

## FROM LARVAE TO MEAL: INSECTS UNDER PROCESSING PRESSURE

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### ABSTRACT

The industrial conversion of insects into high-value protein meals is a cornerstone of the modern circular bioeconomy, where minimizing processing losses is critical for economic viability. This study analyzed the processing losses and mass balance of two economically important insect species – yellow mealworm larvae (*Tenebrio molitor*) and adult house crickets (*Acheta domestica*) – during post-harvest technological processing, including freezing, lyophilization (freeze-drying), and homogenization (mechanical milling). For crickets, three dietary regimens were compared using five replicates each: a traditional plant-protein diet (PP), an insect-protein diet (IP), and a combined-protein diet (CP). For mealworms, two dietary groups were evaluated using five replicates each, specifically a control group (CG) and an insect-protein group (IG). The results showed that losses during freezing and milling did not differ significantly among groups CG and IG ( $p > 0.05$ ). In contrast, losses during lyophilization differed significantly among groups CG and IP ( $p < 0.05$ ). Significant within-species variations in lyophilization losses were also observed, both in *Acheta domestica* and *Tenebrio molitor* ( $p < 0.05$ ). These findings indicate that dietary composition does not affect mechanical processing losses but significantly influences moisture retention and subsequent drying efficiency. Understanding these mass balance parameters allows for the optimization of industrial post-harvest processing of insect biomass.

**Keywords:** *Tenebrio molitor*; *Acheta domestica*; black soldier fly larvae meal; alternative feed; biomass yield; freeze-drying; homogenization

## INTRODUCTION

Insect biorefining is a key driver of the global circular bioeconomy, with the yellow mealworm (*Tenebrio molitor*) and house cricket (*Acheta domestica*) leading EU food and feed applications due to efficient feed conversion and established mass-rearing (Tettamanti et al., 2022; Bordiean et al., 2022; Siddiqui et al., 2024; Conway et al., 2024). While black soldier fly (*Hermetia illucens*, BSF) larvae meal is widely studied as a sustainable replacement for soybean meal in livestock and aquaculture (Tettamanti et al., 2022; Le Boucher et al., 2024), its use as a feed ingredient for rearing other commercial insect species—and its subsequent effect on the technological quality of harvested biomass—remains unexplored.

Diet composition directly dictates insect growth, metabolism, and final biochemical profile, including protein, lipid, and chitin content (Bordiean et al., 2022; Orkusz et al., 2023). Because biochemical composition shapes how biological materials behave during processing, dietary changes likely alter post-harvest characteristics, though this relationship has received minimal scientific focus.

Post-harvest processing ensures microbial safety and product stability (Marzoli et al., 2023). Freezing is standard for ethical extermination and enzyme reduction (Cámara-Ruiz et al., 2023), while freeze-drying preserves nutritional and structural integrity (Krzyżaniak et al., 2022; Yan et al., 2023). The efficiency of freeze-drying depends on matrix properties like moisture distribution and lipid composition, which vary with diet (Krzyżaniak et al., 2022). Finally, milling yields a homogeneous powder (Yan et al., 2023) whose flowability and handling are governed by particle size, residual moisture, and lipids, directly affecting product recovery (Suhag et al., 2024; Weiss et al., 2021).

The aim of the present study was to quantify the biomass losses occurring during successive post-harvest technological processing steps — freezing, lyophilization, and mechanical milling — in two species of edible insects, the house cricket (*Acheta domestica*) and the yellow mealworm (*Tenebrio molitor*), fed diets differing in protein source (plant-based, insect-based, or a combination of both, depending on species), and to evaluate the mass recovery throughout the entire processing chain.

## MATERIAL AND METHODS

### *Experimental animals and dietary treatments*

Two edible insect species were used in this study: the house cricket (*Acheta domestica*) and the yellow mealworm (*Tenebrio molitor*). Insects were reared under controlled conditions at a temperature of 25 °C for crickets and 27 °C for mealworms, relative humidity of 50 % for crickets and 60 % for mealworms, and a photoperiod of 12 hours light and 12 hours dark for both species, for a period of 45 days.

Crickets were divided into three dietary groups (n = 5 replicates per group), differing in their main protein source. The plant-protein diet (PP) was based on

wheat and soybean meal as the primary protein source, supplemented with rapeseed oil and a standard mineral premix (limestone, salt, monocalcium phosphate, sodium bicarbonate). The insect-protein diet (IP) was formulated identically, with the soybean meal fully replaced by black soldier fly (*Hermetia illucens*) larvae meal as the sole protein source. The combined-protein diet (CP) contained a partial replacement of soybean meal with black soldier fly larvae meal (50:50), resulting in a mixed plant- and insect-based protein source. All three diets were formulated to be isonitrogenous and isoenergetic.

**Tab. 1:** Composition of experimental diets for *Acheta domesticus* (%).

| Ingredient (%) | PP            | IP            | CP            |
|----------------|---------------|---------------|---------------|
| Grains         | 48.28         | 47.70         | 47.34         |
| Legumes        | 41.00         | –             | 20.50         |
| Insect protein | –             | 42.40         | 21.40         |
| Oilseeds       | 7.00          | 5.60          | 6.80          |
| Minerals       | 2.72          | 3.30          | 2.96          |
| Additives      | 1             | 1             | 1             |
| <b>Total</b>   | <b>100.00</b> | <b>100.00</b> | <b>100.00</b> |

Mealworms were divided into two dietary groups (n = 5 replicates per group), based on wheat bran as the basal component (78.68%). The control group (CG) received wheat bran combined with a soybean-based protein concentrate (21.32%), identical to the plant-based protein source used in the cricket PP diet. The insect-protein group (IG) received wheat bran combined with black soldier fly (*Hermetia illucens*) larvae meal (21.32%) as the sole protein source, with no soybean-based component included. Mealworms were provided with agar as a water source throughout the experimental period.

**Tab. 2:** Composition of experimental diets for *Tenebrio molitor* (%).

| Ingredient (%) | CG            | IG            |
|----------------|---------------|---------------|
| Grains         | 78.68         | 78.68         |
| Legumes        | 21.32         | –             |
| Insect protein | –             | 21.32         |
| <b>Total</b>   | <b>100.00</b> | <b>100.00</b> |

### Post-harvest processing

At the end of the feeding trial, insects were harvested and subjected to a standardized sequence of post-harvest processing steps: freezing, lyophilization, and mechanical milling. Insects were killed by freezing at -20 °C for 24 hours, which is widely regarded as an ethical killing method that simultaneously reduces enzymatic activity and microbial deterioration prior to further processing. Frozen insect biomass was freeze-dried using a GREGOR Instruments freeze-dryer

(GREGOR instruments, s.r.o., Czech Republic) a shelf temperature of 20 °C to 30 °C, condenser temperature of -55 °C, and chamber pressure of 10 to 50 Pa, for a duration of 72 hours. Freeze-dried samples were mechanically milled using an IKA MultiDrive basic mill (IKA-Werke GmbH & Co. KG, Germany; distributed by VERKON, s.r.o.) to obtain a homogeneous powder suitable for further analysis.

### *Determination of processing losses*

Biomass mass was recorded using an analytical balance (with a readability of 0.001 g) before and after each processing step (freezing, lyophilization, milling). Processing losses (%) at each step were calculated according to the following equation:

$$\text{Loss}(\%) = \left[ (m_1 - m_2) / m_1 \right] \times 100$$

where  $m_1$  is the biomass mass before the given processing step and  $m_2$  is the biomass mass after the given processing step. Mass recovery throughout the entire processing chain was calculated as the cumulative product of the recovery rates of the individual steps.

### *Statistical analysis*

Data are expressed as mean and standard deviation (SD). All statistical analyses were performed using IBM SPSS Statistics (version 25.0). Differences among dietary groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. A significance level of  $p < 0.05$  was considered statistically significant.

## **RESULTS AND DISCUSSION**

### *Losses during freezing and milling*

Losses during freezing (0.18–2.63 %) and milling (0–1.30 %) did not differ significantly among groups ( $p > 0.05$  for both variables, Table 3). This shows that these two steps are relatively unaffected by dietary composition. Their mass losses are also negligible compared with lyophilization. Freezing produced consistently low losses, supporting its use as an ethical and simple killing method. It also inhibits enzymatic activity and microbial spoilage before further processing. Compared with other killing methods, freezing has been shown to better preserve protein quality and limit browning in insect biomass (Leni et al., 2019). Milling losses were similarly low. This is expected, since the material is already largely dehydrated after lyophilization. At this stage, milling mainly fragments the solid matrix rather than causing further loss of mass, moisture, or fine particles (Suhag et al., 2024; Waiss et al., 2021).

### *Lyophilization losses – overall analysis and species effect*

In contrast, losses during lyophilization were highly significant among groups ( $p < 0.001$ ). Tukey's post-hoc test identified three homogeneous subsets: group

CG showed the lowest losses ( $62.25 \pm 1.22\%$ ), group IG an intermediate value ( $65.02 \pm 0.40\%$ ), and groups CP, IP, and PP the highest losses ( $72.60$ – $73.65\%$ ). These three groups did not differ significantly from each other (Table 3).

**Tab. 3:** Biomass losses (% , mean  $\pm$  SD) during post-harvest technological processing across all groups.

| Group   | Freezing (%)    | Lyophilization (%) | Milling (%)     |
|---------|-----------------|--------------------|-----------------|
| CG      | $0.20 \pm 0.06$ | $62.25 \pm 1.22$   | $0.08 \pm 0.09$ |
| IG      | $2.63 \pm 5.57$ | $65.02 \pm 0.40$   | 0               |
| PP      | $0.18 \pm 0.14$ | $72.60 \pm 0.37$   | $0.79 \pm 0.83$ |
| IP      | $0.38 \pm 0.40$ | $73.65 \pm 0.30$   | $1.30 \pm 1.76$ |
| CP      | $0.31 \pm 0.21$ | $73.29 \pm 0.67$   | $0.94 \pm 0.83$ |
| p-value | <b>0.484</b>    | <b>&lt; 0.001</b>  | <b>0.178</b>    |

This pronounced species-level difference ( $62$ – $65\%$  in *Tenebrio molitor* and  $72.6$ – $73.65\%$  in *Acheta domesticus*) likely reflects an intrinsic difference between the two species. Mealworm larvae and adult crickets naturally differ in their initial water and lipid content. Larvae of *T. molitor* contain markedly higher lipid levels ( $\sim 32\%$  of dry weight) than adult *A. domesticus* ( $\sim 15\%$  of dry weight), as shown in a direct comparative analysis of insect lipid profiles (Paul et al., 2017). This difference in lipid content, together with differences in body structure and developmental stage between the two species, may affect how water is bound within the biological matrix, and therefore how available it is for sublimation during lyophilization.

Species-specific differences in the hygroscopic and moisture-related properties of edible insect powders have been reported before, both for house crickets and for black soldier fly larvae (Kamau et al., 2018). Oven-drying has also been shown to affect protein digestibility differently in *T. molitor* and *A. domesticus*, which supports the idea of species-specific responses to dehydration (Hammer et al., 2023). Differences in chitin content between the two species may play a role as well, since chitin content and structure vary considerably between *Coleoptera* and *Orthoptera* (Abenaim & Conti, 2025).

### Lyophilization losses – within-species diet effect

In addition to the species effect, a statistically significant effect of diet was also observed within both species (Tables 4 and 5).

This consistent pattern across both species suggests that replacing plant-based protein with BSF larvae meal systematically increases losses during lyophilization. A plausible mechanism relates to the distinct lipid fraction of BSF meal, which is rich in medium-chain saturated fatty acids, particularly lauric acid; larvae are able to synthesise this fatty acid largely independently of the fatty acid profile of their rearing substrate (Ewald et al., 2020). Nevertheless, the overall fat content and fatty acid profile of BSF larvae can still be modulated by dietary fat sources and levels, and larvae have been shown to bioaccumulate dietary fatty acids in their

**Tab. 4:** Biomass losses (% , mean  $\pm$  SD) during post-harvest technological processing in *Acheta domesticus*.

| Group   | Freezing (%)    | Lyophilization (%) | Milling (%)     |
|---------|-----------------|--------------------|-----------------|
| PP      | 0.18 $\pm$ 0.14 | 72.60 $\pm$ 0.37   | 0.79 $\pm$ 0.83 |
| IP      | 0.38 $\pm$ 0.40 | 73.65 $\pm$ 0.30   | 1.30 $\pm$ 1.76 |
| CP      | 0.31 $\pm$ 0.21 | 73.29 $\pm$ 0.67   | 0.94 $\pm$ 0.83 |
| p-value | <b>0.525</b>    | <b>0.012</b>       | <b>0.801</b>    |

**Tab. 5:** Biomass losses (% , mean  $\pm$  SD) during post-harvest technological processing in *Tenebrio molitor*.

| Group   | Freezing (%)    | Lyophilization (%) | Milling (%)     |
|---------|-----------------|--------------------|-----------------|
| CG      | 0.20 $\pm$ 0.06 | 62.25 $\pm$ 1.22   | 0.08 $\pm$ 0.09 |
| IG      | 2.63 $\pm$ 5.57 | 65.02 $\pm$ 0.40   | 0               |
| p-value | <b>0.357</b>    | <b>0.0013</b>      | <b>0.059</b>    |

tissues (Li et al., 2022). Dietary fat originating from the BSF-based diet may therefore accumulate in insect tissues and subsequently alter the physicochemical structure of the biological matrix, the distribution of bound and free water, and consequently the efficiency of sublimation during lyophilization. A higher lipid fraction within a biological matrix has been reported to negatively affect the efficiency of the freeze-drying process and the physical quality of the resulting product (Bhatta et al., 2020). Furthermore, the drying method and associated thermal history have been shown to interact with the fat and protein fraction of BSF-derived material, affecting its structural and nutritional properties (Huang et al., 2019), while processing-related enzymatic reactions (e.g., phenoloxidase activity) have been reported to alter protein solubility in *T. molitor* and *H. illucens* in a matrix-dependent manner (Janssen et al., 2019). Taken together, these mechanisms indicate that a higher lipid content in the biological matrix may, on the one hand, encapsulate water molecules and slow their evaporation, and on the other hand, alter fat tissue structure such that more water is released during the sublimation phase transition – in either case resulting in a change in overall mass loss relative to a plant-based diet.

## CONCLUSION

This study demonstrated that losses during freezing and mechanical milling were low and largely unaffected by species or dietary composition, confirming that these two processing steps are robust across different rearing conditions. In contrast, lyophilization was the critical step in the post-harvest processing chain, showing both a pronounced species effect — with *Tenebrio molitor* consistently losing less mass than *Acheta domesticus* — and a consistent, statistically significant diet effect within both species, whereby replacing plant-based protein with black soldier fly (*Hermetia illucens*) larvae meal increased lyophilization losses. These

findings indicate that dietary protein source does not influence the mechanical stages of processing but does meaningfully affect moisture-related behaviour during freeze-drying, likely through diet-driven differences in the lipid fraction of the biological matrix. From a practical standpoint, these results suggest that industrial lyophilization protocols for edible insects should be adjusted according to both species and diet formulation to optimise drying efficiency, energy use, and final product recovery. Future research should further investigate the underlying compositional and structural mechanisms — particularly lipid distribution and water-binding capacity — driving these differences and should extend this assessment to a broader range of insect-based diets and species relevant to the circular bioeconomy.

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