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The Effect of Unconventional Technologies on Carbon Emissions During the Convective Drying of Yellow Mealworm (*Tenebrio molitor* L.) Larvae and the Selected Physical Properties Thereof

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Abstract: The drying of insects is an important step in their processing. This research aimed to investigate the impact of a pulsed electric field (PEF), immersion in ethanol (EtOH), and combined (immersion in EtOH followed by PEF) treatment on the convective drying process, the emission of CO_2, and the quality of the dried insects with regard to such elements as water content and activity, rehydration and hygroscopic properties, optical properties, internal structure, and microbiological quality. In applying a PEF, the drying time was made longer (up to 21%), but the rehydration and hygroscopic properties were improved (about 15–16.5% and 8.3–21.7%, respectively) compared to the untreated sample. Using a PEF prior to EtOH treatment improved the rehydration properties (about 3.9–5.9%), while the hygroscopicity was slightly lower compared to the PEF-treated samples. Furthermore, immersion in ethanol (both alone and after PEF) provided a lighter color of dried insects and more outstanding microbiological quality, e.g., the absence of water-borne and food-borne pathogens and anaerobic spore-forming bacteria. This study revealed that combined pretreatment seems to be the most promising method for insects as regards obtaining better rehydration and comparable hygroscopic properties, as well as an attractive color compared to untreated insects, and, above all, in ensuring suitable microbiological quality.

Keywords: edible insects; convective drying; pulsed electric field; ethanol; greenhouse gas emission

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1. Introduction

A growing population and environmental changes, including limited natural resources, have caused us to look for new food sources. Edible insects could be the answer to these needs. Insect farming exhibits a significantly lower impact on the environment than traditional livestock farming, and is considered a solution that would ensure the sustainable use of resources for food production [1,2]. Most research regarding this concept has concentrated on yellow mealworm (*Tenebrio molitor* L.) larvae. In Europe, yellow mealworm larvae are now permitted for human consumption and have high potential in terms of becoming industrialized and produced on a large commercial scale [3]. This insect species has a high quantity of protein (18–64% d.m.), with practically all types of amino acids (lysine, isoleucine, leucine, valine, tyrosine) and fats (18–40% d.m.), and it is rich in mono- and polyunsaturated fatty acids, crude fiber (4–22%), as well as many minerals [3–8].

Despite insects having nutritional and farming advantages, they can pose some chemical hazards, such as allergens, heavy metals, pesticides, and other pollutants, as well as microbiological hazards. The most common microorganisms include the Enterobacteriaceae

family, spore-forming bacteria (*Clostridium* sp. and *Bacillus* sp.), yeasts and molds, as well as food-borne pathogens, e.g., *Listeria* spp., *Escherichia coli*, *Staphylococcus* spp., and *Salmonella* spp. [3,9]. Therefore, insects should be processed before they are consumed. One of the most commonly used processing methods is drying, which can also be applied to them.

Drying can be used not only to preserve food, but also to process and make products that consumers highly desire [10,11]. The most common technique in industrial practice is convective (hot-air) drying. This operation enables the removal of water from the material into the surrounding air [12,13]. Generally, drying ensures microbiological stability and the deceleration of many chemical reactions, and it even limits enzymatic changes [12,14]. However, aside from the low quality of the dried products, this process is the most energy-consuming in the food industry [11,12]. Based on data from the literature, the drying process uses up to 25% of the total energy that is consumed by the whole food industry [15]. Therefore, novel pretreatment methods have been used to attempt to decrease the unsatisfactory effects of convective drying. Pulsed electric fields (PEFs) and immersion in ethanol are among these pretreatment methods.

The application of PEF to food materials, in which short-term pulses with high voltages promote the modification of membrane permeability, destroys the cellular structure, thus enhancing the mass and heat transfer processes [12,16]. In the literature, it has been reported that PEF treatment may accelerate the drying kinetics of many plant materials, e.g., during the freeze-drying of red bell peppers [16] and apples [17], the hot-air drying of zucchini [12], onions [18] and carrots [19], as well as during the vacuum-drying of potatoes [20]. Some findings have been recently provided for edible insects. The PEF treatment of house crickets before oven-drying enhances the drying process and reduces energy consumption by 14.2% [21]. In addition, PEF-treated yellow mealworm larvae were dried faster by 10% when using the infrared-drying process [22]. An entirely novel approach to aiding the drying process of a given material may be its immersion in ethanol (EtOH). Ethanol modifies the structure of the treated material, mainly due to its ability to extract some compounds of cell membranes and walls, and thus reduce their permeability. Ethanol also creates a larger gradient of water/ethanol concentration on the material's surface (Marangoni effect), as it is the first element to evaporate from the material during drying [11,13,23]. The impact of immersion in ethanol on drying-time reduction has been recently demonstrated for melon [24], apple [25], carrot [11,13], and pumpkin [23]. According to the authors' knowledge, no data on insect drying kinetics and energy consumption have been provided.

Therefore, this research aimed to investigate the effects of PEF treatment, EtOH immersion, and a combined treatment (EtOH followed by PEF) on the drying kinetics and greenhouse gas emissions during the convective drying process of yellow mealworm (*Tenebrio molitor* L.) larvae. Furthermore, dried yellow mealworm larvae's quality was evaluated by determining water content, water activity, rehydration and hygroscopic properties, optical properties, microstructures, and microbiological load.

2. Materials and Methods

2.1. Material

The live yellow mealworm (*Tenebrio molitor* L.) larvae, purchased from a Polish producer (Cirwins, Kamień Duży, Poland), were kept under controlled cold conditions (4 ± 1 °C). Before use, the larvae were washed with tap water and gently dried with filter paper.

2.2. Technological Treatment

2.2.1. Pulsed Electric Field (PEF)

For pulsed electric field treatment, larvae were placed directly in the treatment chamber of a PEF Pilot™ Dual system (Elea GmbH, Quakenbrück, Germany) filled with tap water at 21 ± 1 °C and a conductivity of 220 µS/cm. The mass ratio of larvae to tap water was 1:9. The specific energy consumption of 5 and 20 kJ/kg (requiring about 2 and 5 min, respectively) was provided by applying monopolar pulses with a width of 7 µs and a

frequency of 2 Hz. During treatment, the electric field intensity was 1.07 kV/cm. After treatment, the larvae were filtered and gently dried with filter paper. The PEF treatment was performed in duplicate for each sample.

2.2.2. Immersion in Ethanol (EtOH)

The untreated larvae and those previously pretreated with PEF were immersed in 96% ethanol solution at room temperature for 10 min. Larvae weighing 100 g were placed in a beaker and fully submerged in ethanol. After treatment, the larvae were filtered and gently dried with filter paper. The immersion in ethanol was performed in duplicate for each sample.

2.2.3. Convective Drying

The untreated larvae and those pretreated with PEF were arranged to form a layer of about 4 mm in the shape of the perforated tray, providing a sieve load of 0.82 kg/m^2. The larvae were dried using the prototype laboratory dryer constructed at the Warsaw University of Life Sciences (Warsaw, Poland) with airflow parallel to the sample layer of 2 m/s and at a temperature of 90 °C until a constant mass was achieved. The change in mass was recorded continuously every 1 min until the end of the process. The material was dried in duplicate for each variant. After that, the dried insects were packed and stored in air- and light-barrier bags PET/AL/PE (Pakmar, Warsaw, Poland).

The change in mass recorded during drying and the water content were used to describe the drying kinetics of the insect samples, plotted as the ratio of moisture (MR) to drying time. The MR was calculated as follows:

$$\mathrm{MR} = \frac{u_\tau}{u_0}, \quad (1)$$

where u_τ is the water content during drying (kg H_2O/kg d.m.) and u_0 is the initial water content (kg H_2O/kg d.m.).

2.3. CO_2 Emission

Electricity consumption during PEF treatment and convective drying was measured using the R-box 460R-17 m (Pawbol, Sułkowice, Poland) designed for laboratory measurement. Greenhouse gas emissions were calculated based on an equivalent amount of 765 kg CO_2 emitted per 1 MWh of electricity produced [16]. The CO_2 emissions were expressed in kg of CO_2 per 1 kg of dried material.

2.4. Physical Properties

2.4.1. Water Content and Water Activity

The water content was calculated based on dry matter determination, which was analyzed by drying the larvae in a laboratory dryer (Wamed, Warsaw, Poland) for 17 h at a temperature of 105 °C [7]. The results are expressed in grams of water per 1 g of dry matter (g H_2O/g d.m.). The measurement was performed in triplicate for each analyzed sample.

The water activity was measured using a HygroLab C1 hygrometer (Rotronic, Bassersdorf, Switzerland) at 24 ± 1 °C in three replicates for each insect sample.

2.4.2. Rehydration and Hygroscopic Properties

The rehydration properties were measured by placing dried larvae in a beaker containing 100 mL of distilled water for 3 h. Afterward, the larvae were removed and dried gently with filter paper. The rehydration properties were calculated as the ratio of the weight after rehydration to the initial weight of the sample [22]. The rehydration properties were determined in three repetitions for each sample.

The hygroscopic properties were measured by placing dried larvae in the desiccator filled with water (water activity of 1.0) after 1, 2, 3, 6, 9, 12, 24, 48, and 72 h to evaluate the water vapor sorption kinetics [22]. The results of water vapor adsorption were expressed

in grams of water adsorbed by 100 g of dry matter (g H_2O/100 g d.m.). The hygroscopic properties were determined in three repetitions for each insect sample.

2.4.3. Optical Properties

The color parameters (L*, a*, b*) of dried insects were collected using a Konica Minolta CR-5 chromameter (Konica Minolta, Osaka, Japan) working with a D65 standard illuminant light source, CIE 2° Standard Observer, with an 8° angle of viewing and a 30 mm measuring diameter. The color parameters were measured in fifteen repetitions for each insect sample. The total color difference (ΔE) between the untreated and pretreated samples was calculated using the following equation:

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

where ΔL*, Δa* and Δb* are the differences in color parameters (L*, a*, b*) measured for pretreated (PEF, EtOH, combined PEF and EtOH) and untreated (U) insects.

The photos of the dried insects were taken using a Nikon D7000 digital camera (Nikon, Tokyo, Japan).

2.5. Microstructure

The microstructures of dried insects were measured using a SkyScan 1272 microtomograph (Brucker, Kontich, Belgium). Two randomly selected dried insects were placed on a metal table with a diameter of 25 mm. The scans were performed using the following parameters: X-ray source voltage of 40 kV, current of 193 μA, rotation step of 0.3°, and a resolution of 25 μm [16]. The NRecon software (version 2.0, Bruker, Kontich, Belgium) was used for image reconstruction and 3D creation.

2.6. Microbiological Analysis

The microbiological analyses included the testing of total viable count (TVC), Enterobacteriaceae, aerobic spore-forming bacteria, and total yeast and mold count (TYMC). For this purpose, 10 g of the dried insects were weighed, and 90 mL of 0.85% NaCl was added. A homogenizer (Stomacher 400 Circulator, Cambridge, UK) was then used to blend the mixture for one minute. The total viable count (TVC) was counted on plate count agar PCA (Biomaxima, Lublin, Poland) after incubation for 72 h at 30 °C. On Dichloran Glicerol DG 18 agar (Biomaxima, Lublin, Poland), the total yeast and mold count (TYMC) was counted during a 5-day incubation period at 25 °C. In a sterile tube, bacterial endospores were counted by heat-shocking insect dilution for 20 min at 80 °C. Following this, plate count agar (PCA for aerobic and Wilson Blair agar for anaerobic spore-forming bacteria, Biomaxima, Lublin, Poland) was poured onto plates and then incubated at 30 °C for 48 h. Enterobacteriaceae, *L. monocytogenes*, *E. coli*, and *S. aureus* were counted on chromogenic agar accordingly, VRBG, ALOA, TBX, Baird-Parker (Biomaxima, Lublin, Poland) after incubation for 24–48 h at 37–44 °C [26,27]. Salmonella's presence was examined per the Polish Standard [28]. The pre-enrichment was performed by weighing 25 g of the sample and mixing it with buffered peptone water. After that, cultures were transferred to the RVS medium and then incubated on XLD and BGA selective media. If colonies typical of *Salmonella* were present, the next part of the procedure was performed. The ProtoCOL 3-Automatic instrument (Synbiosis, Cambridge, UK) was used to calculate the number of microorganisms detected. Every microbial analysis was performed in triplicate, and the average result was expressed as log CFU/g d.m.

2.7. Mathematical Modeling

The drying kinetics of insects have been described by the simplified Fick's second law for an infinite flat plate using the Table Curve 2D v5.01 software (SYSTAT Software, Inc., Chicago, IL, USA) according to the equation below:

$$\mathrm{MR} = \frac{8}{\pi^2} exp\left[\frac{-D_{eff}\pi^2\tau}{4L^2}\right], \quad (3)$$

where D_{eff} is the effective water diffusion coefficient (m^2/s), L is half of the insect layer on the tray (m), and τ is the drying time (s).

The hygroscopicity kinetics of dried insects have been described by the solution of the second Fick's law for transient diffusion using the Table Curve 2D v5.01 software (SYSTAT Software, Inc., Chicago, IL, USA) according to the below equation [29]:

$$Aexp(-K_\tau) = \frac{u_\tau - u_e}{u_0 - u_e}, \quad (4)$$

where u_τ is the water content during each moment of the adsorption process (g H_2O/100 g d.m.), u_e is the equilibrium water content (g H_2O/100 g d.m.), u_0 is the initial water content (g H_2O/100 g d.m.), A is the shape factor, K is the coefficient linked to water diffusion (1/min), and τ is the sorption time (s).

Fitting the second Fick's kinetic model to the hygroscopicity data was achieved using the Table Curve 2D v5.01 software (SYSTAT Software, Inc., Chicago, IL, USA). The coefficient of determination (R^2) and the root mean square (RMS) were calculated to evaluate the goodness of fit of Fick's second kinetic model:

$$R^2 = 1 - \frac{\sum_{i=1}^{N}\left(u_p - u_{exp}\right)^2}{\sum_{i=1}^{N}\left(u_{exp} - \overline{u_p}\right)^2}, \quad (5)$$

$$\mathrm{RMS} = \sqrt{\frac{\sum_{i=1}^{N}\frac{\left(u_{exp}-u_p\right)^2}{u_{exp}}}{N}}, \quad (6)$$

where u_{exp} is the experimental water content during each moment of the adsorption process (g H_2O/100 g d.m.), u_p is the predicted water content during each moment of the adsorption process (g H_2O/100 g d.m.), $\overline{u_p}$ is the mean predicted water content during each moment of the adsorption process (g H_2O/100 g d.m.) and N is the number of observations.

2.8. Statistical Analysis

The one-way analysis of variance (ANOVA) and the post hoc HSD Tukey's test were utilized to assess significant differences between investigated samples. Statistical analysis was performed using STATISTICA 13.3 (TIBCO Software, Palo Alto, CA, USA). The significance level was set at $\alpha = 0.05$.

3. Results and Discussion

3.1. Drying Process of Yellow Mealworm Larvae

Convective drying curves of the yellow mealworm larvae are shown in Figure 1a,b. The applied pretreatment methods (PEF, EtOH, and combined treatment) impacted the drying process. The lowest time to achieve a dimensionless moisture coefficient (MR) of 0.2 was determined for the untreated sample (U) and protocols EtOH and PEF5_EtOH. The times needed to achieve the target MR coefficients were 128, 128, and 125 min, respectively (Table 1). PEF input energies of 5 and 20 kJ/kg caused extensions of the drying time (to achieve MR = 0.2) to 155 and 150 min, respectively. Also, immersion in EtOH followed by PEF at 20 kJ/kg prolonged drying time to achieve MR = 0.2 to 148 min. This could be related to changes in the transmembrane potential of cell structures and their subsequent opening

due to PEF application [30], resulting in the entry of water into the interior, which could not be removed once they were closed. Furthermore, PEF treatment promotes the unfolding of the protein chain, its aggregation, and, consequently, a greater ability to bind water [31]. The water diffusion coefficient calculated based on the simplified Fick's equation ranged from 0.856×10^{-9} to 1.22×10^{-9} m^2/s, and reached the highest values in the untreated sample (U) and EtOH and PEF5_EtOH (Table 1), indicating faster water removal during convective drying. Many researchers have observed a beneficial effect of PEF pretreatment on the drying rates of plant materials [12,16,17,20]. However, studies on the use of PEF as a pretreatment for insect larvae remain limited.

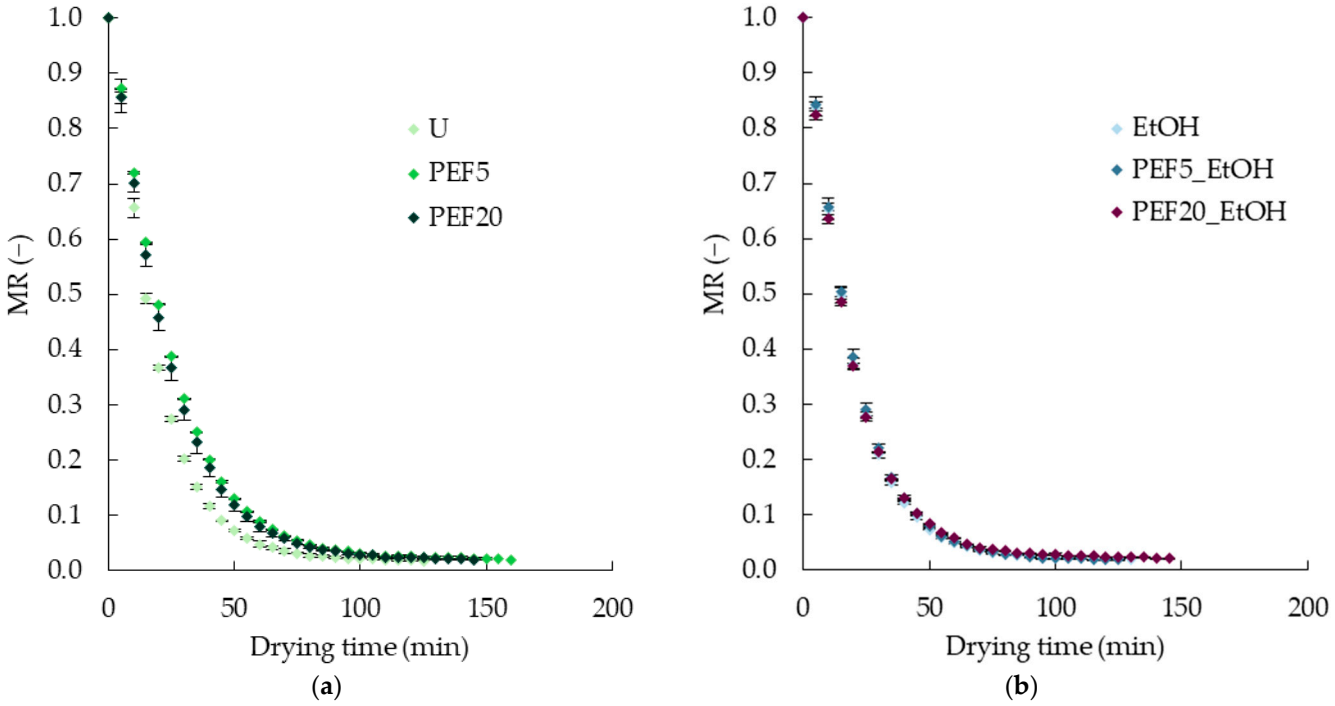

Figure 1. Drying kinetics of yellow mealworm larvae: (**a**) untreated (U) and pretreated with PEF (PEF5 and PEF20—pulsed electric field with specific energy consumption of 5 and 20 kJ/kg, respectively), and (**b**) combined pretreatment with ethanol (EtOH) followed by PEF.

Table 1. The drying time, effective diffusion coefficient, water content, and water activity of dried yellow mealworm larvae: without pretreatment (U), pretreated with PEF (PEF5 and PEF20—pulsed electric field with specific energy consumption of 5 and 20 kJ/kg, respectively), and combined pretreatment with ethanol (EtOH) followed by PEF.

Treatment	Drying Time to MR = 0.02 (min)	Effective Diffusion Coefficient D_{eff} ($\times 10^{-9}$ m^2/s)	Water Content (g H_2O/g d.m.)	Water Activity (–)
U	128 a [1] ± 4	1.122 b ± 0.009	0.028 a ± 0.001	0.234 c ± 0.004
PEF5	155 b ± 7	0.856 a ± 0.001	0.034 b ± 0.001	0.202 b ± 0.003
PEF20	150 b ± 7	0.865 a ± 0.028	0.034 b ± 0.001	0.226 c ± 0.004
EtOH	128 a ± 4	1.120 b ± 0.020	0.028 a ± 0.001	0.211 b ± 0.008
PEF5_EtOH	125 a ± 0	1.088 b ± 0.141	0.028 a ± 0.001	0.188 a ± 0.008
PEF20_EtOH	148 b ± 4	1.099 b ± 0.009	0.030 a ± 0.001	0.210 b ± 0.004

[1] The same letters in columns denote no significant differences between mean values (Tukey's HSD, $p < 0.05$).

The effect of PEF treatment at a level of 5 and 20 kJ/kg on the course of the infrared-convective drying process of yellow mealworm and black soldier fly larvae has been analyzed previously [22]. The study revealed that the effect of PEF on the rate of the drying

process was small and did not exceed a few percentage points. Applying PEF at 20 kJ/kg reduced infrared drying time by 6.5%, whereas PEF at 5 kJ/kg had no impact on the drying time. The authors highlighted that the drying time required to achieve lower MR values was not consistently influenced by the PEF treatment. The impact of PEF pretreatment on the convective drying kinetics of other insect larvae has been previously described by Alles et al. [32] and Shorstkii et al. [33]. These researchers found that applying PEF can shorten the drying time of black soldier fly larvae by up to 30% during drying at 90 °C. The researchers did not observe this effect during drying at 70 °C. The studies conducted in this work show that PEF did not shorten the drying time of the yellow mealworm larvae. The larvae treated with PEF (PEF5, PEF20, PEF20_EtOH) took significantly longer to dry than larvae without pretreatment or those treated with ethanol (U, EtOH, PEF5_EtOH). The immersion of insects in ethanol also did not significantly shorten the drying time compared to insects without pretreatment. The values of the water diffusion coefficient calculated based on Fick's second equation confirm the unfavorable effect of PEF treatment on the rate of the convective drying process of yellow mealworm larvae (Table 1).

3.2. Water Content and Water Activity of Dried Yellow Mealworm Larvae

The water content of the tested samples was in the range of 0.028 to 0.034 g H_2O/g d.m. The lowest water content (0.028 g H_2O/g d.m.) was observed for the untreated (U), EtOH, and PEF5_EtOH samples. A significantly higher water content was found for insects treated with PEF (PEF5 and PEF20). The results might be due to the opened internal structure of insect tissue, which provides greater leakage of soluble solids into the treatment medium (water) [34], as well as the greater penetration of water molecules into it [35]. In the case of immersion in ethanol, the used solvent could cause the partial dissolution of cell structures and the leakage of their components into the medium [25]. The mechanism of the utilized pretreatment methods is not well known and explained yet, so further research is needed. Furthermore, we do not know the water content in each larva from the portion taken for the drying process. Therefore, the differences in the obtained results are also related to the initial dry matter content in the yellow mealworm larvae taken.

Water activity refers to the free water available in the material, and provides information on the susceptibility of the material to microbial growth and the occurrence of chemical reactions [36]. Although all samples had a value below 0.6, indicating microbial stability, the treatment applied prior to drying affected the values obtained. Treatment with PEF caused a decrease (significant only after PEF at 5 kJ/kg) in water activity values as a result of the greater ability to bind water by the unfolded and aggregated protein chains [31], and the lower free water content. The immersion of insects in ethanol resulted in significantly lower (0.188–0.211) water activities. The lowest water activity was obtained for the PEF5_EtOH treatment. This is probably due to the lower surface tension of ethanol compared to that of water, which results in a less shrunken structure of the insect tissue and, therefore, better water removal during convective drying.

3.3. Greenhouse Gas Emission During Processing of Yellow Mealworm Larvae

The drying process is widely recognized as one of the most energy-intensive operations in food technology [11]. Therefore, the pretreatment steps are often applied to enhance drying and reduce CO_2 emissions. The drying process caused CO_2 emissions in the range of 6.7 to 8.3 kg/kg of dried material, depending on the pretreatment method used (Figure 2). Interestingly, the highest emission of CO_2 was not observed for the untreated insects. The variants where PEF was used as a pretreatment were associated with higher emissions of CO_2—about 21.1% and 17.2% for PEF at 5 and 20 kJ/kg, respectively. The combined treatment marked as PEF5_EtOH was efficient, with the lowest CO_2 emissions (6.7 kg/kg dried material). In turn, the PEF20_EtOH treatment insignificantly reduced the CO_2 emissions (only by 1.3%) in comparison to those treated with PEF at 20 kJ/kg (PEF20).

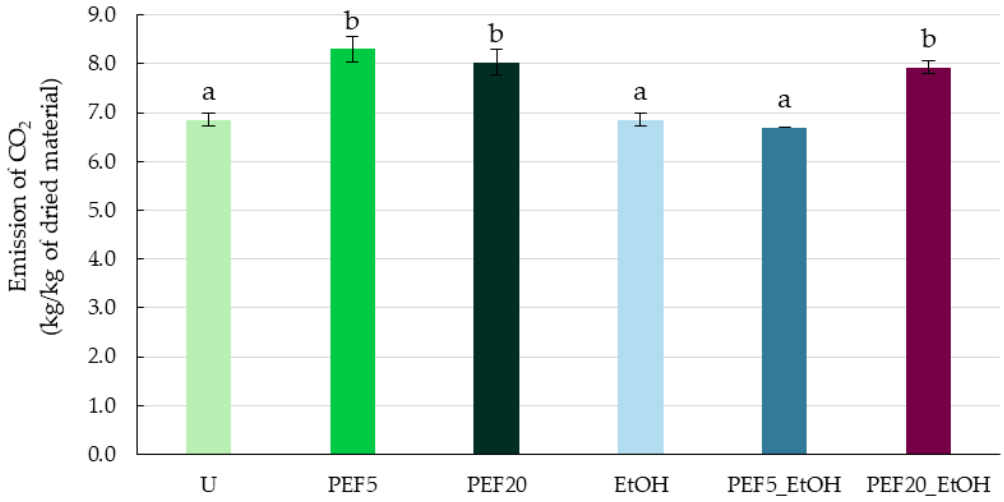

Figure 2. The emission of CO_2 during the drying of yellow mealworm larvae: without pretreatment (U), pretreated with PEF (PEF5 and PEF20—pulsed electric field with specific energy consumption of 5 and 20 kJ/kg, respectively), and combined pretreatment with ethanol (EtOH) followed by PEF, a,b; the same letters above columns denote no significant differences between mean values (Tukey's HSD, $p < 0.05$).

The results of CO_2 emissions obtained during the convective drying of yellow mealworm larvae are unexpected. PEF treatment, by causing structural changes, was expected to improve water diffusion during the drying process, shorten its duration, and thus reduce energy consumption and CO_2 emissions. This was observed so far for certain materials, such as freeze-dried and vacuum-dried Chilean abalone [37] and freeze-dried red bell pepper [16]. Also, for freeze-dried house crickets pretreated with PEF before convective drying, a 14.2% reduction in energy consumption was observed [21]. However, as in our study, energy consumption during the convective drying of Chilean abalone after PEF pretreatment was higher [34]. It therefore appears that certain modifications in protein or chitin structure after PEF and, consequently, a greater ability to bind water may have had a greater impact than structural physical changes in insect tissue.

3.4. Rehydration and Hygroscopic Properties of Dried Yellow Mealworm Larvae

Rehydration properties are directly linked to the ability to absorb water, indirectly providing information about the dried material's quality and the changes occurring during its processing and drying [16,38]. The results regarding the rehydration properties of tested dried yellow mealworm larvae are presented in Table 2. Among the samples studied, the lowest rehydration ability was obtained for the untreated sample. Even though there are no significant differences, a higher capacity was observed for materials immersed in ethanol (EtOH) before drying, followed by PEF-treated insects (PEF5, PEF20). A significantly higher rehydration ability was noted for materials treated with PEF combined with immersion in ethanol.

Hygroscopic properties are directly linked to the ability to adsorb water vapor, indirectly providing information about the dried material's quality and its behavior during storage [13,39]. The lowest hygroscopicity after 72 h above water vapor was obtained for the untreated sample (Table 2), followed by PEF5 and EtOH materials. The highest hygroscopicity and water content after the adsorption process were observed for the PEF20 treatment. When looking at the adsorption kinetics of water vapor in the dried insects analyzed, it can be seen that the highest hygroscopicity was achieved by the insects subjected to PEF at 20 kJ/kg (Figure 3). The kinetics process for this sample clearly differ from those of other samples throughout the water vapor adsorption process. In the initial stage (up to 12 h) of water vapor adsorption, the untreated sample presented a higher adsorption capacity than all samples immersed in ethanol. After this time, the water vapor adsorption

intensity for the untreated sample was the lowest, and it remained consistent until the end of the measurement.

Table 2. Rehydration properties and hygroscopic properties (after 72 h) of dried yellow mealworm larvae: without pretreatment (U), pretreated with PEF (PEF5 and PEF20—pulsed electric field with specific energy consumption of 5 and 20 kJ/kg, respectively), and combined pretreatment with ethanol (EtOH) followed by PEF.

Treatment	Rehydration Properties (−)	Hygroscopic Properties (g H_2O/100 g d.m.)
U	1.33 a [1] ± 0.04	35.37 a ± 0.23
PEF5	1.55 ab ± 0.17	38.32 abc ± 2.22
PEF20	1.53 ab ± 0.07	43.04 d ± 1.26
EtOH	1.45 ab ± 0.08	36.85 ab ± 2.00
PEF5_EtOH	1.62 b ± 0.01	37.43 bcd ± 2.45
PEF20_EtOH	1.61 b ± 0.06	38.01 cd ± 3.28

[1] The same letters in columns denote no significant differences between mean values (Tukey's HSD, $p < 0.05$).

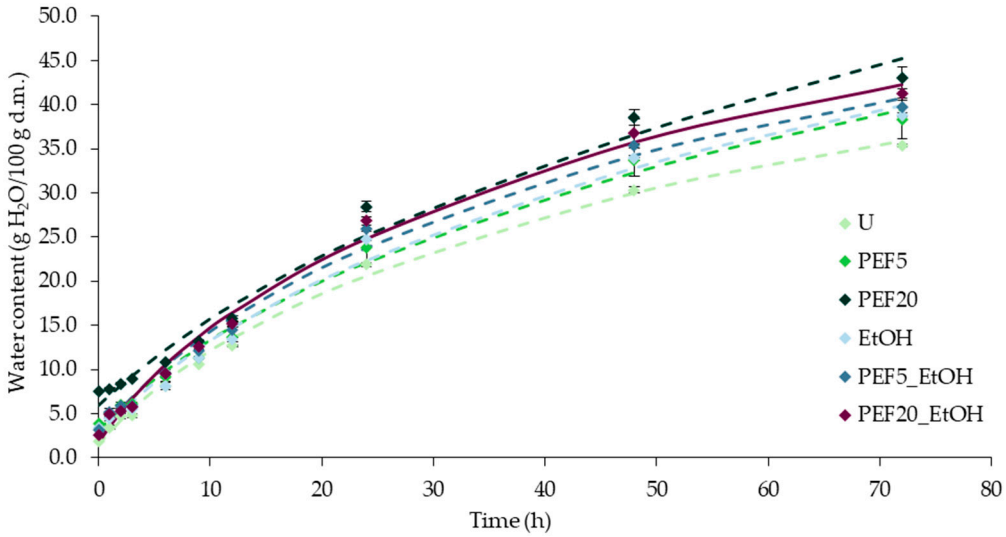

Figure 3. The hygroscopic kinetics of dried yellow mealworm larvae: without pretreatment (U), pretreated with PEF (PEF5 and PEF20—pulsed electric field with specific energy consumption of 5 and 20 kJ/kg, respectively), and combined pretreatment with ethanol (EtOH) followed by PEF. Dotted lines represent values obtained from mathematical modeling.

The application of the unsteady diffusion equation to describe the water vapor sorption curves (Figure 3) allowed us to determine the parameters that are important concerning the material's behavior during storage (Table 3). The equilibrium water content (u_e) means the water content that the tested material would reach after an infinitely long storage time, while the coefficient K gives information about the rate of water diffusion in the material [29]. Changes in the rehydration and hygroscopic properties of dried insects pretreated with PEF and immersion in ethanol are associated with both physical and chemical adjustments. Physical changes refer to changes in the material's cellular structure, such as the rupture of membranes due to electroporation phenomena [30,40], which increases microporosity and the possibility of entry of more water molecules, which are then bound. PEF treatment can also promote certain modifications in protein or chitin structure [31,35]. As Rostamabadi et al. [31] highlighted, PEF treatment promotes the unfolding of the protein chain, its aggregation, and, consequently, a greater ability to bind water. Such a tendency was observed in the case of the rehydration and hygroscopic properties of yellow mealworm larvae given PEF treatment before infrared–convective [22] and freeze-drying [26], especially when utilizing a higher specific energy of PEF. Ethanol can cause the partial

dissolution of cell structures (membranes and cell walls), which promotes swelling during rehydration and the binding of more water or water vapor [41]. In addition, it has a lower surface tension than that of water, helping to minimize the material's shrinkage during drying [25]. The immersion of the insects in ethanol allowed the porosity obtained from PEF treatment to be largely preserved, which contributed to the higher rehydration ability and hygroscopicity of the samples.

Table 3. Parameters of sorption kinetics of dried yellow mealworm larvae: without pretreatment (U), pretreated with PEF (PEF5 and PEF20—pulsed electric field with specific energy consumption of 5 and 20 kJ/kg, respectively), and combined pretreatment with ethanol (EtOH) followed by PEF.

Treatment	Equilibrium Water Content u_e (g H_2O/100 g d.m.)	Coefficient K ($\times 10^{-6}$ 1/min)	R^2 (−)	RMS (%)
U	46.08 a [1] ± 1.31	2.16 cd ± 0.09	0.996	7.89
PEF5	58.14 c ± 2.70	1.64 b ± 0.19	0.992	7.80
PEF20	80.80 d ± 1.52	0.98 a ± 0.03	0.981	9.34
EtOH	55.77 bc ± 0.23	1.75 bc ± 0.02	0.982	10.68
PEF5_EtOH	51.95 b ± 1.79	2.21 cd ± 0.32	0.990	10.83
PEF20_EtOH	52.10 b ± 3.53	2.42 d ± 0.16	0.992	12.06

[1] The same letters in columns denote no significant differences between mean values (Tukey's HSD, $p < 0.05$).

3.5. Optical Properties of Dried Yellow Mealworm Larvae

Color is an important quality differentiator related to consumer behavior and product selection [24]. Considering the obtained results, it can be stated that PEF-treated insects were darker than the untreated sample. In contrast, insects immersed in ethanol were characterized by a lighter color than the others (Table 4, Figure 4). Moreover, insects immersed in ethanol demonstrated higher redness (a* color parameter) and yellowness (b* color parameter) than the other samples. This is a positive result since, among consumers, these colors are desirable and favorably influence product choice [42].

Table 4. Color parameters and total color difference of dried yellow mealworm larvae: without pretreatment (U), pretreated with PEF (PEF5 and PEF20—pulsed electric field with specific energy consumption of 5 and 20 kJ/kg, respectively), and combined pretreatment with ethanol (EtOH) followed by PEF.

Treatment	L*	a*	b*	ΔE
U	27.8 c [1] ± 0.7	3.7 a ± 0.2	9.3 b ± 0.7	–
PEF5	26.9 b ± 0.4	4.0 b ± 0.3	8.9 ab ± 0.6	1.1 a ± 0.7
PEF20	25.7 a ± 0.4	3.6 a ± 0.2	8.4 a ± 0.5	2.3 b ± 0.5
EtOH	28.2 cd ± 0.8	4.8 c ± 0.3	10.3 c ± 0.7	1.7 b ± 0.7
PEF5_EtOH	29.6 e ± 0.8	6.0 e ± 0.1	12.5 e ± 0.4	4.4 c ± 0.1
PEF20_EtOH	28.5 d ± 0.4	5.1 d ± 0.3	11.4 d ± 0.5	1.8 b ± 0.5

[1] The same letters in columns denote no significant differences between mean values (Tukey's HSD, $p < 0.05$).

The calculated total color difference (ΔE) values indicate a notable impact of the employed pretreatment methods on the dried insects' color (Table 4). In general, an increase in the ΔE value corresponds to a greater visibility of color differences for an inexperienced observer [43]. The ΔE values were higher than 2.0 only for PEF20 and PEF5_EtOH samples, corresponding to the changes in color recognized by an inexperienced observer [43]. The highest ΔE value was observed for the PEF5_EtOH sample (ΔE = 4.4), which means that inexperienced observers can notice color differences between samples. The color change that occurred could be related not only to non-enzymatic and thermal browning, or to the fat oxidation process [7,26], but also to the different degree of reflection of the light source from the material, which was related to the different water content of the dried insects. Furthermore, due to the PEF treatment and increased porosity, higher light retention inside

the sample may have appeared during the reflectance measurement, and thus, a lower lightness of materials [22].

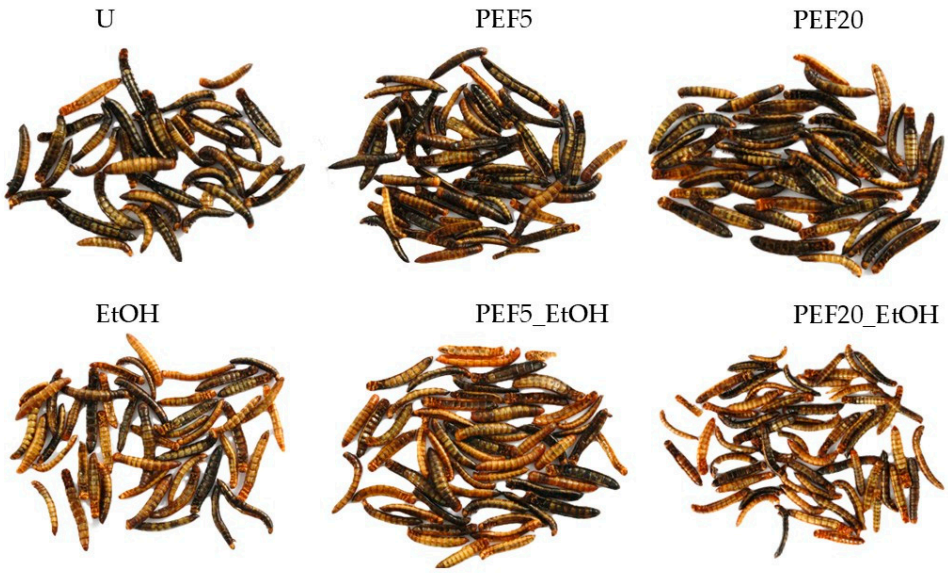

Figure 4. Photos of dried yellow mealworm larvae: without pretreatment (U), pretreated with PEF (PEF5 and PEF20—pulsed electric field with specific energy consumption of 5 and 20 kJ/kg, respectively), and treated with combined pretreatment of ethanol (EtOH) followed by PEF.

3.6. Microstructure of Dried Yellow Mealworm Larvae

The pretreatment and drying process may cause various structural changes in the material. Considering images obtained via X-ray microtomography (Figure 5), the impact of PEF on the internal structure of dried yellow mealworm larvae can be seen. The partial destruction of cellular structures, and thus reduced internal density, was noted for samples subjected to PEF. Also, the partial delamination of the exoskeleton (composed mainly of chitin and proteins) was observed due to the electroporation phenomenon.

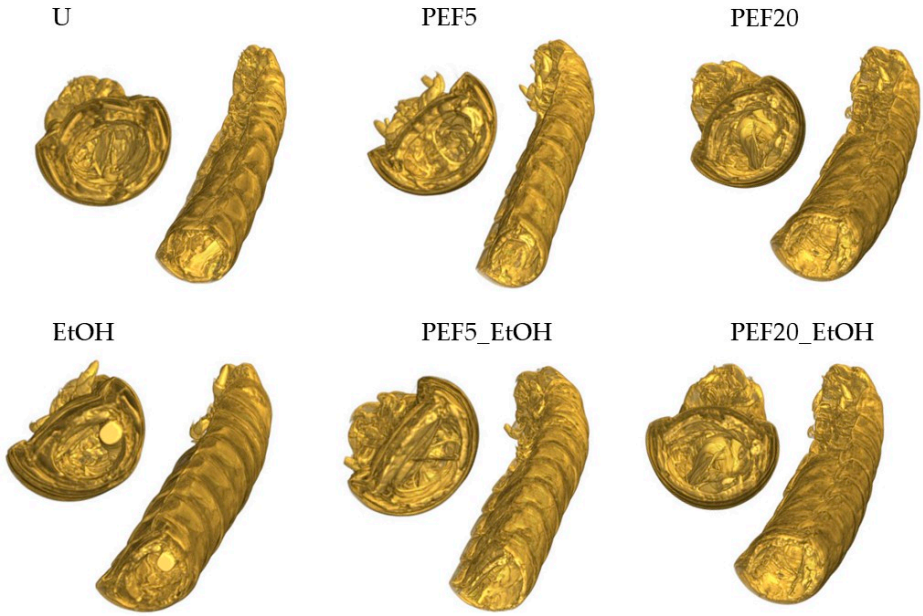

Figure 5. Microstructure of dried yellow mealworm larvae: without pretreatment (U), pretreated with PEF (PEF5 and PEF20—pulsed electric field with specific energy consumption of 5 and 20 kJ/kg, respectively), and treated via combined pretreatment with ethanol (EtOH) followed by PEF.

The additional immersion in ethanol deepened these changes. As is known, ethanol can cause the partial dissolution of cell structures. The damage caused by the PEF application allowed the better penetration of ethanol into the exoskeleton, leading to more visible changes. In our preceding study, we investigated the influence of PEF pretreatment on the microstructure of freeze-dried yellow mealworm larvae, and proved that the use of PEF at 20 kJ/kg resulted in greater changes in the density of internal structures and the fibrous structure of the exoskeleton [26]. In general, the applied pretreatment caused structural changes to the insect tissue. However, further research is needed, as it may depend on factors related to the insect, such as the size or developmental stage, as well as technical factors related to the treatment parameters.

3.7. Microbiological Quality of Dried Yellow Mealworm Larvae

Microbiological quality is an important criterion of food safety, which determines the acceptance for consumption or the necessity of using processes to improve this parameter. This study evaluated the effect of PEF treatment and immersion in ethanol (both alone and combined) on reducing the number of both pathogenic and saprophytic microorganisms transmitted by water and food. The yellow mealworm larvae examined in the study demonstrated good microbiological quality. In fresh larvae, the total count of microorganisms at the level of 4.8 log CFU/g d.m., and the total count of fungi at 3.8 log CFU/g d.m. (Table 5), were observed. Enterobacteriaceae were found only in fresh larvae; the application of thermal treatment affected the reduction in these bacteria below the detection level. To reduce the count of fungi below the detection level, it was necessary to use the combined pretreatment (EtOH followed by PEF). These treatments caused a decrease in the total count of microorganisms and aerobic spore-forming bacteria by more than 2.5 cycle log CFU/g d.m. The EtOH immersion used in this study acted on the surface, while PEF works volumetrically, but with the used parameters, it does not reduce the microorganism load to an acceptable level. Hence, it was impossible to completely reduce the number of microorganisms due to their occurrence in the digestive tracts of the larvae [9,26]. Nevertheless, it can be stated that pretreatment methods may help to reduce the number of microorganisms, and thus ensure consumer safety [44].

Table 5. Saprophytic microorganisms in dried yellow mealworm larvae: without pretreatment (U), pretreated with PEF (PEF5 and PEF20—pulsed electric field with specific energy consumption of 5 and 20 kJ/kg, respectively), and combined pretreatment with ethanol (EtOH) followed by PEF.

Treatment	Microbial Load (log CFU/g d.m.)				
	Total Viable Count (TVC)	Enterobacteriaceae	Aerobic Spore-Forming Bacteria	Anaerobic Spore-Forming Bacteria	Total Yeast and Mold Count (TYMC)
FRESH	4.83 ± 0.12	3.28 ± 0.11	4.65 ± 0.34	≤1.00	3.76 ± 0.22
U	3.15 ± 0.17	≤1.00	3.82 ± 0.15	≤1.00	2.82 ± 0.19
PEF5	2.77 ± 0.33	≤1.00	3.05 ± 0.11	≤1.00	2.55 ± 0.09
PEF20	2.39 ± 0.27	≤1.00	2.77 ± 0.17	≤1.00	2.30 ± 0.14
EtOH	2.84 ± 0.13	≤1.00	3.14 ± 0.25	≤1.00	1.57 ± 0.26
PEF5_EtOH	2.12 ± 0.05	≤1.00	2.45 ± 0.07	≤1.00	≤1.00
PEF20_EtOH	2.17 ± 0.08	≤1.00	2.11 ± 0.03	≤1.00	≤1.00

Insect larvae may also contain pathogenic microorganisms, both on the surface due to contamination from transport or processing steps and in the digestive tract because of the consumption of poor-quality feed [45]. The Regulation (EU) 2022/169 establishes the acceptable levels of microbiological load of yellow mealworm larvae [46]. The examined larvae revealed no water- or food-borne pathogenic microorganisms, e.g., *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* sp. (Table 6). Similarly to our study, the absence of *Salmonella* sp., *Listeria* sp., and *Staphylococcus* sp. in fresh and processed yellow mealworm larvae was found in many studies [9,45,47–49].

Table 6. Pathogenic microorganisms in dried yellow mealworm larvae: without pretreatment (U), pretreated with PEF (PEF5 and PEF20—pulsed electric field with specific energy consumption of 5 and 20 kJ/kg, respectively), and with combined pretreatment via ethanol (EtOH) followed by PEF.

Treatment	Microbial Load (log CFU/g d.m.)			
	Listeria monocytogenes	*Escherichia coli*	*Staphylococcus aureus*	Presence of *Salmonella*
FRESH	≤1.00	≤1.00	≤1.00	absence in 25 g
U	≤1.00	≤1.00	≤1.00	absence in 25 g
PEF5	≤1.00	≤1.00	≤1.00	absence in 25 g
PEF20	≤1.00	≤1.00	≤1.00	absence in 25 g
EtOH	≤1.00	≤1.00	≤1.00	absence in 25 g
PEF5_EtOH	≤1.00	≤1.00	≤1.00	absence in 25 g
PEF20_EtOH	≤1.00	≤1.00	≤1.00	absence in 25 g

Both fresh and dried yellow mealworm larvae revealed a relatively low microbial load (Table 5). In the studies of Yan et al. [9,50], performed on fresh or unprocessed yellow mealworm larvae, TVC was found at 6.4–9.3 log CFU/g, Enterobacteriaceae > 4.2 log CFU/g, fungi 2.6−2.9 log CFU/g, and aerobic bacterial endospores 3.4−5.3 log CFU/g, while *Listeria* sp. and *Salmonella* sp. were absent. In turn, yellow mealworm powder obtained from boiled larvae [9] exhibited a higher TVC (4.4−5.7 log CFU/g) and number of fungi (up to 3.4 log CFU/g), a comparable number of aerobic bacterial endospores (2.1−2.7 log CFU/g), and a notable presence of Enterobacteriaceae (up to 3.6 log CFU/g), compared to the microbial load in the current study (Table 5). Therefore, it can be concluded that the microbiological testing of feeds, breeding environments, and the drying of insects before being approved for consumption are all still essential [9].

4. Conclusions

This research investigated the impacts of pulsed electric field (PEF), immersion in ethanol (EtOH), and combined (immersion in ethanol followed by pulsed electric field) treatment on the convective drying processes and emission of CO_2, along with the physical properties of the resulting dried yellow mealworm larvae. The outcomes exhibit that the PEF application extended the drying time (from about 128 min to about 155 min) due to the better binding of water molecules by the other compounds due to PEF treatment, and the impact on their structure. Microscopic analysis has not identified distinct differences in the internal structure; nevertheless, it cannot be denied that they did not affect the drying process.

Differences in water content, water activity, and rehydration and hygroscopic properties were observed between insect samples, emphasizing the impacts of pretreatment on dried product attributes. PEF-treated insects were characterized by a significantly higher water content (0.034 g H_2O/g d.m.), while immersion in EtOH did not impact the water content compared to untreated ones. In turn, insects pretreated with PEF (alone and combined with EtOH) exhibited significantly lower water activity and higher rehydration and hygroscopic properties than untreated ones. Nonetheless, greater rehydration and hygroscopic properties were found for insects pretreated with a combined method. All insects immersed in ethanol were characterized by a lighter, less brownish color compared to the other samples.

The number of microorganisms in the tested insects revealed a good microbiological quality. Before drying, pretreatment reduced the microbial load of tested insects; nevertheless, a greater effect was observed for combined treatment than for single-PEF treatment or immersion in ethanol. Besides the number of aerobic spore-forming bacteria (≥ 2 log CFU/g d.m.), insects subjected to combined treatment have an ensured quality that meets the microbiological criteria for dried insects and insect-based food.

Based on the obtained results, future studies should research the use of different parameters of PEF or its combination with other non-thermal methods to shorten the time of drying, and to ensure the obtaining of dried insects of high quality.

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