

Article

Comparison of the Effect of Drying Treatments on the Physicochemical Parameters, Oxidative Stability, and Microbiological Status of Yellow Mealworm (*Tenebrio molitor* L.) Flours as an Alternative Protein Source

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Abstract: The increasing production of edible insects on an industrial scale makes it crucial to implement appropriate technologies after harvesting to process safe and high quality insect products. The aim of this work was to compare the impact of different drying treatments used in the production of flour from *Tenebrio molitor* larvae. The larvae were subjected to freeze-drying (FD), conventional drying (CD), microwave drying (MWD), microwave drying without freezing prior blanching (MWDL), and microwave drying with addition of 0.1% butylated hydroxytoluene (BHT) during the blanching of the larvae (MWDA). The studied parameters included water activity (a_w), instrumental colour, chemical composition, lipid oxidative processes, antioxidant activity, as well as microbiological status. The freeze-drying and conventional drying of the larvae reduced the a_w of the derived flours ($p < 0.0001$); however, their nutritional profile revealed lower protein ($p < 0.0001$) and considerably higher fat content ($p < 0.0001$) compared to the flours after microwave treatments. The conventional drying and microwave treatment with BHT induced significantly darker colour ($p < 0.0001$) in comparison to the other methods. Despite the advantages of the microwave drying as a fast and energy efficient method, it displayed some negative effects associated with low lipid stability such as higher acid value (AV) and secondary products of lipid oxidation (TBARS) ($p < 0.0001$). This was also observed in the MWDA flour, indicating a certain pro-oxidative effect of the BHT. Regardless of the drying method, all the flours had a low microbial load.

Keywords: yellow mealworm; drying treatments; flours; nutrients; lipid oxidation; antioxidant activity; safety



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1. Introduction

The agriculture industry is alarmed by a future shortage of food products due to the increasing global population [1]. In recent decades, there has been a continuous increase in the demand for proteins worldwide [2]. It is known that insects are a complete source of proteins, vitamins, and minerals of organic origin. According to Wageningen University, insect protein can replace 10% of the animal protein in the animal feeds and 20% in human diets in 2025 [3]. Insects have high reproductive capacity. Furthermore, the low resource utilization (land and water) and feed for their cultivation makes them a suitable alternative to meat and dairy products, against the backdrop of growing global needs for nutritious food [4]. While in many countries in Asia, Africa and Latin America insects are used as a major protein source, in Western societies people still resist eating them and they have greater potential as animal feed rather than human [5]. Researchers have studied

the aversion to insects to find ways to overcome it and have found that the species of insects chosen for consumption is considered a significant factor [6]. For example, some species are considered more acceptable and visually appealing than others [7]. Yellow mealworms (the larvae of *Tenebrio molitor*) are consumed as a part of a regular diet or for medicinal purposes in some countries outside the EU. The yellow mealworms were the first insects approved by EFSA for human consumption [8], considering the consumption of mealworms as generally safe, as the larvae can be consumed “both as a whole, dried insect and in powder form” [9]. Consumers in Europe are more inclined to consume insects when the body parts are not visible [10]. Therefore, the development of new food products including insects is expected to attract consumer interest. With the increase in production of edible insects on an industrial scale, it is crucial to implement appropriate technologies after their harvesting and processing. Thus, the safety, preservation, quality improvement, fractionation, and storage of the insects and insect products will be guaranteed.

In its report, the World Bank announced inflation in domestic food prices ranging from 5 to 30% in the majority of countries worldwide [2]. This, in turn, necessitates the search for cheaper and more efficient methods for food processing and production. Conventional food and feed processing may include several individual operations. The pathways for insect processing may vary depending on the nature of the initial material and the desired end product. In recent years, new food processing technologies (e.g., high-pressure processing, ultrasound, or microwave waves) have shown potential as alternatives or synergies to conventional technologies. The main challenges in insect processing are the development of efficient, environmentally friendly, and low-cost processing technologies, waste minimization, utilization, and incorporation of by-products. Drying is the most common technology used to extend the shelf life of foods and feeds. Dehydration enhances the microbiological stability, reduces oxidation, and possibly improves the colour and texture of end products. From traditional sun drying to innovative technologies such as freeze-drying or microwave drying, these methods have been studied to increase shelf life and safety. Despite their effectiveness, some pre-treatments are recommended before drying to eliminate or reduce the overall microbiological load and to inactivate enzymes, initiators of lipid and protein oxidation. Blanching is the most commonly used method, both at a traditional and industrial level, as it has safety advantages, reduces lipid oxidation, and inactivates enzymes, contributing to extending shelf life. Microwave drying, in combination with various types of freezing, has been studied by Vlahova-Vangelova [11]. Microwave drying takes less time to dry insects compared to freezing and conventional drying. Finally, drying affects the final quality of the proteins and lipids and the extraction of other metabolites with potential health benefits. Despite the research on the methods of processing insects for their further use in foods and feeds, the information on suitable modes and methods of drying, as well as the qualities of the derived products, including flours, still remains rather scarce. This work was designed to compare the impact of different drying treatments applied in the production of flour from *Tenebrio molitor* larvae as an alternative protein source. This will enable the clarification of their advantages or disadvantages in regards to maximal preservation of the nutritional parameters of the insect flours and selection of the best method for further processing and implementation in the strategies of animal and human nutrition.

2. Materials and Methods

2.1. Larvae Rearing

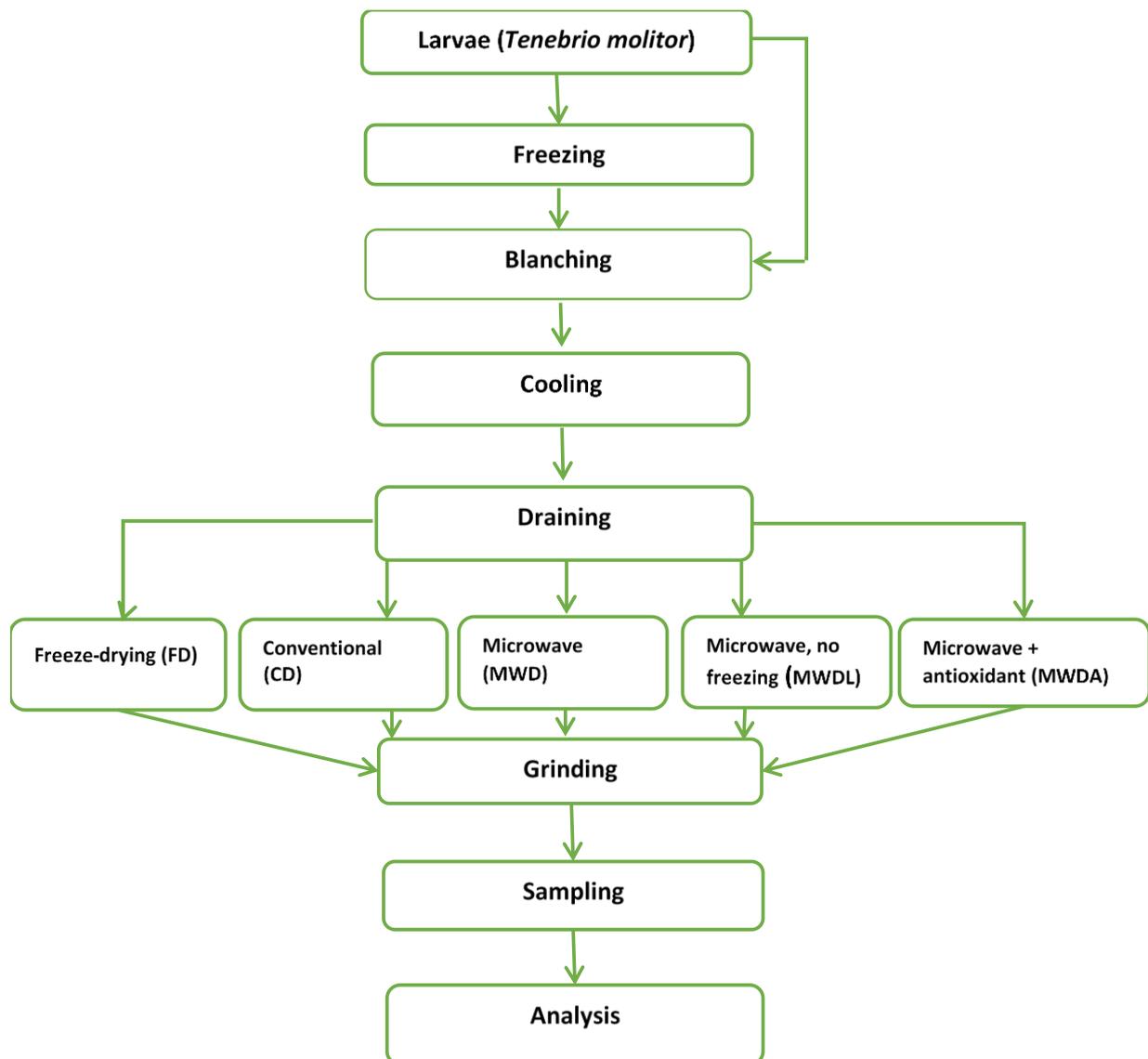
The mealworm larvae were reared in plastic boxes (600 × 400 × 120 mm), at an insect farm located in Petrich, Bulgaria (Vi Bi Ef PRO Ltd.) in controlled microclimate (T = 25 ± 2 °C, RH = 50–70%). The insects were fed on wheat bran up to day 70. Fruits and vegetables were also added 2–3 times a week. Three days prior to harvest, the mealworms were not fed. The proximate composition of the larvae after harvest and before any treatment is presented in Table 1:

Table 1. Proximate composition of the larvae after harvesting.

Protein, %	Fat, %	Ashes, %	Moisture, %	Carbohydrates, %
14.85	1.20	1.02	77.30	5.63

2.2. Drying Treatments

After harvesting, the larvae were separated into five groups (1 kg each). Four of the groups were frozen at $-18\text{ }^{\circ}\text{C}$ and kept at this temperature for 24 h. All the larvae were blanched ($100\text{ }^{\circ}\text{C}$, 45 s) and cooled in cold water prior to drying (Figure 1). For the purpose of the study, five drying treatments were applied:

**Figure 1.** Scheme of the drying treatments applied to *Tenebrio molitor* larvae.

Freeze-drying (FD)—the larvae were freeze-dried ($T = -45\text{--}40\text{ }^{\circ}\text{C}$; 0.400 mBar) for 12–18 h using laboratory freeze dryer CRYODOS—50 (Telstar Industrial, S.L., Terrassa, Spain).

Conventional drying (CD)—the conventional drying was carried out in a hot air rack oven (KC-100/200, Zalmed, Warsaw, Poland), for 6 h ($T = 60\text{ }^{\circ}\text{C}$, RH = 17%, air speed 1 m/s).

Microwave drying (MWD)—the frozen and blanched larvae were dried in microwave (MWD307/WH, Whirlpool, Benton Harbor, MI, USA) at 825 W for 7 min.

Microwave drying without freezing (MWDL)—the live directly blanched larvae were dried in the microwave oven at 825 W for 7 min.

Microwave drying + antioxidant (MWDA)—after freezing, the larvae were blanched in water containing 0.1% BHT and dried in the microwave at 825 W for 7 min.

After drying, the insect flours were prepared by grinding the larvae using a Nutribullet blender (NB-WL046A-02, Capital Brands, Los Angeles, CA, USA). Each flour was divided into three samples of approximately 200 g. The samples were stored in sealed plastic bags until analysis.

2.3. Water Activity (a_w) and Instrumental Colour Measurements

Water activity was determined using an a_w meter LabSwift-aw (Novasina AG, Lachen, Switzerland) at 20 °C.

Colour measurements of the flours were conducted using a Konica Minolta CR-400 chromameter (Konica Minolta Holding, Inc., Ewing, NJ, USA) by measuring the lightness (L^*), redness (a^*), and yellowness (b^*). The chromameter had the following settings: aperture = 8 mm, standard observer 2°, and illuminant D65. The instrument was calibrated using a standard white plate ($Y = 94.3$, $x = 0.3134$ and $y = 0.3197$).

2.4. Proximate Composition

The total nitrogen was determined by the AOAC [12], and the protein content was calculated by nitrogen-to-protein conversion factor 4.76, as proposed by Janssen et al. [13] to compensate for the presence of chitin-derived nitrogen.

The fat content was determined after extraction performed by Soxhlet apparatus [14].

Total ash content was measured after incineration of the insect flour [15].

The moisture content of the flours was calculated after drying at 104–105 °C using a KERN MLS 65 3A—moisture analyser (Kern & Sohn GmbH, Balingen, Germany) until constant weight [16]. The carbohydrate content was calculated using the following equation [17]:

$$\text{Carbohydrates} = 100 - \text{Protein} - \text{Fat} - \text{Ash} - \text{Moisture, \%}$$

2.5. Acid Value (AV)

The degree of lipolysis was described by the acid value of the extracted lipids and measured following the method of Kardash and Tur'yan [18]. The extraction of lipids was performed according to the method of Bligh and Dyer [19]. After extraction, 1 g of lipid was dissolved in 20 cm³ neutral alcohol–ether mixture with added phenolphthalein. The mixture was titrated with 0.01N KOH. The volume of the used KOH was recorded. The acid value was calculated as $AV = (V \times F \times 5.6104)/m$, mg KOH/g, where:

V—volume of KOH used for titration, g.

F—factor of 0.1 nKOH = 0.996.

m—the weight of the sample, g.

2.6. Peroxide Value (PV)

Peroxide value was determined according to Shantha and Decker [20]. Briefly, the extracted lipid (0.1 g) was mixed in a glass tube with 50 µL iron (II) solution, 50 µL NH₄ SCN (300 mg/mL), and CHCl₃:CH₃OH (3:5, v/v) for a final volume of 10 mL. The samples were incubated for 5 min at room temperature and the absorbance was determined by spectrophotometer at 507 nm against a blank, containing all the reagents except the sample. The PV was calculated through a standard curve set up using Fe³⁺ chloride standard solution (10 µg/mL). The results were presented as meqO₂/kg lipid.

2.7. TBARS Content

The content of the thiobarbituric acid reactive substances was determined as described by Botsoglou et al. [21] with slight modifications. Ten grams of flour were homogenized with 50 mL NaCl (0.9%), and left for 5 min. Furthermore, 50 mL of trichoroacetic acid (10%) were added and the samples were filtered through (Filtrax, Grade 391). The filtered samples (4 mL) were then mixed with 1 mL 2-thiobabituric acid (1%) and were incubated at 70 °C for 30 min. After cooling to room temperature, the absorbance of the samples was determined at 532 nm against a blank, containing distilled water instead of the sample. TBARS concentrations were calculated using 1, 1, 3, 3 tetraethoxypropane as standard. The results were expressed as mg MDA/kg product or TBARS units.

2.8. Antioxidant Activity

The antioxidant activity of the flours was measured through DPPH assay according to the method of Brand-Williams et al. [22], modified by Dinkova et al. [23]: 250 µL of the sample extract (in methanol 1/10, *w/v*) was mixed with 2250 µL of methanolic solution of DPPH (6×10^{-5} M) in a UV-macro cuvette. The cuvettes were sealed and left in the dark for 15 min at room temperature. Absorbance was measured at 515 nm against a blank of pure methanol. The results were expressed as µmol TE (Trolox Eq)/100 g sample.

2.9. Microbiological Assay

All of the preparation and decimal dilutions of sample suspensions were conducted according to ISO 6887-4:2017 [24]. The microbiological status was presented by the Total Plate Count (TPC), Coliforms count, and *E. coli* count, following the procedure of ISO 4833-1:2013/Amd 1:2022 [25].

All of the analyses were performed in triplicates.

2.10. Statistical Evaluation

The statistical evaluation was performed through one way ANOVA procedure and post-hoc comparisons (Tukey HSD, $p < 0.05$) using JMP v. 7 statistical software [26].

3. Results

3.1. Water Activity and Instrumental Colour

The drying treatments significantly affected the a_w and they differed among all the flours. The lowest value of this parameters was measured in the CD flour, whereas the MWDA flour exhibited the highest a_w (Table 2). The freezing of the larvae prior to blanching for the microwave treatment decreased the water activity, and as a result the MWD flour had lower a_w when compared to MWDL flour.

Table 2. Water activity and instrumental colour parameters of the flours derived from *Tenebrio molitor* larvae according to the drying treatment.

Treatment	a_w	L*	a*	b*
FD	0.35 ^d	52.76 ^b	6.55 ^c	11.79 ^b
CD	0.14 ^e	49.09 ^c	6.17 ^d	9.95 ^c
MWD	0.50 ^c	51.74 ^b	7.29 ^a	12.94 ^a
MWDL	0.54 ^b	54.70 ^a	6.63 ^c	13.27 ^a
MWDA	0.63 ^a	49.54 ^c	7.00 ^b	13.37 ^a
SEM	0.01	0.44	0.05	0.34
<i>p</i>	<0.0001	<0.0001	<0.0001	<0.0001

FD, freeze-drying; CD, conventional drying; MWD, microwave drying; MWDL, microwave drying without freezing prior blanching; MWDA, microwave drying + 0.1% BHT; SEM, standard error of the mean. The means connected with different letters in a row are significantly different ($p < 0.05$).

The drying treatments had a significant effect on the lightness of the flours (Table 2). The darkest colour was observed in the CD and MWDA flours, whereas the MWDL flour

was the lightest. The freeze-drying and microwave drying of the insect larvae also resulted in the lighter colour of the FD and MWD flours, when compared to the oven dried and MWDA flours. In addition to the lower L^* , the CD flour also had lower a^* and b^* , when compared to the flours derived after the other studied methods of drying. Higher a^* values were observed in the MWD and MWDA flour. These flours, together with the MWDL flour, exhibited higher b^* values in comparison to the FD and CD samples.

3.2. Proximate Composition

The freeze-drying and conventional drying of the *Tenebrio molitor* larvae led to lower protein content of the derived flours (Table 3). The highest protein content was measured in the MWDL followed by MWD flours. Contrary to protein content, the fat percentage was significantly higher in FD and CD flours when compared to the rest. FD, CD, and MWDA flours had ash content within the range of 4.16–4.23%, which was significantly higher than MWD and MWDL.

Table 3. Proximate composition of the flours derived from *Tenebrio molitor* larvae according to the drying treatment.

Treatment	Protein, %	Fat, %	Ash, %	Moisture, %	Carbohydrate, %
FD	41.21 ^d	20.82 ^a	4.17 ^a	6.07	27.73 ^c
CD	41.42 ^d	21.11 ^a	4.23 ^a	5.62	27.62 ^c
MWD	47.14 ^b	12.71 ^b	2.87 ^c	5.71	31.57 ^{ab}
MWDL	49.99 ^a	11.35 ^b	3.83 ^b	5.18	29.65 ^{bc}
MWDA	44.97 ^c	11.42 ^b	4.17 ^a	6.29	33.15 ^a
SEM	0.75	0.55	0.10	0.46	0.97
<i>p</i>	<0.0001	<0.0001	<0.0001	0.1034	0.0001

FD, freeze-drying; CD, conventional drying; MWD, microwave drying; MWDL, microwave drying without freezing prior blanching; MWDA, microwave drying + 0.1% BHT; SEM, standard error of the mean. The means connected with different letters in a row are significantly different ($p < 0.05$).

The latter differed in regard to the ash content as MWD displayed lower values of this parameter in comparison to MWDL flour. With regard to the carbohydrates, their percentage was lower in the FD and CD than in MWD and MWDA flours, while the carbohydrate content of the MWDL flour had an intermediate position.

3.3. Lipid Stability

The hydrolysis in lipids of the derived flours were presented through the acid value (Table 4).

Table 4. Acid value (AV), peroxide value (PV), and TBARS value in the lipids of flours derived from *Tenebrio molitor* larvae according to the drying treatment.

Treatment	AV, mgKOH/g	PV, meqO ₂ /kg	TBARS, mgMDA/kg
FD	1.94 ^c	1.27 ^a	0.05 ^c
CD	1.69 ^d	1.22 ^{ab}	0.06 ^c
MWD	2.58 ^b	0.97 ^{bc}	0.12 ^a
MWDL	2.55 ^b	0.91 ^c	0.10 ^b
MWDA	3.10 ^a	1.16 ^{abc}	0.10 ^b
SEM	0.04	0.10	0.005
<i>p</i>	<0.0001	0.0080	<0.0001

FD, freeze-drying; CD, conventional drying; MWD, microwave drying; MWDL, microwave drying without freezing prior blanching; MWDA, microwave drying + 0.1% BHT; SEM, standard error of the mean. The means connected with different letters in a row are significantly different ($p < 0.05$).

Lower AV was found in the FD and CD flours, while the microwave drying in all three modes stimulated the lipid hydrolysis.

The content of the primary products of lipid oxidation also depended on the drying treatment. The highest levels of peroxides were measured in the flours derived after freeze-drying and conventional drying of the *Tenebrio molitor* larvae. Microwave drying; however, was associated with lower content of peroxides, except for the MWDA flour.

The analysis of the secondary products of lipid oxidation showed that FD and CD flours had significantly lower TBARS levels when compared to all the flours derived from microwave dried insects.

3.4. Antioxidant Activity

Not surprisingly, MWDA displayed the highest radical scavenging activity (Figure 2). It was followed by the MWDL and MWD flours. The lowest radical scavenging activity was observed in the FD and CD samples.

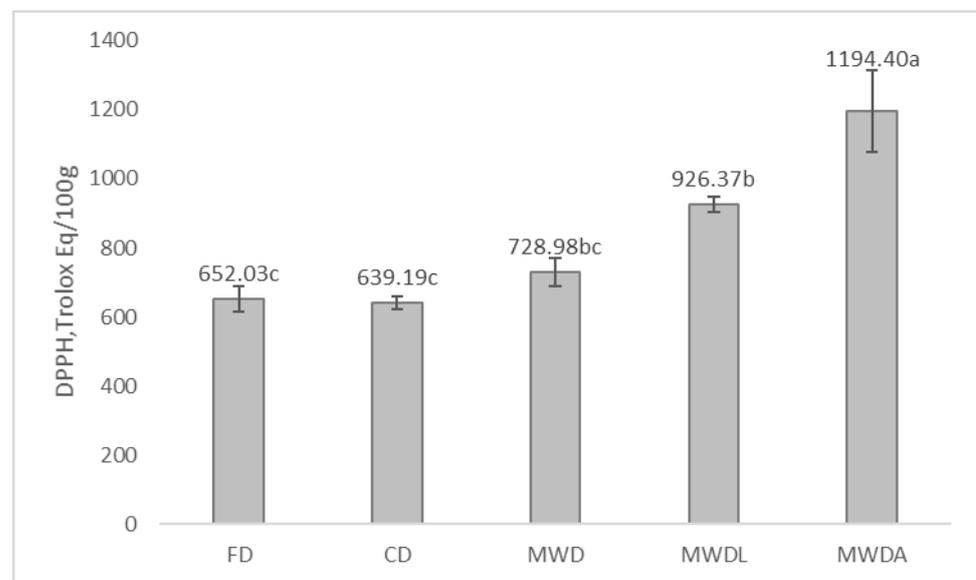


Figure 2. Antioxidant activity of the flours derived from *Tenebrio molitor* larvae according to the drying treatment. FD, CD, MWD, MWDL, and MWDA represent freeze-drying, conventional drying, microwave drying, microwave drying without freezing prior blanching, and microwave drying + 0.1% BHT, respectively. The means connected with different letters in a row are significantly different ($p < 0.05$).

3.5. Microbiological Status

The total plate count was higher in the FD flours in comparison to all the other flours in this study; however, the differences were not significant (Table 5).

Table 5. Microbiological status of the flours derived from *Tenebrio molitor* larvae according to the drying treatments.

Treatment	TPC, log ₁₀ CFU/g	Coliforms, log ₁₀ CFU/g	Yeasts Moulds, log ₁₀ CFU/g	<i>E. coli</i> , log ₁₀ CFU/g
FD	4.52	2.93	N.D.	N.D.
CD	3.91	3.19	N.D.	N.D.
MWD	3.93	2.97	N.D.	N.D.
MWDL	3.97	2.87	N.D.	N.D.
MWDA	3.99	2.90	N.D.	N.D.
SEM	0.62	0.50		
<i>p</i>	0.7279	0.9411		

FD, freeze-drying; CD, conventional drying; MWD, microwave drying; MWDL, microwave drying without freezing prior blanching; MWDA, microwave drying + 0.1% BHT; SEM, standard error of the mean; TPC, total plate count; N.D.—not detected.

The coliform content varied within 2.87–3.19 log₁₀CFU/g, with no statistical difference among the drying treatments. Yeasts, moulds, and *E. coli* were not detected.

4. Discussion

Drying of the larvae is of crucial importance for the further processing of the insects so that the nutritional quality and safety are guaranteed. Some studies have already reported changes in the quality characteristics of edible insects or insect flours due to different drying methods [11,15,27–29]. The results of this study confirmed the effect of freeze-drying, oven drying, and microwave drying of the larvae on the water activity of the flours that was observed by Krönke et al. [28] and Vlahova-Vangelova et al. [11]. The freeze-drying and conventional drying considerably reduced the water activity of the flours, while it was higher after microwave drying. Substances with higher water activity tend to support microbial growth; most bacteria usually require a_w at least 0.91 [30], while some moulds and yeasts need a_w in the range of 0.61–0.65 [31]. Colour is one of the most important quality attributes. It is a crucial factor influencing the consumer's decision when buying a new product, but also can indicate chemical changes that occur in food [32]. Colour can change during drying due to chemical and biochemical reactions [33]. In our study, the lowest L* were observed for the flours derived after conventional drying and microwave drying of the larvae blanched in the presence of BHT. The other microwave treatments led to a lighter colour, compared to conventional drying and freeze-drying. In their study, Trukhanova et al. [34] observed lighter colour of the microwave dried larvae of *Tenebrio molitor*, when compared to the larvae dried through convection, which is in line with our results. The lower L* values observed in the CD flour can be attributed to Maillard reaction, due to the longer drying process (6 h). Usually, this type of browning occurs in foods that have high lipid content and where reactions between products of lipid oxidation with amino acids, amines, and proteins occur [32]. Maillard reaction and lipid oxidation are interrelated and should be considered simultaneously in regard to colour changes [35]. The microwave treated flours also exhibited elevated content of secondary products of lipid oxidation, which was considerably higher in comparison to FD and CD flour. Furthermore, the significantly darker colour of the MWDA flour suggests a certain pro-oxidative effect of the BHT in the amount used to treat the larvae for the present experiment. Some phenolic compounds might act as pro-oxidants depending on the conditions of the environment (such as high pH, presence of oxygen molecules, and high concentration of transition metals) [36].

There was considerable variation in the chemical components of the flours according to the drying treatment. Generally, the flours derived from microwave dried larvae had higher protein content when compared to the FD and CD flours. Additionally, the flours derived from larvae that were not frozen prior to blanching exhibited the highest protein content of all the flours. This indicates the negative effect of freezing, most likely associated with the denaturation of proteins in the other flours in the study [37]. The higher protein content in the MWD, MWDL, and MWDA flours corresponded to their lower fat percentage when compared to the FD and CD flours. The latter did not differ in regard to the protein and fat content. Our results coincided with those of Selaledi and Mabelele [1], who did not observe any differences in the protein content of *Tenebrio molitor* larvae when freeze-dried and oven dried. In contrast to our data, Krönke et al. [38] found that rack oven dried mealworm larvae had higher protein and fat content than freeze-dried larvae. The authors also reported dramatic increase in moisture after freeze-drying, whereas in our study, we failed to observe any significant difference in the moisture content among the flours. The moisture content only tended to be higher in the MWDA flour and corresponded to its highest a_w .

When comparing the different drying treatments applied in this study, we can conclude that the conventional drying is associated with lowest degree of free fatty acid accumulation, whereas the microwave drying stimulated the hydrolytic changes in the lipid fraction of the insect flours. Other studies also reported a similar effect of the microwave treatment on

the lipid stability in foods, associated with a considerable degree of hydrolysis [39,40]. The freezing prior to blanching of the *Tenebrio molitor* larvae did not affect the lipid hydrolysis in the flours derived from the microwave treated larvae. The comparison of the flours derived after the different microwave treatments showed that in the BHT addition there was a 20% increase in the free fatty acid content, again indicating the potential pro-oxidative effect of BHT. According to Pérez-Torres et al. [41], the high concentrations of strong antioxidants such as BHT in the food matrices exert a pro-oxidative effect. Hence, the higher degree of hydrolysis of the lipids in the MWDA flour might be due to the higher dose of BHT. The elevated AV of the flours derived after microwave treatment corresponded to their higher TBARS values. Usually, with advancing of lipid oxidation, triacylglycerols are converted into fatty acids and glycerol thus increasing the acid value, which is in line with our results. On the other hand, we observed a decrease in the PV of the MWD and MWDL flours, corresponding to the elevated levels of TBARS in these flours. The lipid peroxides are unstable and susceptible to decomposition [42] and formation of other products, such as alcohols, ketones, and aldehydes. The freeze-drying and conventional drying were associated with a significant increase in the peroxide content in the insect flours and the same was observed in the MWDA samples. In all the samples regardless of the drying treatment, PV remained below 2 meq/O₂, which is considered as a threshold recommended by EFSA [43].

Despite the higher degree of lipid oxidation, the flours derived of microwave dried larvae displayed higher antioxidant activity in comparison to the FD and CD flours. Not surprisingly, the MDWA flour exhibited the highest antioxidant activity. As reported in previous studies, the antioxidant content correlates positively with the radical scavenging activity [44]. On the other hand, the higher antioxidant activity measured in the MWD, MWDL and MWDA flours contradicts to the higher degree of lipid oxidation. It can be suggested that the products from lipid oxidation have reacted with DPPH, presenting falsely increased antioxidant activity [45].

Microbiological status is crucial for the safety of the foods. In our study, we observed a low microbial load of the flours. In their study, Bußler et al. [46] observed a high microbial contamination in *Tenebrio molitor* larvae before processing (8.1 log CFU/g) that reduced to 4.3 log CFU in the high protein fraction. Kluder et al. [47] found a low microbial load in whole *Tenebrio molitor* larvae after boiling and roasting (<1.7 log CFU/g); however, after crushing, the TVC was considerably augmented (2.5–4.8 log CFU/g). The analysis of the results showed that the total plate count measured in the FD flours was higher than the CD, MWD, MWDL, and MWDA flours. Caparros Megido et al. [48] reported data about different treatments on the microbial load in edible insects. Similar to our results, the authors measured the total aerobic count in freeze-dried mealworm and house crickets as 4.47 and 4.05 log CFU/g, respectively, significantly higher than the microbial load in the sterilized insects. Messina et al. [49] presented a microbiological profile of powders prepared using mealworm and house crickets after prolonged storage. They identified seven microbial populations in the *Tenebrio molitor* powder, consisting of LAB cocci, enterococci, pseudomonads, CPS, and members of the Bacillaceae family, while yeasts and moulds were not detected. The flours presented in this study showed insignificant differences in regard to the coliforms. Yeast, moulds, and *E. coli* were not detected, showing the high level of microbial safety of the applied drying treatments.

5. Conclusions

The drying treatments caused considerable changes in the qualities of the flours prepared from *Tenebrio molitor* larvae. The flours obtained by freeze-dried and conventionally dried larvae had lower water activity and showed considerably higher fat content than the microwave treated flours; however, their nutritional profile was less favourable with lower protein. The conventionally dried flour, as well as the flour derived from microwave treated insects with addition of BHT, exhibited a darker colour that can probably be attributed to the Maillard reaction and lipid oxidation. Although known as a fast and energy efficient

method, in this study, the microwave drying displayed some negative effects associated with a higher degree of lipid oxidation in the flours. Increased oxidation was also observed after BHT treatment, indicating that the dose of the antioxidant used in the study (0.1%) is not recommended for obtaining a good quality in the flours treated with microwaves. Showing both positive and negative effects of the applied treatments, this study contributes to the development of optimal drying modes for preparation of insect flours. However, further experiments are needed to determine the most appropriate drying technique that will help to preserve the best nutritional qualities of the flours while restricting the processes that will deteriorate them.

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References

- Selaledi, L.; Mabelebele, M. The influence of drying methods on the chemical composition and body color of yellow mealworm (*Tenebrio molitor* L.). *Insects* **2021**, *12*, 333. [CrossRef]
- Food Security Update. World Bank Response to Rising Food Insecurity. The World Bank. Available online: <https://thedocs.worldbank.org/en/doc/40ebbf38f5a6b68bfc11e5273e1405d4-0090012022/related/Food-Security-Update-XCV-11-9-23.pdf> (accessed on 11 December 2023).
- Insects as Replacement of Animal Protein Gets a Boost. Available online: <https://www.wur.nl/en/newsarticle/insects-as-replacement-of-animal-protein-gets-a-boost.htm> (accessed on 21 January 2024).
- Smarzyński, K.; Sarbak, P.; Musiał, S.; Jezowski, P.; Piątek, M.; Kowalczewski, P. Nutritional analysis and evaluation of the consumer acceptance of pork pâté enriched with cricket powder—Preliminary study. *Open Agric.* **2019**, *4*, 159–163. [CrossRef]
- Kim, T.K.; Yong, H.I.; Kim, Y.B.; Kim, H.W.; Choi, Y.S. Edible Insects as a protein source: A review of public perception, processing technology, and research trends. *Food Sci. Anim. Resour.* **2019**, *39*, 521–540. [CrossRef] [PubMed]
- Schäufele, I.; Albores, E.B.; Hamm, U. The role of species for the acceptance of edible insects: Evidence from a consumer survey. *Br. Food J.* **2019**, *121*, 2190–2204. [CrossRef]
- Fischer, A.R.H.; Steenbekkers, B. All insects are equal, but some insects are more equal than others. *Br. Food J.* **2018**, *120*, 852–863. [CrossRef] [PubMed]
- Turck, D.; Castenmiller, J.; De Henauw, S.; Hirsch Ernst, K.; Kearney, J.; Maciuk, A.; Knutsen, H. Safety of dried yellow mealworm (*Tenebrio molitor* larva) as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA J.* **2021**, *19*, 6343. [CrossRef]
- Novel Food: Insects—Food of the Future?! Available online: https://www.eurofins.de/food-analysis/food-news/food-testing-news/novel-food_insects-as-food-of-the-future/ (accessed on 30 January 2024).
- Hartmann, C.; Shi, J.; Giusto, A.; Siegrist, M. The psychology of eating insects: A cross-cultural comparison between Germany and China. *Food Qual. Prefer.* **2015**, *44*, 148–156. [CrossRef]
- Vlahova-Vangelova, D.; Balev, D.; Kolev, N.; Stoyanov, V. Effect of drying regimes on the quality and safety of alternative protein source (*Tenebrio molitor* L.). *Acta Sci. Pol. Technol. Aliment.* **2023**, *22*, 217–225. [CrossRef]
- AOAC. *Official Methods of Analysis of AOAC International: AOAC 984.13 1994 Protein (Crude) in Animal Feed and Pet Food*; AOAC: Rockville, MD, USA, 1996.
- Janssen, R.; Vincken, J.; van den Broek, L.; Fogliano, V.; Lakemond, C. Nitrogen to protein conversion factors for three edible insects: *Tenebrio molitor*, *Alphitobius diaperinus*, and *Hermetia illucens*. *J. Agric. Food Chem.* **2017**, *65*, 2275–2278. [CrossRef]
- ISO 1444:1996; Meat and Meat Products—Determination of Free Fat Content. ISO: Geneva, Switzerland, 1996.
- Lenaerts, S.; Van Der Borght, M.; Callens, A.; Van Campenhout, L. Suitability of microwave drying for mealworms (*Tenebrio molitor*) as alternative to freeze drying: Impact on nutritional quality and colour. *Food Chem.* **2018**, *254*, 129–136. [CrossRef]

16. Vandeweyer, D.; Lenaerts, S.; Callens, A.; Van Campenhout, L. Effect of blanching followed by refrigerated storage or industrial microwave drying on the microbial load of yellow mealworm larvae (*Tenebrio molitor*). *Food Control* **2017**, *71*, 311–314. [[CrossRef](#)]
17. Joymak, W.; Ngamukote, S.; Chantarasinlapin, P.; Adisakwattana, S. Unripe papaya by-product: From food wastes to functional ingredients in pancakes. *Foods* **2021**, *10*, 615. [[CrossRef](#)]
18. Kardash, E.; Tur'yan, Y.I. Acid value determination in vegetable oils by indirect titration in aqueous-alcohol media. *Croat. Chem. Acta* **2005**, *78*, 99–103.
19. Bligh, E.G.; Dyer, W.J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911–917. [[CrossRef](#)]
20. Shantha, N.C.; Decker, E.A. Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. *J. AOAC Int.* **1994**, *7*, 421–424. [[CrossRef](#)]
21. Botsoglou, N.A.; Fletouris, D.J.; Papageorgiou, G.E.; Vassilopoulos, V.N.; Mantis, A.J.; Trakatellis, A.G. Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. *J. Agric. Food Chem.* **1994**, *42*, 1931–1937. [[CrossRef](#)]
22. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT* **1995**, *28*, 25–30. [[CrossRef](#)]
23. Dinkova, R.; Heffels, P.; Shikov, V.; Weber, F.; Schieber, A.; Mihalev, K. Effect of enzyme-assisted extraction on the chilled storage stability of bilberry (*Vaccinium myrtillus* L.) anthocyanins in skin extracts and freshly pressed juices. *Food Res. Int.* **2014**, *65*, 35–41. [[CrossRef](#)]
24. *ISO 6887 4:2017; Microbiology of the Food Chain—Preparation of Test Samples, Initial Suspension and Decimal Dilutions for Microbiological Examination—Part 4: Specific Rules for the Preparation of Miscellaneous Products*. ISO: Geneva, Switzerland, 2017.
25. *ISO 4833 1:2013/Amd 1:2022; Microbiology of the Food Chain—Horizontal Method for the Enumeration of Microorganisms—Part 1: Colony Count at 30 °C by the Pour Plate Technique—Amendment 1: Clarification of Scope*. ISO: Geneva, Switzerland, 2013.
26. *JMP, Version 7*; SAS Institute Inc.: Cary, NC, USA, 2007.
27. Fombong, F.T.; Van Der Borght, M.; Vanden Broeck, J. Influence of freeze-drying and oven-drying post blanching on the nutrient composition of the edible insect *Ruspolia differens*. *Insects* **2017**, *8*, 102. [[CrossRef](#)] [[PubMed](#)]
28. Kröncke, N.; Bösch, V.; Woyzichowski, J.; Demtröder, S.; Benning, R. Comparison of suitable drying processes for mealworms (*Tenebrio molitor*). *Innov. Food Sci. Emerg. Technol.* **2018**, *50*, 20–25. [[CrossRef](#)]
29. Yisa, N.K.; Osuga, I.M.; Subramanian, S.; Ekesi, S.; Emmambux, M.N.; Duodu, K.G. Effect of drying methods on the nutrient content, protein and lipid quality of edible insects from East Africa. *J. Insects Food Feed* **2022**, *9*, 647–659. [[CrossRef](#)]
30. Allen, L.V., Jr. Quality control: Water activity considerations for beyond-use dates. *Int. J. Pharm. Compd.* **2018**, *22*, 288–293. [[PubMed](#)]
31. Tapia, M.S.; Alzamora, S.M.; Chirife, J. Effects of Water Activity (aw) on Microbial Stability: As a Hurdle in Food Preservation. In *Water Activity in Foods: Fundamentals and Applications*; Barbosa-Cánovas, G.V., Fontana, A.J., Jr., Schmidt, S.J., Labuza, T.P., Eds.; Blackwell Publishing Ltd.: Oxford, UK, 2008; pp. 239–271. [[CrossRef](#)]
32. Hidalgo, F.J.; Zamora, R. The role of lipids in nonenzymatic browning. *Grasas Aceites* **2000**, *51*, 35–49. [[CrossRef](#)]
33. Bonazzi, C.; Dumoulin, E. Quality changes in food materials as influenced by drying processes. In *Modern Drying Technology*; Tsotsas, E., Mujumdar, A.S., Eds.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2011; pp. 1–20.
34. Trukhanova, K.A.; Mechtaeva, E.V.; Novikova, M.V.; Sorokoumov, P.N.; Ryabukhin, D.S. Influence of drying and pretreatment methods on certain parameters of yellow mealworm larvae (*Tenebrio molitor*). *Theory Pract. Meat Process.* **2022**, *7*, 247–257. [[CrossRef](#)]
35. Zamora, R.; Hidalgo, F.J. Coordinate contribution of lipid oxidation and Maillard reaction to the nonenzymatic food browning. *Crit. Rev. Food Sci. Nutr.* **2005**, *45*, 49–59. [[CrossRef](#)]
36. Cotoras, M.; Vivanco, H.; Melo, R.; Aguirre, M.; Silva, E.; Mendoza, L. In vitro and in vivo evaluation of the antioxidant and prooxidant activity of phenolic compounds obtained from grape (*Vitis vinifera*) pomace. *Molecules* **2014**, *19*, 21154. [[CrossRef](#)] [[PubMed](#)]
37. Lee, S.; Jo, K.; Jeong, H.G.; Choi, Y.-S.; Kyoung, H.; Jung, S. Freezing-induced denaturation of myofibrillar proteins in frozen meat. *Crit. Rev. Food Sci. Nutr.* **2024**, *64*, 1385–1402. [[CrossRef](#)] [[PubMed](#)]
38. Kröncke, N.; Grebenteuch, S.; Keil, C.; Demtröder, S.; Kroh, L.; Thünemann, A.F.; Benning, R.; Haase, H. Effect of different drying methods on nutrient quality of the yellow mealworm (*Tenebrio molitor* L.). *Insects* **2019**, *10*, 84. [[CrossRef](#)]
39. Chandrasekaran, S.; Ramanathan, S.; Basak, T. Microwave food processing—A review. *Food Res. Int.* **2013**, *52*, 243–261. [[CrossRef](#)]
40. Jiang, H.; Liu, Z.; Wang, S. Microwave processing: Effects and impacts on food components. *Crit. Rev. Food Sci. Nutr.* **2018**, *58*, 2476–2489. [[CrossRef](#)]
41. Pérez-Torres, I.; Guarner-Lans, V.; Rubio-Ruiz, M.E. Reductive stress in inflammation-associated diseases and the pro-oxidant effect of antioxidant agents. *Int. J. Mol. Sci.* **2017**, *18*, 2098. [[CrossRef](#)] [[PubMed](#)]
42. Talbot, G. The stability and shelf life of fats and oils. In *The Stability and Shelf Life of Food*; Subramaniam, P., Ed.; Elsevier: Amsterdam, The Netherlands, 2016; pp. 461–503. [[CrossRef](#)]
43. European Food Safety Authority (EFSA). Scientific opinion on fish oil for human consumption. Food hygiene, including rancidity. *EFSA J.* **2010**, *8*, 1874. [[CrossRef](#)]

44. Kumar, S.; Sandhir, R.; Ojha, S. Evaluation of antioxidant activity and total phenol in different varieties of *Lantana camara* leaves. *BMC Res. Notes* **2014**, *7*, 560. [[CrossRef](#)] [[PubMed](#)]
45. Jeong, M.K.; Yeo, J.D.; Jang, E.Y.; Kim, M.-J.; Lee, J.H. Aldehydes from oxidized lipids can react with 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals in isooctane systems. *J. Am. Oil Chem. Soc.* **2012**, *89*, 1831–1838. [[CrossRef](#)]
46. Bußler, S.; Rumpold, B.A.; Jander, E.; Rawel, H.M.; Schlüter, O.K. Recovery and techno-functionality of flours and proteins from two edible insect species: Meal worm (*Tenebrio molitor*) and black soldier fly (*Hermetia illucens*) larvae. *Heliyon* **2016**, *2*, e00218. [[CrossRef](#)]
47. Klunder, H.C.; Wolkers-Rooijackers, J.; Korpela, J.M.; Nout, M.J.R. Microbiological aspects of processing and storage of edible insects. *Food Control* **2012**, *26*, 628–631. [[CrossRef](#)]
48. Caparros Megido, R.; Desmedt, S.; Blecker, C.; Béra, F.; Haubruge, É.; Alabi, T.; Francis, F. Microbiological load of edible insects found in Belgium. *Insects* **2017**, *8*, 12. [[CrossRef](#)]
49. Messina, C.M.; Gaglio, R.; Morghese, M.; Tolone, M.; Arena, R.; Moschetti, G.; Santulli, A.; Francesca, N.; Settanni, L. Microbiological profile and bioactive properties of insect powders used in food and feed formulations. *Foods* **2019**, *8*, 400. [[CrossRef](#)]

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