

Impact of drying techniques on protein denaturation in *Tenebrio molitor*: A thermal analysis via differential scanning calorimetry

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The increasing global demand for protein, driven by rapid population growth, calls for sustainable production methods with minimal environmental impact. *Tenebrio molitor* (mealworms) offer a promising, sustainable protein source for both, feed and food, applications. In industrial mealworm production, optimized drying techniques, essential for maintaining product quality, are critical. The key to selecting the drying technology and process conditions is the protein content of the final product. This study, therefore, compares drying time and product quality of *T. molitor* from freeze-drying and oven drying at 40°C and 60°C and investigates the potentials and limits of differential scanning calorimetry (DSC) to quantitatively assess protein denaturation of the dried product.

Keywords: Protein denaturation, sustainable protein source, mealworm, *Tenebrio molitor*, freeze-drying, oven drying, differential scanning calorimetry (DSC)

INTRODUCTION

According to a report by the United Nations, the global population is expected to reach at least 11.5 billion by 2050, leading to an increase in global food demand by up to 70% compared to the current situation [1]. Traditional sources of animal protein, such as beef, pork, and chicken may not be sufficient to meet this demand. Therefore, the development of sustainable protein production methods with minimal negative environmental impact is regarded as a potential route to overcome the expected gap [2, 3]. Novel protein sources include single-cell proteins, fish protein concentrates, and edible insects. Since 2010, the Food and Agriculture Organization (FAO) has recognized the potential of insects as human food and animal feed to ensure food security [4, 5].

Entomophagy, or consumption of insects, has been practiced for many years across various regions worldwide. Insect-based foods are consumed by over two billion people globally and are readily available in the U.S. and European markets. Over 2,000 insect species have been identified as edible. Various cultures use insects, particularly their larvae, as everyday food. Examples include termites in Kenya, mopane worms in Zimbabwe, and grasshoppers in Mexico. These insects are often regarded as delicacies due to their flavor and high nutritional value [3, 10]. Edible insects offer relatively high nutritional values, have a high biodiversity and allow efficient production with

lower environmental pollution at the same time [6]. Insects demonstrate high feed conversion capacity and produce fewer greenhouse gas emissions compared to traditional livestock, while requiring less water and land. Consequently, the consumption of insects contributes positively to environmental sustainability, food and nutrition security, and a healthy lifestyle [3].

Insects are nutrient-dense, with a generally high protein and fat content, a high biological value, and good digestibility. Additionally, they provide micronutrients, including minerals and vitamins. Thus, insects represent a potential supplement for various commercial foods. Despite the widespread consumption of whole insects in different regions, many consumers remain hesitant to accept them due to their unappealing appearance. This issue can be addressed by processing insects into less recognizable forms. The use of insect flour, which renders the insects nearly invisible when incorporated into dishes, is expected to enhance acceptance by an increasing number of consumers [6].

The mealworm, which is the larval stage of the flour beetle, is gaining attention as a suitable candidate for alternative protein sources for large-scale production and integration into the European food and feed industry. The primary bioactive compounds in mealworms that offer health benefits include bioactive peptides, omega-3 fatty acids, oleic acid, and chitosan [11]. They contain 52.35%

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protein, 24.7% fat, and approximately 2% carbohydrates and fiber, providing a balanced nutritional profile that meets WHO recommendations for adults. Nutrition by mealworms fulfills human amino acid requirements. They are rich in both monounsaturated and polyunsaturated fatty acids, as well as trace elements such as magnesium, copper, iron, and zinc [5, 10, 12, 13].

Processing conditions, particularly heat treatment, can lead to conformational changes associated with the physicochemical properties of proteins and other ingredients. Hot air drying and freeze-drying are the commonly used processing methods that extend shelf life and facilitate the incorporation of mealworms into food products that are more easily perceived by consumers. Hot air drying can be realized with relatively simple equipment. The drying rate, and thus, process time and throughput, are essentially controlled by the applied temperature. However, already relatively low temperatures of 40°C significantly deteriorate mealworm quality and protein content [7]. Freeze-drying, instead, preserves both, color and protein content, but yields long drying times and low energy efficiency [8].

Differential scanning calorimetry (DSC) is a thermal analysis technique used to measure the heat flow associated with material transitions as a function of temperature. In DSC, a sample is heated (or cooled) at a constant rate, and the heat absorbed or released by the sample is recorded. This allows for the determination of thermal properties such as melting points, glass transition temperatures, and protein denaturation. DSC is particularly valuable for evaluating protein stability and quality in food processing, as it can provide insights into protein unfolding, aggregation, and overall thermal behavior. It is widely used to study the impact of different processing techniques, such as drying or cooking, on the structural integrity of proteins and other biomolecules.

This study aims to compare drying rates and quality parameters of mealworm subjected to freeze-drying and oven drying at 40 °C and 60 °C. Differential scanning calorimetry was employed to evaluate the thermal properties and protein stability of mealworms post-drying, providing a quantitative assessment of product quality.

EXPERIMENTAL

Sample collection

Tenebrio molitor larvae used in this study were supplied by SAHAWA UG, Germany, as well as from ENTAVA Insektenshop, Germany. During

rearing, the larvae were primarily fed a diet consisting of wheat bran, grains, and NaCl. The larvae were delivered frozen and packaged in breathable mesh and stored at -20 °C in a freezer until use.

Drying procedures

Moisture content before and after drying was determined by thermogravimetric analysis (TGA) using a moisture analyzer (Model MA100, Sartorius, Germany).

Drying experiments were conducted on chopped mealworms using two techniques: oven drying and freeze-drying. During the drying process, weight loss was monitored by interrupting the process at regular intervals and weighing the samples with a precision balance (Secura225D-1S, Sartorius, Germany). Drying curves were generated based on these measurements. Drying was terminated once the drying rate approached zero, i.e., when no further mass loss was observed.

- *Oven drying* was performed using an oven dryer (Mettler, Germany) at two temperature settings: 40 °C and 60 °C.

- *Freeze-drying* was carried out using a freeze dryer (Model BETA 1-16, Martin Christ Gefriertrocknungsanlagen GmbH, Germany). The drying parameters were as follows: pressure 0.1 mbar, ice condenser temperature 50 °C, and shelf temperature 20 °C.

After drying, all mealworms were stored in a desiccator. Experiments with SAHAWA UG samples were repeated once for validation, while those with ENTAVA larvae were not repeated.

DSC characterization

Differential scanning calorimetry (DSC) was used to characterize the thermal behavior of the samples, both before and after drying, using a DSC 92 calorimeter (SETARAM, France). For analysis, the dried worms were ground in a coffee grinder until a fine flour was obtained. Undried worms were crushed using a pestle, analogous to the preparation before drying. The samples were inserted into open aluminum crucibles and compressed using a die tool (punch) for measurements. The weight of the sample varied between 10 and 20 mg. Calibration of the device was carried out with aluminum oxide powder. After each run, the samples were weighed to determine the mass loss during the measurement.

Due to the relatively unknown thermal behavior of mealworms, it is advisable to scan at different measurement rates and across various temperature intervals, starting at a lower temperature, to assess the unfolding kinetics and to determine the optimal settings for conducting the measurements [14].

To identify the appropriate DSC settings for subsequent measurements, initial experiments were conducted with untreated mealworms, as well as mealworms dried in an oven at 60 °C and primarily those dried in a freeze dryer, across different temperature intervals and scan rates. The measurements were performed using liquid nitrogen as a coolant, facilitating a temperature range of 0-100 °C or 0-120 °C. Different scan rates of 1, 2, and 10 K/min were employed to evaluate the kinetics of protein unfolding and to establish optimal measurement settings. The measurement sequence and various setting parameters for the DSC trial are presented in Figure 1.

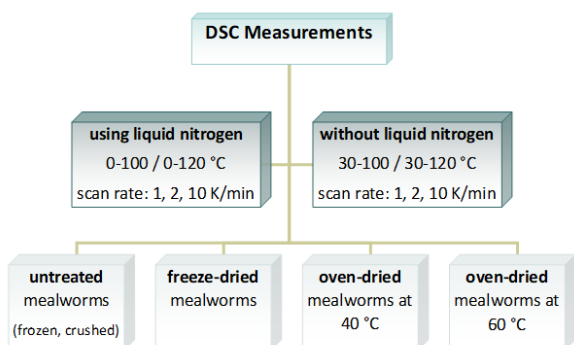


Figure 1. Block diagram of DSC measurements

RESULTS AND DISCUSSION

Comparison of drying processes

- *Drying curves.* The drying curves of the three studied cases (40°C, 60°C, freeze-drying) are given in Fig. 2. As expected, drying at 60°C is fastest at the start of drying. However, the scarce, measurement-dependent data do not allow any conclusion to be drawn about drying periods. Nevertheless, the curves suggest that a first drying period did not occur in any of these experiments (40°C, 60°C, freeze-drying).

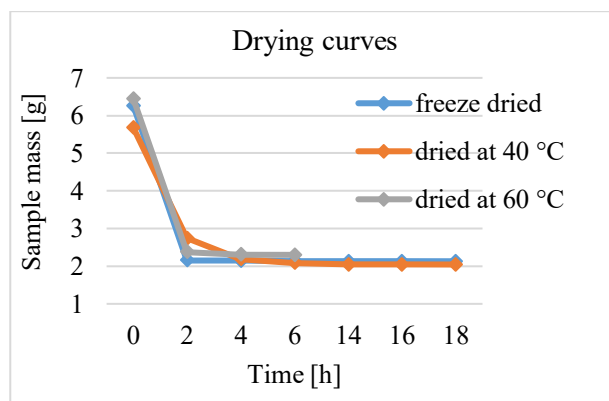


Figure 2. Drying curves for different drying conditions. The overall drying times are indicated in the plots.

Surprisingly, freeze-drying is almost as fast as drying at 40°C. The drying rates at the start are very similar in both cases.

Residual moisture content

The residual moisture content was measured for each one experiment with a TGA. Non-dried mealworms have a moisture content of approximately 65%, which aligns with the values reported in the literature (59-68%) [15]. As can be seen from the data collected in Table 1, on average, all dried samples had a residual moisture content of less than 5 m% (TGA-based value). Table 1 indicates that higher process temperatures yield lower values.

Table 1. Residual moisture contents of SAHAWA UG larvae.

	Result from TGA (m %)
Untreated	65.04
Oven drying at 40°C	4.12
Oven drying at 60°C	3.62
Freeze-drying	4.90

Visual appearance

After drying, a noticeable difference in color was observed, as shown in Fig. 3. Mealworms dried at 40 °C and 60 °C exhibit a visibly darker color compared to those dried in the freeze-dryer. The color of the samples from the freeze-dryer is closer to that of the non-dried samples. Non-enzymatic browning reactions (Maillard reaction) could apparently be avoided by the selected freeze-drying conditions, whereas they strongly affected drying at 40°C and 60°C [7]. As shown in Fig. 3, both oven samples exhibit a very similar color, indicating a similar degree of deterioration. According to literature, Maillard reaction and protein denaturation reactions can result in a reduction of protein, amino acid and peptide contents of up to 20 % [5, 7, 15].

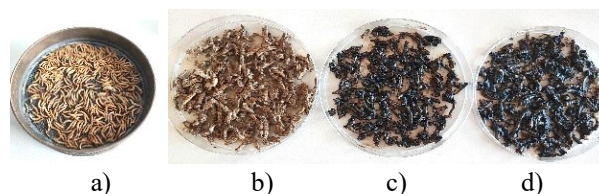


Figure 3. Visual appearance of mealworms: a) before drying, b) after freeze-drying, c) after oven drying at 40°C, d) after oven drying at 60°C.

- *Determining optimal DSC parameters.* Differential scanning calorimetry (DSC) measurements were conducted to analyze the thermal properties in terms of protein denaturation

of mealworms subjected to various drying conditions. Firstly, the optimal settings for conducting the measurements were determined. The measurements were conducted at different scan rates of 1, 2, and 10 K/min within the temperature range of 30-120 °C, in consultation with the literature [14]. Among the scan rates tested, 2 K/min provided the most defined thermogram. At 1 K/min, the denaturation peak shifted to lower temperatures, while 10 K/min was too fast to resolve unfolding kinetics. A scan rate of 10 K/min was too rapid to adequately monitor the unfolding kinetics of the protein; consequently, no endothermic characteristic peak is observed in the resulting curve, which is typically expected during protein denaturation [6, 16].

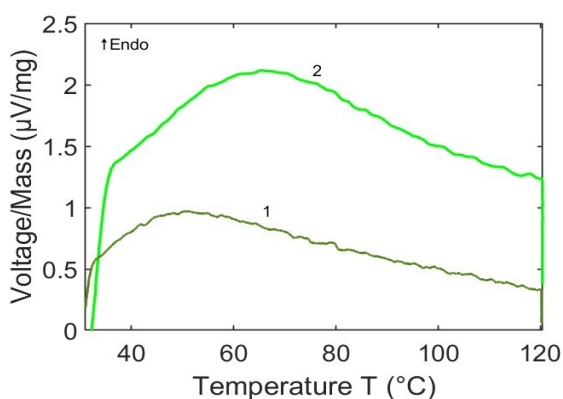


Figure 4. DSC thermogram of mealworms dried in a freeze dryer at a scan rate of 1 K/min (curve 1) and 2 K/min (curve 2)

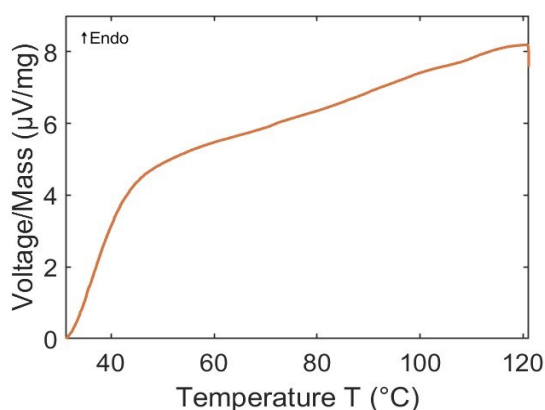


Figure 5. DSC thermogram of mealworms dried in a freeze dryer at a scan rate of 10 K/min

- *Thermal behavior of freeze-dried mealworms.* According to Fig. 4, the measurement is conducted using freeze-dried mealworms within a temperature range of 30 to 120 °C. The graph shows that the peak begins at temperatures below 30 °C. Since setting the appropriate temperature range is crucial for obtaining a complete thermogram when

measuring unknown samples, the sample should be retested over a wider temperature range to fully capture the heat transfer dynamics [14]. Therefore, the measurement with freeze-dried mealworms was conducted in the presence of a coolant (liquid nitrogen), to achieve a broader measurement temperature range of 0 to 120 °C.

The results show that the use of a coolant positively affects the complete formation of the endothermic peak. Therefore, it was decided that all DSC measurements in this study will be conducted in the presence of liquid nitrogen as a coolant, starting at a temperature of 0 °C.

- *Comparison between drying methods.* The results obtained (Fig. 6) indicate that there is no clearly defined peak; however, an endothermic thermal process is observed in the range of 40 to 80 °C, immediately followed by an exothermic region. To investigate how the curve develops over a broader temperature range, a measurement was conducted from 0 to 200 °C.

The expansion of the measurement temperature range to 200 °C (Fig. 7) reveals that the curve in the exothermic region continues to rise with increasing temperature without reaching an exothermic peak. Therefore, it was concluded that heating to such high temperatures is unnecessary, as other studies indicate that the protein denaturation temperature in mealworms lies within the range of 55 °C to 80 °C [6]. Thus, a temperature range of 0 to 100 °C is deemed optimal for the measurements. For this reason, it was decided to conduct all measurements in this study in the presence of liquid nitrogen as a coolant, within a temperature range of 0 to 100 °C at a scan rate of 2 K/min.

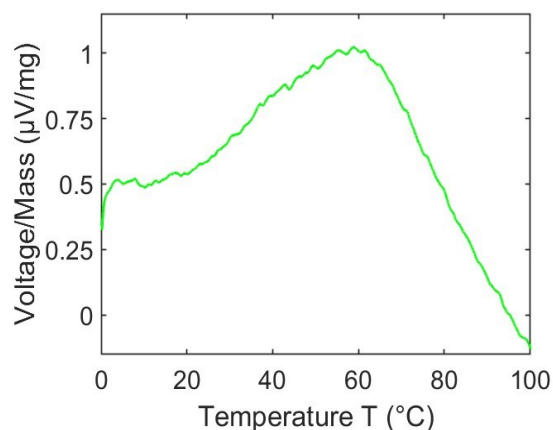


Figure 6. Measurement in the temperature range of 0 to 100 °C with liquid nitrogen as a coolant.

According to Fig. 6, calorimetric measurements reveal an endothermic event occurring in the range

of 30–80 °C, with a temperature peak around approx. 60 °C, though a well-defined peak is absent.

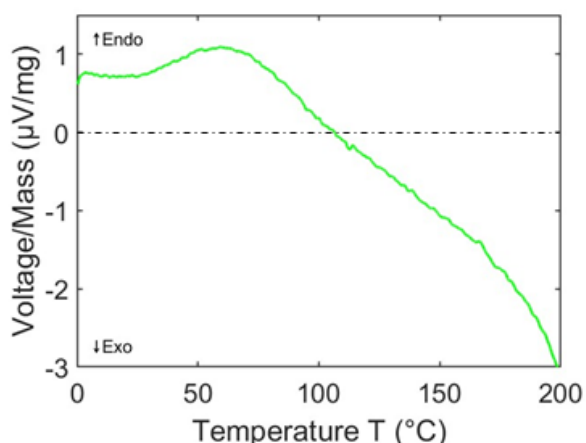


Figure 7. Expansion of the measurement temperature range to 200 °C.

This endothermic phenomenon may be attributed to protein denaturation, as it falls within the temperature range for protein denaturation in mealworms reported in other studies (55 °C to 80 °C) [6].

For the DSC measurements, the entire form of *T. molitor* was used, meaning that the sample also contained various complex components such as hemolymph, skeletal muscle, enzymes, chitin, oil, and other substances [17]. This sample complexity may explain the lack of a distinct peak. In other studies [6, 17], pre-treatment of the mealworm sample, such as defatting of mealworm flour using ethanol or hexane followed by protein extraction was conducted. The resulting protein extract was then processed into a dilute protein solution, potentially resulting in more pronounced peaks in DSC thermograms.

- *Discussion of protein denaturation and aggregation.* Protein unfolding during thermal denaturation involves the absorption of heat to break intramolecular bonds (non-covalent and, in some cases, disulfide bonds) and is therefore an endothermic process. When hydrogen bonds or other hydrophilic interactions that maintain the tertiary structure of the proteins are disrupted by heating, initially buried hydrophobic regions within the proteins are exposed on the protein surface and interact with hydrophobic regions in other protein molecules, leading to aggregation [18].

Thus, the exothermic region in the thermogram (Fig. 7) can be attributed to protein aggregation, which is known to be an exothermic event, as reported in other studies [14, 19]. Additionally, one study has demonstrated that the presence of such an exothermic region is due to the degradation of chitin

components in the composition of insects, occurring at 300-400 °C [8]. There is also evidence of lipid oxidation contributing to exothermic regions in the DSC thermogram [20]. However, this is not the focus of the current study.

The graph (Fig. 8) illustrates the DSC thermogram of untreated mealworms (frozen, not dried). Fig. 8 shows a significant endothermic peak, which may be attributed to the denaturation process. According to Akhtar *et al.*, the protein denaturation temperature in mealworms ranges from 55 °C to 80 °C [6]. Figure 8 shows a peak at approximately 70 °C. However, future investigations should focus on measuring water-solubilized samples with moisture levels similar to those of frozen mealworms. Nevertheless, the detected peak might also be associated with the effects of water loss from the sample during the measurement (from 0 to 100 °C). According to the literature, fresh mealworms have a moisture content ranging from 59% to 68% [15], aligning with the values measured in this study. The change in the amount of absorbed heat flow due to the denaturation of proteins is much less significant than the change caused by the breaking of hydrogen bonds or water loss [21]. This is because water possesses an extensive network of hydrogen bonds, resulting in a considerably higher heat capacity compared to organic compounds like proteins. Furthermore, water is more ordered and densely packed near the protein surface. Since water cannot form hydrogen bonds with nonpolar entities, the hydrogen bonding between water molecules at the protein's surface is maximized. As the temperature rises, the ordered water shell around the protein begins to dissipate, leading to increased heat absorption during the measurement [21, 22].

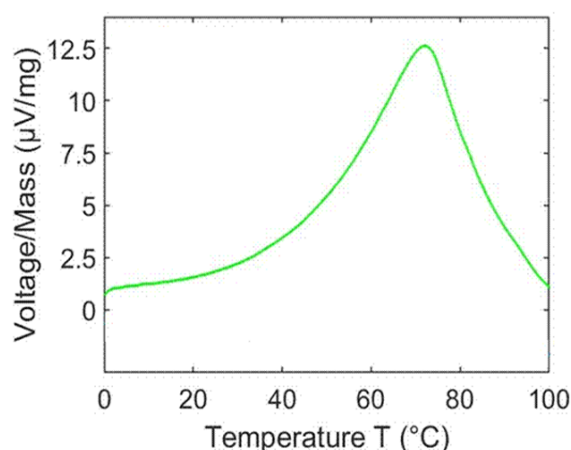


Figure 8. DSC thermogram of untreated mealworms (frozen, not dried).

The following graph (Fig. 9) presents a comparison of the measurement results for

mealworm samples dried in a freeze-dryer, in an oven at 40 °C, and in an oven at 60 °C. This graph clearly shows that the peak temperatures of endothermic processes decrease with increasing drying temperature. While the peak temperature for freeze-dried samples is approximately 60 °C, it drops to around 55 °C for samples dried in an oven at 40 °C, and further to about 50 °C for samples dried at 60 °C.

As illustrated in the graph, the freeze-dried samples require the largest amount of absorbed heat flow for protein unfolding in the mealworms. This may be due to the preservation of native protein in the freeze-dried mealworm samples [8], although residual moisture in the samples could also play a role.

However, the measured signal for samples dried in an oven at 40 °C is reduced by about one-third compared to the lyophilized samples, possibly due to a lower amount of remaining native protein and the presence of aggregates following the drying process, as reported in other studies [15, 16]. It should also be noted that mealworms dried at 40 °C contain less residual moisture, which may also influence the results.

Mealworms dried at 60 °C absorb the least heat flow. This may be because the drying temperature is close to the denaturation temperature of proteins in mealworms, potentially resulting in a significant portion of the native protein already being denatured. Additionally, the low residual moisture could contribute to the smaller signal observed in the thermogram.

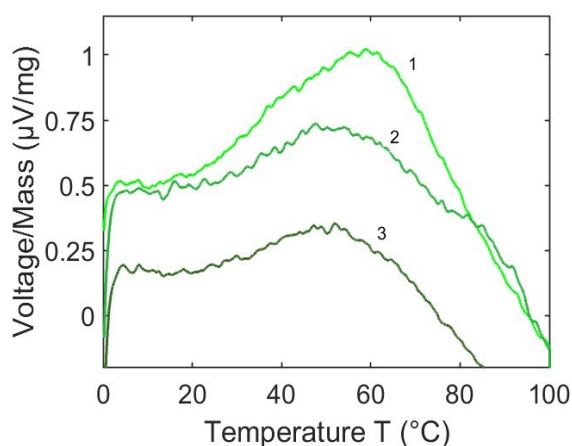


Figure 9. Comparison of the DSC thermograms of mealworms dried in a freeze-dryer (curve 1), in an oven at 40 °C (curve 2), and in an oven at 60 °C (curve 3)

Figure 9 shows a progressive reduction in peak temperature and enthalpy with increasing drying temperature, supporting the hypothesis that higher

thermal exposure induces partial protein denaturation during processing.

CONCLUSION

Drying is a critical step in mealworm processing, yet it can negatively affect the functionality of native proteins [5]. This study investigated how different drying methods influence the denaturation temperature and protein quality of the samples. Differential scanning calorimetry (DSC) measurements were performed on both untreated (frozen, undried) mealworms and those subjected to freeze-drying or oven drying at 40 °C and 60 °C.

The results indicate that both freeze-drying and drying in an oven at 40 °C and 60 °C have a significant impact on the protein denaturation of *T. molitor* larvae. The gentler effect of freeze-drying on the color of edible insects can be attributed to the lower drying temperatures, which help prevent thermal browning reactions, while the elevated temperatures in the oven promote non-enzymatic browning of the samples [7].

As the drying temperature increases, the heat capacity peak associated with mealworm protein denaturation decreases and shifts to lower temperatures. This shift is likely due to conformational changes in the proteins that occur during the drying process. Moreover, the thermograms obtained show a significantly higher endothermic heat quantity for freeze-dried mealworms compared to those dried in an oven at 40 °C and 60 °C. This increase in absorbed heat in the lyophilized samples may be linked to a more native protein structure, but it may also be influenced by residual moisture in the samples.

The DSC measurements suggest that the complexity of the samples leads to a lack of defined peaks in the thermograms. Therefore, a pre-treatment of the mealworm samples, such as defatting mealworm flour and protein extraction [6, 17] is recommended to achieve more distinct peaks in the thermograms [14].

In conclusion, freeze-drying is more suitable for preserving the native protein structure in mealworms, while oven drying at higher temperatures leads to partial denaturation and potential loss of functionality. For more precise thermal characterization, further work using purified protein fractions is recommended.

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