ± 0.01</td>
<td>1.10 ± 0.01</td>
<td>1.44 ± 0.02</td>
</tr>
<tr>
<td>20 kJ/kg</td>
<td>1.35 ± 0.15</td>
<td>1.13 ± 0.01</td>
<td>1.48 ± 0.06</td>
</tr>
<tr>
<td>T. molitor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kJ/kg</td>
<td>1.52 ± 0.35</td>
<td>1.06 ± 0.01</td>
<td>1.73 ± 0.07</td>
</tr>
<tr>
<td>5 kJ/kg</td>
<td>1.46 ± 0.25</td>
<td>1.04 ± 0.01</td>
<td>1.73 ± 0.05</td>
</tr>
<tr>
<td>20 kJ/kg</td>
<td>1.76 ± 0.18</td>
<td>1.05 ± 0.02</td>
<td>1.89 ± 0.14</td>
</tr>
</tbody>
</table>

* The same letters in columns (lowercase for H. illucens larvae, uppercase for T. molitor larvae) denote the same homogeneous groups, which do not differ statistically (p < 0.05).

The hygroscopic properties of freeze-dried H. illucens and T. molitor larvae are shown in Figure 2 and in Table 2 (which presents the results of hygroscopicity after 72 h). The PEF-treated insects more intensively adsorbed water vapor before freeze drying (Figure 2a). The pretreated samples presented similar or higher hygroscopicity than the untreated ones. In the case of H. illucens larvae, it was stated that during the adsorption time the pattern of changes for PEF-treated samples was similar, i.e., samples treated by PEF showed a statistically higher hygroscopicity than untreated samples. Meanwhile, for T. molitor larvae, the effect of pre-treatment on hygroscopicity was found only for the sample subjected to 20 kJ/kg, which showed the highest hygroscopic properties throughout the measurement (Figure 2b). Even though after 72 h the hygroscopicity for this sample was higher by 8.4% in comparison to untreated sample, the changes were still not statistically significant (Table 2). Changes in the hygroscopicity may be related to the electroporation phenomenon, which may lead to cellular structural disruption and the release of certain polar compounds into
the surrounding environment. Furthermore, some changes in the structure of biopolymers related to water binding or also some still unrecognized interactions between the insects’ chemical components [38] may have influenced it.

Figure 2. Hygroscopic kinetics of freeze-dried untreated (0 kJ/kg) and PEF-treated (5 and 20 kJ/kg) (a) H. illucens and (b) T. molitor larvae.

3.2.3. Color of Freeze-Dried Edible Insects Larvae

The L*, a*, b* color parameters, and total color difference (ΔE) between fresh and freeze-dried edible insects are presented in Table 3. The changes in the color parameters were dependent on the insect species and pretreatment application. The L*, a*, b* color parameters for fresh H. illucens larvae were 39.4 ± 0.8, 7.9 ± 0.8, and 24.0 ± 0.2, respectively, while for T. molitor larvae they were 39.9 ± 1.7, 3.1 ± 0.2, and 17.1 ± 1.0, respectively.

Table 3. The color parameters (L*, a*, b*), and total color difference (ΔE, in comparison to raw material) of fresh and freeze-dried untreated (0 kJ/kg) and PEF-treated (5 and 20 kJ/kg) H. illucens and T. molitor larvae.

<table>
<thead>
<tr>
<th>Sample</th>
<th>L* [-]</th>
<th>a* [-]</th>
<th>b* [-]</th>
<th>ΔE [-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. illucens</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>39.4 ± 0.8 b</td>
<td>7.9 ± 0.8 c</td>
<td>24.0 ± 0.2 c</td>
<td>-</td>
</tr>
<tr>
<td>0 kJ/kg</td>
<td>42.1 ± 1.3 c</td>
<td>5.6 ± 0.3 b</td>
<td>17.8 ± 0.7 b</td>
<td>7.3</td>
</tr>
<tr>
<td>5 kJ/kg</td>
<td>34.9 ± 2.2 a</td>
<td>4.8 ± 0.4 a</td>
<td>15.1 ± 1.1 a</td>
<td>10.6</td>
</tr>
<tr>
<td>20 kJ/kg</td>
<td>34.7 ± 1.0 a</td>
<td>4.5 ± 0.3 a</td>
<td>14.9 ± 0.7 a</td>
<td>10.8</td>
</tr>
<tr>
<td>T. molitor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>33.8 ± 0.8 A</td>
<td>10.8 ± 0.5 C</td>
<td>20.7 ± 0.6 C</td>
<td>10.5</td>
</tr>
<tr>
<td>5 kJ/kg</td>
<td>33.5 ± 0.6 A</td>
<td>9.7 ± 0.3 B</td>
<td>19.4 ± 0.5 B</td>
<td>9.5</td>
</tr>
<tr>
<td>20 kJ/kg</td>
<td>34.3 ± 0.3 A</td>
<td>9.4 ± 0.3 B</td>
<td>18.9 ± 0.4 B</td>
<td>8.6</td>
</tr>
</tbody>
</table>

* The same letters in columns (lowercase for H. illucens larvae, uppercase for T. molitor larvae) denote the same homogeneous groups, which do not differ statistically (p < 0.05).

The lightness (L*) of freeze-dried insects ranged from 34.7 to 42.1 and from 33.5 to 34.3 for H. illucens and T. molitor larvae, respectively. The utilization of PEF prior to drying affected the color parameters of the freeze-dried material. For H. illucens larvae, the lightness was significantly lower than for untreated dried insects, while for T. molitor larvae treated by PEF at an energy intake of 20 kJ/kg, the L* value was slightly higher. The higher L* value can be explained by irreversible electroporation and a greater number of pores, which may promote the transfer of fat globules to the surface of insect tissue [36].
Furthermore, this may also be related to the measurement procedure, which is based on measuring the reflected beams of light from the sample surface. Dried edible insects have a lower moisture content, so light was reflected differently from the dry and porous surface, and therefore these samples are probably characterized by a higher lightness [56]. In turn, for *H. illucens* larvae, a lower lightness during measurement may be associated with the greater porosity of the dried material and higher light retention inside its tissue [41].

The dried insects were characterized by an *a*⁺ color parameter from 4.5 to 5.6 (*H. illucens* larvae) and from 9.4 to 10.8 (*T. molitor* larvae). Regardless of insect species, increasing the PEF specific energy intake significantly decreased the *a*⁺ values. In turn, the values of the *b*⁺ color parameter of the dried insects ranged from 14.9 to 17.8 and from 18.9 to 20.7 for *H. illucens* and *T. molitor* larvae, respectively. It was observed that the *a*⁺ and *b*⁺ color parameter values for the PEF-treated and freeze-dried materials were significantly lower than for the intact, dried material. Furthermore, *H. illucens* larvae have demonstrated lower *b*⁺ values and higher *a*⁺ values in comparison to *T. molitor* larvae. The differences in color parameter values for PEF-treated samples can be explained by the electroporation phenomenon. Some compounds, such as fat, proteins or bioactive components, may be flushed out as a result of water penetration in the disrupted tissue [54] and then undergo enzymatic and non-enzymatic changes as well as oxidation process [43,57].

The total color difference (ΔE) between raw and freeze-dried insects ranged from 7.3 to 10.6 and from 8.6 to 10.5 for *H. illucens* and *T. molitor* larvae, respectively. For *H. illucens* larvae, after PEF treatment and freeze drying, the total color difference was higher than for untreated samples. However, the application of PEF treatment prior to drying of *T. molitor* larvae led to an inverse relationship; a lower ΔE was observed when increasing the PEF energy intake. Overall, for both species of dried insects, the ΔE values were higher than 3.5, which means that color changes are visible and can be recognized by the human eye [42].

### 3.3. Structure of Freeze-Dried Edible Insects Larvae

The photographs and structure of the freeze-dried *H. illucens* and *T. molitor* larvae obtained by microtomography are shown in Figure 3. Changes in internal structure were dependent on insect species and pre-treatment application. Regarding *H. illucens* larvae, the images demonstrated that the sample pretreated with 5 kJ/kg was characterized by the most compact structure. In turn, the internal structure of untreated and PEF-treated at 20 kJ/kg samples was similar, i.e., it presented a visible distance between the exoskeleton and the main body, which was located in the abdominal part of the larva (bottom of the cross-section images). Nevertheless, the PEF-treated sample was characterized by a more collapsed structure, which may be related to the partial destruction of cellular structures due to the electroporation phenomenon. For the freeze-dried *T. molitor* larvae, the images showed no noticeable deformation of the internal structure. On the cross-section of the analyzed samples, visible voids were formed probably in locations where there could be a digestive tract and other internal organs. However, freeze-dried *T. molitor* larvae pretreated with 20 kJ/kg were characterized by the reduced density of the fibrous structure of the exoskeleton, especially a procuticle (a thick layer of insect exoskeleton), which is mainly composed of chitin and proteins [53]. The current study is in accordance with the results demonstrated by Alles et al. [36], who also observed the destruction of the exoskeleton structure of *H. illucens* larvae as a result of PEF application.

### 3.4. Chemical Composition of Freeze-Dried Edible Insect Larvae

The chemical composition of the freeze-dried *H. illucens* and *T. molitor* larvae is summarized in Table 4. The highest amount of components for both insects, *H. illucens* and *T. molitor* larvae, is protein content. Also, the insects are a good source of fat and the fat content of *H. illucens* larvae is notably elevated (similar amount to protein content), while for *T. molitor* larvae, a higher content of protein is noted, but quite a high amount of fat is still obtained. These differences are associated with the insect species. The effect of PEF treatment for protein and fat extraction yield is not observed for *H. illucens* larvae.
Nevertheless, the sample treated with 20 kJ/kg is characterized by a slightly higher protein content and lower fat content, probably due to cellular structural damage and an enhanced capability of the solvent to thoroughly permeate the material. The study demonstrated by Alles et al. [36] also confirmed the lack of increase in the oil extraction yield from convective-dried H. illucens larvae after PEF treatment. In turn, the freeze-dried T. molitor larvae present a significant decrease in protein content. Such behavior can be linked to the electroporation phenomenon, which may promote some structural changes in the proteins and in the results, may lead to a reduction in protein extractability, especially when the higher electric field strength was used [36]. Also, molecular rearrangements during drying can change the protein content, especially due to proteolysis and oxidative stress [9] as well as the lack of enzyme inactivation during a freeze-drying process [58]. Furthermore, the reason may also be the fat composition of T. molitor larvae. This fat contains a large amount of unsaturated fatty acids, which may cause its oxidation process and thus foster protein oxidation [9].

![Photos and structure of freeze-dried untreated (0 kJ/kg) and PEF-treated (5 and 20 kJ/kg) H. illucens and T. molitor larvae.](image)

**Table 4.** Chemical composition of freeze-dried untreated (0 kJ/kg) and PEF-treated (5 and 20 kJ/kg) H. illucens and T. molitor larvae.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 kJ/kg</td>
<td>5 kJ/kg</td>
<td>20 kJ/kg</td>
<td></td>
</tr>
<tr>
<td>H. illucens</td>
<td>16.80 ± 2.33</td>
<td>35.73 ± 1.00</td>
<td>34.52 ± 1.83</td>
<td>4.57 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>12.51 ± 0.51</td>
<td>35.73 ± 1.00</td>
<td>34.52 ± 1.83</td>
<td>4.57 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>16.77 ± 2.01</td>
<td>37.38 ± 0.87</td>
<td>33.33 ± 2.47</td>
<td>4.30 ± 0.02</td>
</tr>
<tr>
<td>T. molitor</td>
<td>6.69 ± 0.25</td>
<td>48.02 ± 0.54</td>
<td>26.78 ± 0.21</td>
<td>3.82 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>4.19 ± 1.05</td>
<td>48.03 ± 0.49</td>
<td>26.83 ± 0.80</td>
<td>4.09 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>4.15 ± 0.11</td>
<td>45.40 ± 0.59</td>
<td>26.90 ± 0.70</td>
<td>3.93 ± 0.01</td>
</tr>
</tbody>
</table>

* The same letters in columns (lowercase for H. illucens larvae, uppercase for T. molitor larvae) denote the same homogeneous groups, which do not differ statistically (p < 0.05).
The moisture and ash content in freeze-dried larvae has been dependent on the insect species and PEF treatment. The lower moisture content determined for PEF-treated samples might be due to the electroporation phenomenon. Due to the formation of induced pores and a more open cellular structure, heat and mass transfer processes are enhanced, which allows an easier and faster removal of the water. An interesting phenomenon has been noted for the dried *H. illucens* larvae subjected to PEF at 20 kJ/kg. This sample was characterized by a higher moisture content than the sample pretreated at 5 kJ/kg. This observation can be explained by the phenomenon called 'overtreatment'.

Furthermore, some changes were noted in the chemical structures on the basis of the FTIR spectra of the freeze-dried *H. illucens* and *T. molitor* larvae (Figure 4). The absorbance peak observed close to 3280 cm\(^{-1}\) contributes to the stretching vibrations of O–H bond of hydrophilic compounds, e.g., amino acids or reducing sugars as well as N–H bond stretching vibrations from the amide A group \([59,60]\). A weak peak around 3000 cm\(^{-1}\) in dried *T. molitor* may have contributed to the acetamide functional group of chitin fraction \([61]\) and also the O–H bond stretching vibrations of polysaccharides \([45]\), which are a characteristic component of *T. molitor*. Moreover, the current study presented the PEF treatment as influencing the FTIR absorbance. It is noted that when PEF energy input increased, the absorbance decreased, which may indicate partial structural damage of these compounds. A peak found around 2945 cm\(^{-1}\) may be related to aromatic, aliphatic, as well as charged amino acids \([62]\). As before, the effect of PEF utilization on the absorbance value of the *T. molitor* FTIR spectra was observed. The strong peaks found at 2920 and 2850 cm\(^{-1}\) are responsible for the symmetric and asymmetric C–H bond stretching vibrations of –CH\(_2\) group, characteristic of polysaccharides and lipids \([45,61]\). The peak at 2924 cm\(^{-1}\) is related to the C–H bond of the amide B group \([60,63]\). For dried *H. illucens*, a peak around 1744 cm\(^{-1}\) may indicate the C=O bond stretching vibrations of ester carbonyl groups, which are characteristic of lipids \([60]\). Around 1720 cm\(^{-1}\), vibrations of the stretching C=O bond of the carbonyl group were seen, probably associated with free fatty acids \([64]\). Strong absorbance peaks in the range of 1640–1620 and at around 1540 and 1400 cm\(^{-1}\) are known to be related to the amide I, II, and III, respectively. In the case of amide I and II, regardless of insect species, the highest absorbance was observed for dried material without PEF pretreatment. Furthermore, Queiroz et al. \([60]\) and Bolat et al. \([63]\) provided that the absorbance peak at around 1255 cm\(^{-1}\) may also indicate the amide III and it was detected in the current study as a peak in the range between 1220 and 1250 cm\(^{-1}\). The region between 900 and 1200 cm\(^{-1}\) is called the fingerprint region, which is generally associated with nonstructural carbohydrates and stretching vibrations of C–C, C–H and C–O bonds \([60,64]\).

### 3.5. Microbiological Quality of Freeze-Dried Edible Insects Larvae

Changes in the total viable count (TVC), spore-forming bacteria, and total fungi count of fresh and freeze-dried *H. illucens* and *T. molitor* larvae are presented in Figure 5. The initial number of TVC on the fresh larvae is 7.3–7.7 log CFU/g, and it is comparable to the literature data \([18,25]\). For both insect species, after the utilization of PEF and freeze drying, a decrease in TVC is observed, in the range of 6.3–6.5 and 6.2–7.0 for *H. illucens* and *T. molitor*, respectively. All processed samples had TVC values lower than the advised limit of 7.0 log CFU/g for edible insects for food purposes \([18]\). In turn, according to the Commission Implementing Regulation (EU) 2022/169 of 8 February 2022, the TVC limit for *T. molitor* larvae should be below 5.0 log CFU/g; nevertheless, all freeze-dried insects exceeded this limit.
To reduce the microbiological hazards, raw materials should be processed before consumption [27,28]. Various processing methods are used for this purpose, and one of efficient use of PEF, one must set some key parameters for it, including the process (e.g., Tm) and the dose of intake energy. The obtained results might be explained by a higher resistance of bacteria spores to PEF application, the use of a different dose of energy (5 and 20 kJ/kg) did not change the number of these microorganisms, whereas the pretreatment method for the injurious microorganisms; its presence in general exceeds this limit. Nevertheless, to make promising pretreatment method for the injurious microorganisms, it seems to be a result of the forming of fungi spores [69].

According to the Com- position [66], it seems to be a higher decrease was noted for fresh and freeze-dried larvae (from 4.7 to 4.3 log CFU/g) than for a sterilization effect [65], due to the possibility of microorganisms exhibiting resistance to PEF.

Changes in the total viable count (TVC) are presented in Figure 4. FTIR spectra of freeze-dried untreated (0 kJ/kg) and PEF-treated (5 and 20 kJ/kg) H. illucens and T. molitor larvae are shown. The initial number of total fungi count in the fresh insects was approximately 5.6 log cycles for T. molitor larvae and 5.5 log cycles for H. illucens larvae, which are difficult to eliminate using thermal processing [11,12]. Treatment with PEF and proceeded edible insects. These bacteria are generally present in soil and dust [11], and are a serious problem for food products since they can produce resistant endospores [37,38].

Microbial load [log CFU/g]

<table>
<thead>
<tr>
<th>Microbial load [log CFU/g]</th>
<th>0.0</th>
<th>1.0</th>
<th>2.0</th>
<th>3.0</th>
<th>4.0</th>
<th>5.0</th>
<th>6.0</th>
<th>7.0</th>
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<tr>
<td>Fresh</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kJ/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 kJ/kg</td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>20 kJ/kg</td>
<td></td>
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</table>

Figure 4. FTIR spectra of freeze-dried untreated (0 kJ/kg) and PEF-treated (5 and 20 kJ/kg) (a) H. illucens and (b) T. molitor larvae.

Figure 5. Microbiological quality of fresh, and freeze-dried untreated (0 kJ/kg) and PEF-treated (5 and 20 kJ/kg) (a) H. illucens and (b) T. molitor larvae. The same letters above columns for each group of microorganisms denote the same homogeneous groups, which do not differ statistically (p < 0.05).
Aerobic spore-forming bacteria like *Bacillus* sp. might be frequently found in fresh and proceeded edible insects. These bacteria are generally present in soil and dust [11], and are a serious problem for food products since they can produce resistant endospores which are difficult to eliminate using thermal processing [11,12]. Treatment with PEF and freeze-drying of insects may limit the growth of spore-forming bacteria. The application of these treatments caused a reduction in the number of microorganisms by 1.0–1.5 log cycles for fresh and dried larvae (Figure 5a,b). A more effective impact of PEF application in lowering the number of these microorganisms was noted for *H. illucens* larvae (from 4.7 to 4.3 log CFU/g) than for *T. molitor* larvae. What is interesting is that for *T. molitor* larvae, a dose of intake energy had no impact on a reduction in the number of these bacteria. The obtained results might be explained by a higher resistance of bacteria spores to PEF application, and thus treatment only with PEF could be applied rather for a pasteurization than for a sterilization effect [65], due to the possibility of microorganisms exhibiting recovery after PEF treatment [66].

The initial number of total fungi count in the fresh insects was approximately 5.6 log CFU/g (Figure 5a,b). A decrease in the number of fungi was observed in the studied insects after the freeze drying. A slightly higher decrease was noted for *H. illucens* larvae by 2.3–2.6 log cycles than for *T. molitor* larvae (1.9–2.3 log cycles). With reference to the PEF application, the use of a different dose of energy (5 and 20 kJ/kg) did not change the number of fungi probably due to their morphological structure, as well as the cell wall composition [67,68]. Moreover, for *T. molitor* larvae (Figure 3b), the microbial load was higher than would seem to be a result of the forming of fungi spores [69]. According to the Commission Implementing Regulation (EU) 2022/169 of 8 February 2022, the number of total fungi count for freeze-dried *T. molitor* larvae should be lower than 2.0 log CFU/g; meanwhile, in the current study, all freeze-dried insects exceeded this limit.

To reduce the microbiological hazards, raw materials should be processed before consumption [27,28]. Various processing methods are used for this purpose, and one of them, freeze drying, is used in this study. During the freezing and freeze-drying process, formed ice crystals cause disruptions in microorganisms’ cellular structure, loss of cell viability, and then their death [70,71]. Furthermore, PEF might be used to inactivate some microorganisms. Taking into account the volumetric effect of PEF [72], it seems to be a promising pretreatment method for the injurious microorganisms; its presence in general is higher in the digestive tract than on the skin of insects [11,18]. Nevertheless, to make efficient use of PEF, one must set some key parameters for it, including the process (e.g., electric field strength, pulse width, frequency, and duration), the product (e.g., type, chemical composition, pH), and the microorganism characteristics (e.g., type, species, strains, and cell size) [65,66,73]. The decrease in the number of microorganisms was not found to be dependent on the application of PEF (decrease below 1.0 log cycle). Therefore, more studies should be performed to explain how PEF treatment can be useful in inactivating microorganisms present in insects. However, there was a reduction of more than 2.0 log cycles of total fungi count, although this still exceeded the accepted microbiological criteria for dried insect larvae.

One of the reasons for the microbial load of the freeze-dried insects can be taken as their high hygroscopicity properties and consequently higher exposure to microbial growth [28,43]. Hence, a suitable drying process and pretreatment are necessary steps to minimize this risk. It is also necessary to control external conditions, i.e., temperature and humidity during production and storage stages, to prevent against secondary contamination [74].

4. Conclusions

The conducted research indicates that the application of PEF treatment did not improve the freeze-drying process of insect biomass and failed to yield microbiological quality suitable for food purposes. Notably, only fungi displayed increased susceptibility to PEF treatment compared to bacteria, resulting in a reduction of 1.9 to 2.6 log cycles. The
utilization of pulsed electric field (PEF) treatment prior to freeze drying of insect biomass induced subtle modifications in protein and fat content. Notably, only *T. molitor* larvae subjected to higher PEF energy input (20 kJ/kg) exhibited a reduction in protein content. Moreover, microtomography images of freeze-dried *H. illucens* and *T. molitor* larvae reveal distinct structural changes based on species and pre-treatment. For example, *T. molitor* larvae exhibit minimal internal structural deformation except for reduced exoskeletal fibrous density after 20 kJ/kg pre-treatment. However, the differences in rehydration and hygroscopic characteristics for both larvae species were not apparent across the studied samples. Samples subjected to higher PEF energy input displayed a slightly higher ability to absorb moisture from the ambient environment. Moreover, the color of the PEF-treated larvae was significantly darker for *H. illucens*, and similar for *T. molitor* compared to the untreated samples. Also, both species presented lower values of *a* and *b* color parameters which influence the higher total color difference (ΔE). Nevertheless, to explain all the changes occurring during insect biomass processing, further studies and more information are needed.

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

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66. Li, L.; Yang, R.; Zhao, W. The Effect of Pulsed Electric Fields (PEF) Combined with Temperature and Natural Preservatives on the Quality and Microbiological Shelf-Life of Cantaloupe Juice. *Foods* 2021, 10, 2606. [CrossRef] [PubMed]


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