COMPARATIVE ANALYSIS OF DRYING METHODS ON *IN VITRO* PROTEIN DIGESTIBILITY OF HOUSE CRICKET (*ACHETA DOMESTICUS*)

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Abstract
Insects can be considered high-quality protein sources for human diet; however, processing methods can influence protein digestibility, which is a critical factor for their nutritional value. This study evaluates the effect of different blanching and drying techniques on the protein digestibility of house cricket (*Acheta domesticus*) *in vitro*. Samples were water blanched (60 °C for 90 s; 100 °C for 60 s) or steam blanched (60 s, 90 s) using a non-pressurized domestic steam cooker operating at approximately 100 °C, before drying in a conventional dryer (at 85 °C and 100 °C), freeze-dryer, or microwave-vacuum dryer. Protein content was determined using the Kjeldahl method (kp = 5.33), and digestibility was assessed through *in vitro* gastrointestinal simulation according to the INFOGEST protocol. A total of 21 samples were processed. Results indicated no statistically significant differences in protein digestibility across drying methods ($p > 0.05$), suggesting that drying technique selection can be based on industrial feasibility, energy efficiency, and cost, without compromising nutritional quality. Blanching method and duration also had no significant impact on protein digestibility, raising questions about the necessity of this pre-treatment step. This study further highlights the importance of using accurate nitrogen-to-protein conversion factors, as conventional calculations (kp = 6.25) may overestimate protein content in insects due to chitin interference. By applying a corrected factor (kp = 5.33), more precise protein values were obtained. These findings contribute to optimizing insect processing for food applications, supporting sustainability and efficiency in edible insect production. The research aligns with broader efforts to improve alternative protein quality and develop evidence-based food labelling standards.
Keywords: *Acheta domesticus*, insect protein, *in vitro* digestibility, drying, blanching.

Introduction

The global interest in edible insects as an alternative protein source is increasing due to insects' nutritional value, complete protein and sustainability of raw material production (Mohamad et al., 2025). Insects are consistently a rich source of proteins across their various life stages, making protein the predominant nutritional component. The content of raw protein often ranges from 40 to 75 g 100 g^{-1} in insect dry matter (Lampová et al., 2024).

The studies have reported protein digestibility values for various edible insect species, stages, and processing methods applied (Oonincx & Finke, 2021). Protein digestibility is a significant factor influencing the bioavailability of amino acids – the building blocks of proteins – within the human body. High digestibility ensures that proteins are effectively broken-down during digestion, realising amino acids to be readily absorbed and utilized for various physiological functions, including tissue repair, enzyme production, and immune responses (Ajomiwe et al., 2024).The bioavailability of amino acids from dietary proteins depends on the degree of protein hydrolysis during digestion and the intestines capacity to utilize amino acids. Therefore, assessing protein digestibility is crucial to determine the nutritional value of protein sources, including edible insects (Rodríguez-Rodríguez et al., 2022).

Processing methods applied in the food industry significantly influence the protein digestibility of edible insects. For instance, blanching – brief exposure to boiling water or steam – can inactivate enzymes raising spoilage and preserving the colour and flavour of the product, thereby enhancing protein digestibility (Rodríguez-Rodríguez et al., 2022). Several studies have demonstrated that certain cooking methods, such as boiling and roasting, can decrease protein digestibility in edible insects. For instance, Mohd Zaini et al. (2023) observed that boiling insects led to a loss of protein due to leaching into the boiling water, resulting in a decrease protein digestibility *in vitro* up to 25%. Similarly, Manditsera et al. (2019) found that boiling and roasting significantly influence protein digestibility in *Tenebrio molitor* and *Gryllus assimilis*, with drying emerging as the most effective method for enhancing digestibility. Therefore, a selection of appropriate processing techniques is crucial to optimize the nutritional benefits of insect-based foods.

Analysing the protein content of insects like house cricket (*Acheta domesticus*), process involves measuring total nitrogen content and applying a nitrogen-to-protein conversion factor. The conventional factor of 6.25, based on the assumption that proteins contain 16% nitrogen, is widely used in protein calculation (Janssen et al., 2017).

Applying this calculation, chitin nitrogen is rated as protein nitrogen, too. Chitin consists of 6.89 g 100 g^{-1}. During Kjeldahl or Dumas nitrogen analysis, this nitrogen generally falsifies the protein content resulting in an overestimation of data (Sudwischer et al., 2025).

Recent studies have proposed more accurate, species-specific conversion factor for *Acheta domesticus* protein. Ritvanen et al. (2020) calculated a factor of 5.09 for house crickets. Similarly, Janssen et al. (2017) suggested a factor of 4.76 for the same species. Boulos et al. (2020) in the study of 'Nitrogen-to-Protein Conversion Factors' confirmed that insect protein content has been significantly overestimated. Conclusively, the conversion factor *kp* = 5.33, which was determined as an average from 7 different batches of 3 insect species, reflects a more accurate true protein content.

This study evaluates the effect of different blanching and drying techniques on the protein digestibility of house cricket (*Acheta domesticus*) *in vitro*.

Materials and Methods

The house crickets (*Acheta domesticus*) were obtained from the supplier 'Eurocrickets' UAB (Lithuania). Crickets are farmed for human consumption and fasted for 24 hours before being subjected to humane freeze treatment. Crickets were delivered frozen in dry ice packaging and stored in the freezer at -18 °C until further processing. Frozen insects were thawed at room temperature for 10 min, weighed for 10 g, and processed accordingly.

Blanching was performed using two methods:

- water blanching: 60 °C for 90 s and 100 °C for 60 s (according to Cacchiarelli et al., 2022);
- steam blanching: 60 s and 90 s (adapted from Bawa et al., 2020).

Water blanching was performed by first bringing water to a boil in a stainless-steel pot. Insect samples were placed in a metal sieve and fully submerged in the boiling water for the specified time.

Samples were blanched using a domestic electric steam cooker (Philips Daily Collection HD9126/90, 900 W, Philips, Netherlands) equipped with three baskets and a freestanding reservoir. Steam cooker was not sealed and pressure not measured, water maintained at boiling temperature (approximately 100 °C).

After blanching, insects were submerged in an ice bath for 60 seconds, placed on filter paper to remove excess moisture, and kept for 4 minutes.

Blanched crickets were dried using three drying methods:

- conventional drying in oven (Memmert, UF55, Germany) at 85 °C and 100 °C till constant weight achieved (adapted from Purschke, et al. (2018) and Liang et al., 2024);
- freeze-drying – in a vacuum chamber of a lyophilization device FT33 (Armfield Ltd, UK) at a pressure of 6.4 Pa for 24 h. The temperature in the condensation chamber was -45±3 °C;
- microwave-vacuum drying (Musson 1, Russia): number of magnetrons 13–7–10, pressure 7.47–10.67 kPa, drum rotation speed 6 rpm, drying time 35 min, sample total weight before drying 700 g.

Dried samples were milled using a blade grinder (Stollar SKD600), producing a particle size distribution ranging from 300 to 1,000 μm, depending on the milling duration and sample properties. Samples for further analysis were kept in a plastic 15 mL tube with a lid at room temperature (21±2 °C).

The chemicals used in the study were purchased from Sigma Aldrich (supplied by a local company) and were the analytical grade. Protein digestibility was assessed using *in vitro* digestion simulation following the standard gastrointestinal digestion protocol INFOGEST according to Minekus et al. (2014).

In vitro protein digestibility protocol steps:

1. Gastric phase: 3.8 mL deionized water to 0.5 g sample was added, added 0.08 g pepsin (P7000, ≥ 250 U mL^{-1}), 6.2 mL simulated gastric fluid (SGF, pH 3.0) reaching the total volume 10 mL. The samples were incubated on a Premium Hot Plate Stirrer SMHS-3 (Germany) at 37 °C and rotation speed 80 rpm for two hours. pH checked in one hour after incubation started. Two hours after the gastric phase, pH was raised to 7 with 0.5 M NaOH.

2. Intestinal phase: to 10 mL digested gastric sample added simulated intestinal fluid (SIF, pH 7.0), 0.01g pancreatin (P1750, Sigma-Aldrich, 4xUSP), 0.0125 g chymotrypsin (C3142, Sigma-Aldrich,≥40 U mL^{-1}) and 0.083 g bile salts (48305, Sigma-Aldrich, 10 mM). The samples were incubated on a Premium Hot Plate Stirrer SMHS-3 (Germany) at 37 °C and 80 rpm for two hours. pH checked in one hour after incubation started. After two hours of incubation, samples were transferred to an ice bath, left for 15 min, and centrifuged ELMI Centrifuge CM-6MT SkyLine (USA) for 15 min, 3500 rpm. The supernatant was carefully separated from sediments, kept in the refrigerator till placed for further processing of protein analysis on the digestor (FOSS Tecator™, Denmark).

The samples were processed on a FOSS Tecator™ (Denmark) and Kjeltec™ 2100 (Denmark). The protein content was determined using the Kjeldahl method (AOAC, 2019), and the digestibility was calculated by measuring the nitrogen released after enzymatic hydrolysis.

Nitrogen content (%) was calculated using the formula:

$$N = \frac{(a-b)*k*14.007*100}{c}, \quad (1)$$

where:

N – nitrogen, %;

a – 0.1 M HCl used for sample titrating, mL;

b – 0.1 M HCl used for control titrating, mL;

c –sample weight, mg;

k – HCl molarity, 0.1.

Protein digestibility (PD) was calculated using the formula (Almeida et al., 2015):

$$PD = \frac{Ns-Nb}{Ns} * 100, \quad (2)$$

where:

PD – protein digestibility, %;

Ns – nitrogen content in the sample before digestion, %;

Nb – nitrogen content in the sample after digestion, %.

In this study, the conversion factor k_p = 5.33 is used to determine the protein content.

Data were analysed using Microsoft Excel (version 16.78.3.) and ANOVA paired-sample t-test was used to compare the digestibility differences across drying methods. Significance was set at $p < 0.05$.

Results and Discussion

The choice of an appropriate nitrogen-to-protein conversion factor significantly affects the reported protein content in edible insects. In this study, a paired-sample t-test was performed to calculate protein content for comparison using two different conversion factors (see Table 1). The factor k_p=6.25 is commonly used by insect farmers to determine the protein content in insect samples, but k_p=5.33 is a corrected factor suggested by Boulos et al. (2020). The results of ANOVA, comparing the data using factor k_p=6.25 and k_p=5.33, showed a statistically significant difference ($p < 0.0001$), leading to the conclusion that insect protein content is overestimated using a regular conversion factor of 6.25 (Formula 1). The protein content is overestimated by approximately 15–16% when using the 6.25 nitrogen factor instead of 5.33.

This finding is consistent with previous studies suggesting that a nitrogen factor closer to 5.33 may be more accurate for insects due to the presence of chitin and a unique protein profile.

These results highlight the need to reconsider the choice of nitrogen factor when reporting protein content in edible insects, especially in nutrition labels. Future studies should explore species-specific nitrogen factors to improve the accuracy of protein quantification and ensure standardization in the edible insect production. The slight differences in protein content may stem from natural variability in the sampled crickets (sample size per drying method 10 g), such as the male-to-female ratio. Female *Acheta domesticus* tend to be larger, potentially influencing protein and chitin content (Kulma et al., 2019).

Oven drying at 85 °C and 100 °C generally produced consistent results (see Table 1), with protein SDs mostly below ±2.5%. One steam-blanched sample dried at 100 °C showed higher variability (SD ±4.8%). Freeze-dried steam-blanched samples displayed moderate variation, with SDs up to ±3.7%. During freeze-drying, a visible structural difference was observed, which may have influenced protein uniformity in the final powder. Microwave-vacuum drying exhibited the highest variability in protein content. The observed standard deviation may have resulted from uneven moisture reduction (Zhang et.al., 2006), as microwave-assisted drying can lead to localized differences in drying intensity, potentially affecting protein conformation and retention.

However, the minimal variations were observed in digestibility results, these differences are not statistically significant ($p > 0.05$) and are unlikely to affect overall conclusions.

Table 1

The protein content of samples, calculated using two conversion coefficients: kp6.25 and kp5.33

Drying method	Sample	Nitrogen, %	Protein, % (k_p=6.25)	Protein, % (kp=5.33)
Convential drying	60 °C water blanch for 90 s, dried at 85 °C	10.17 ± 0.28	63.54 ± 1.77	54.18 ± 1.51
	100 °C water blanch for 60 s, dried at 85 °C	10.39 ± 0.38	64.96 ± 2.38	55.40 ± 2.03
	Thawed (no processing), dried at 85 °C	10.03 ± 0.13	62.69 ± 0.84	53.47 ± 0.71
	60 °C water blanch for 90 s, dried at 100 °C	10.17 ± 0.38	63.59 ± 2.35	54.23 ± 2.00
	100 °C water blanch for 60 s, dried at 100 °C	10.10 ± 0.14	63.15 ± 0.85	53.85 ± 0.73
	Thawed (no processing), dried at 100 °C	10.13 ± 0.36	63.30 ± 2.27	53.98 ± 1.93
	60 s steam blanch, dried at 85 °C	10.30 ± 0.22	64.41 ± 1.36	54.93 ± 1.16
	90 s steam blanch, dried at 85 °C	10.49 ± 0.40	65.55 ± 2.50	55.90 ± 2.13
	60 s steam blanch, dried at 100 °C	10.21 ± 0.33	63.82 ± 2.05	54.42 ± 1.75
	90 s steam blanch, dried at 100 °C	10.99 ± 0.90	68.68 ± 5.65	58.57 ± 4.82
Freeze-drying	Frozen (no processing), freeze dried	10.37 ± 0.35	64.83 ± 2.20	55.28 ± 1.86
	60 °C water blanch for 90 s, freeze dried	10.40 ± 0.47	64.98 ± 2.96	55.41 ± 2.53
	100 °C water blanch for 60 s, freeze dried	10.46 ± 0.44	65.35 ± 2.76	55.73 ± 2.35
	60 s steam blanch, freeze dried	9.96 ± 0.70	62.26 ± 4.35	53.09 ± 3.70
	90 s steam blanch, freeze dried	10.47 ± 0.37	65.44 ± 2.32	55.80 ± 1.98
	Thawed (no processing), frozen, freeze dried	10.28 ± 0.25	64.25 ± 1.53	54.79 ± 1.31
Microwave-vacuum drying	60 s steam blanch, microwave-vacuum dried	10.66 ± 0.69	66.63 ± 4.32	56.83 ± 3.68
	90 s steam blanch, microwave-vacuum dried	10.76 ± 0.63	67.25 ± 3.92	57.35 ± 3.34
	60 °C water blanch for 90 s, microwave-vacuum dried	10.81 ± 1.12	67.54 ± 6.98	57.60 ± 5.96
	100 °C water blanch for 60 s, microwave-vacuum dried	9.99 ± 0.45	62.41 ± 2.81	53.22 ± 2.40
	Thawed (no processing), microwave-vacuum dried	10.18 ± 0.27	63.64 ± 1.72	54.27 ± 1.46

Protein digestibility is an important factor in determining its quality. All samples in this study showed high protein digestibility (see Table 2), calculated according to Formula 2. The data differed slightly, which could be due to a small sample size, uneven grinding, or weighing inaccuracies. Statistical data analysis (ANOVA, $p = 0.1505$) showed no statistical difference between all samples, in each

repetition, regardless of the blanching or drying method applied. Statistical data analysis was also performed between individual drying methods, yielding results (ANOVA p = 0.07 - 0.79), which confirmed that the data were not statistically significant among themselves.

Table 2
Protein digestibility across samples

Drying method	Sample	Digestibility, %
Conventional drying	60 °C water blanch for 90 s, dried at 85 °C	76.53 ± 2.62
	100 °C water blanch for 60 s, dried at 85 °C	77.35 ± 1.80
	60 s steam blanch, dried at 85 °C	77.20 ± 3.74
	90 s steam blanch, dried at 85 °C	79.25 ± 4.30
	Thawed (no processing), dried at 85 °C	77.86 ± 3.17
	60 °C water blanch for 90 s, dried at 100 °C	78.17 ± 5.25
	100 °C water blanch for 60 s, dried at 100 °C	76.97 ± 2.99
	60 s steam blanch, dried at 100 °C	77.70 ± 3.59
	90 s steam blanch, dried at 100 °C	79.20 ± 2.46
	Thawed (no processing), dried at 100 °C	76.98 ± 3.20
Freeze-drying	60 °C water blanch for 90 s, freeze dried	77.93 ± 5.81
	100 °C water blanch for 60 s, freeze dried	77.79 ± 3.85
	60 s steam blanch, freeze dried	78.00 ± 5.40
	90 s steam blanch, freeze dried	77.98 ± 2.05
	Thawed (no processing), frozen, freeze dried	78.17 ± 4.58
	Frozen (no processing), freeze dried	75.97 ± 3.87
Microwave-vacuum drying	60 °C water blanch for 90 s, microwave-vacuum dried	78.91 ± 4.40
	100 °C water blanch for 60 s, microwave-vacuum dried	79.44 ± 5.71
	60 s steam blanch, microwave-vacuum dried	78.07 ± 3.85
	90 s steam blanch, microwave-vacuum dried	79.19 ± 3.29
	Thawed (no processing), microwave-vacuum dried	78.84 ± 4.49

However, the standard deviation in digestibility values varies across samples, primarily due to inconsistencies in sample handling for Kjeldahl analysis. These variations were not caused by experimental errors but rather by unavoidable circumstances that prevented a uniform methodology for storing and processing the digested samples. Future studies should ensure consistent handling procedures, preferably performing Kjeldahl analysis immediately after *in vitro* digestion, to minimize variability while maintaining the high-quality data integrity demonstrated in this study.

The *in vitro* protein digestibility of *Acheta domesticus* observed in this study aligns with existing literature, which reports digestibility values ranging from 79 to 93% (Hammer et al., 2023). These results confirm that crickets are a highly digestible protein source suitable for human nutrition.

According to Sánchez-Velázquez et al. (2023) data, different drying methods (conventional, freeze-drying and microwave) do not affect the protein digestibility. Similarly, Kröncke et al. (2018) concluded that drying methods affect only insect protein solubility. However, other studies showed contradictory results. For instance, Purschke et al. (2018) concluded that drying temperature above 100 °C affects protein digestibility, as protein denaturation occurs. In turn, Lier et al. (2024) indicate that microwave drying negatively affects the protein quality of black soldier fly larvae.

The absence of significant differences in digestibility data between drying methods is of specific relevance. These findings suggest that insect farmers can select any drying method based on its industrial feasibility, costs and energy efficiency rather than protein digestibility. This research contributes to the development of improved processing methods, showing that insect protein digestibility remains consistent regardless of the drying method used, enabling manufacturers to choose their processing methods to be more resource and environmentally sustainable, as well as revenue generating, without the fear of protein loss.

Conclusions

1. The study results reveal the need to reconsider the choice of conversation factor when reporting protein content in edible insects, especially in nutrition labels.
2. The results of *in vitro* protein digestibility across different drying methods – oven drying at 85 °C and at 100 °C, freeze drying, and microwave-vacuum drying revealed no statistically significant differences in house cricket (*Acheta domesticus)* protein digestibility values ($p > 0.05$)
3. Additionally, blanching or steam blanching did not significantly impact digestibility outcomes when followed by drying.
4. Based on these findings, it is recommended to assess the necessity and time for blanching, to minimize processing steps for improved sustainability.

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