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Short communication

Centrifugal fractionation of mealworm larvae (*Tenebrio molitor,* L.) for protein recovery and concentration

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ABSTRACT

The utilization of edible insect protein as a novel source and potential substitute for conventional proteins used in food/feed formulations has recently drawn attention in industrial countries. The development of efficient processes for the recovery of insect protein is of crucial importance and will help to gain industrial applicability. However, systematic studies about important unit operations of insect protein recovery procedures are scarce. The present study evaluates a centrifugal fractionation concept by investigating the impact of solubilization pH (7.0–10.5), centrifugation speed/force (4000-8850 min^{-1}; 1860–9170 *g*) and centrifugation time (10–30 min) on the protein recovery of mealworm larvae (*Tenebrio molitor* L.). Therefore, a 2^3 full factorial screening design was applied. The statistical evaluation identified the centrifugation force and solubilization pH as key parameters determining the protein content and recovery in the supernatant fraction. Within the experimental campaign, protein content was enhanced from initially 39 g/100 g dry base (db) in the larvae to 56–65 g/100 g db in the extracts while the recovery ranged from 31 to 58%. The results of this study elucidated the main factors determining the efficiency of the centrifugal separation step for mealworm protein concentration and recovery and provide a database for the design of industrial separation processes.

1. Introduction

The inclusion of edible insects in the Western food and feed industry as an alternative source of animal-derived protein and fat has recently moved into the centre of attention (FAO, 2013; van Huis, 2013). Insect-derived proteins are of particular interest as a potential substitute or replacer of conventional proteins commonly used in food and feed applications. Beside few other species, mealworm larvae (*Tenebrio molitor* L.) are highly discussed for the integration in European food and feed industry due to existing large-scale rearing know-how and promising composition in terms of protein (50 g/100 g db) and fat (30 g/100 g db) content (Adámková, Kouřimská, Borkovcová, Kulma, & Mlček, 2016; Cortez Ortiz et al., 2016; Osimani et al., 2016; Rumpold & Schlüter, 2013).

The development of efficient and scalable processes for the recovery of standardized, nutritious and techno-functional insect-derived protein will be inevitable in order to meet the requirements of the highly industrialized Western food sector and consumer demands (Balzan, Fasolato, Maniero, & Novelli, 2016; Gere, Székely, Kovács, Kókai, & Sipos, 2017; Hartmann, Shi, Giusto, & Siegrist, 2015). Several protocols for the recovery of insect protein, especially for mealworm larvae, have already been investigated and resulted in protein concentrations and recoveries of 60–80 g/100 g db and 30–80%, respectively (Azagoh et al., 2016; Buβler, Rumpold, Jander, Rawel, & Schlüter, 2016; Yi, Van Boekel, & Lakemond, 2017; Yi et al., 2013; Zhao, Vázquez-Gutiérrez, Johansson, Landberg, & Langton, 2016). These procedures are inspired by conventional plant protein extraction routes including the following unit operations: (1) cell disruption, (2) defatting (3) aqueous protein solubilization (4) mechanical separation of insoluble fractions, (5) protein precipitation and (6) drying. Comprehensive evaluation of the relevant process parameters acting in the aforementioned unit operations will help to find optimal process windows with regard to protein recovery and separation efficiency of lipid and chitin fraction. To date, only systematic studies for the defatting of mealworm larvae either via solvent (Zhao et al., 2016) or supercritical CO_2 extraction (Purschke, Stegmann, Schreiner, & Jäger, 2017) and for the solubilization step (Xing-Qian, Dong-Hong, & Cui, 2001; Yi et al., 2017; Zhao et al., 2016) are available.

To the best of our knowledge, the present study provides the first systematic investigation on the impact of centrifugation on the protein

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Table 1
2^3 full factorial screening design for the centrifugal fractionation of mealworm larvae including independent variables solubilization pH (X_1), centrifugation speed/force (X_2) and time (X_3) and observed responses protein content (Y_C) and recovery (Y_R) in the supernatant.

Coded variables			Uncoded variables			Responses[a]	
X_1	X_2	X_3	X_1 [-]	X_2 [min^{-1}]/[g]	X_3 [min]	Y_C [g/100 g db]	Y_R [%]
0	0	0	8.75	6425/4800	20	61.9 ± 1.4	37.7 ± 3.0
−1	−1	−1	7.00	4000/1860	10	56.1 ± 0.8	30.9 ± 0.4
−1	−1	+1	7.00	4000/1860	30	58.9 ± 2.3	32.6 ± 1.3
−1	+1	−1	7.00	8850/9107	10	61.3 ± 0.4	36.1 ± 0.3
−1	+1	+1	7.00	8850/9107	30	64.7 ± 3.5	38.7 ± 2.1
+1	−1	−1	10.50	4000/1860	10	62.6 ± 1.3	58.7 ± 1.2
+1	−1	+1	10.50	4000/1860	30	59.8 ± 1.5	53.3 ± 1.3
+1	+1	−1	10.50	8850/9107	10	63.2 ± 0.3	54.3 ± 0.3
+1	+1	+1	10.50	8850/9107	30	63.0 ± 0.6	55.1 ± 0.5

[a] Results are means ± standard deviation of triplicate determinations.

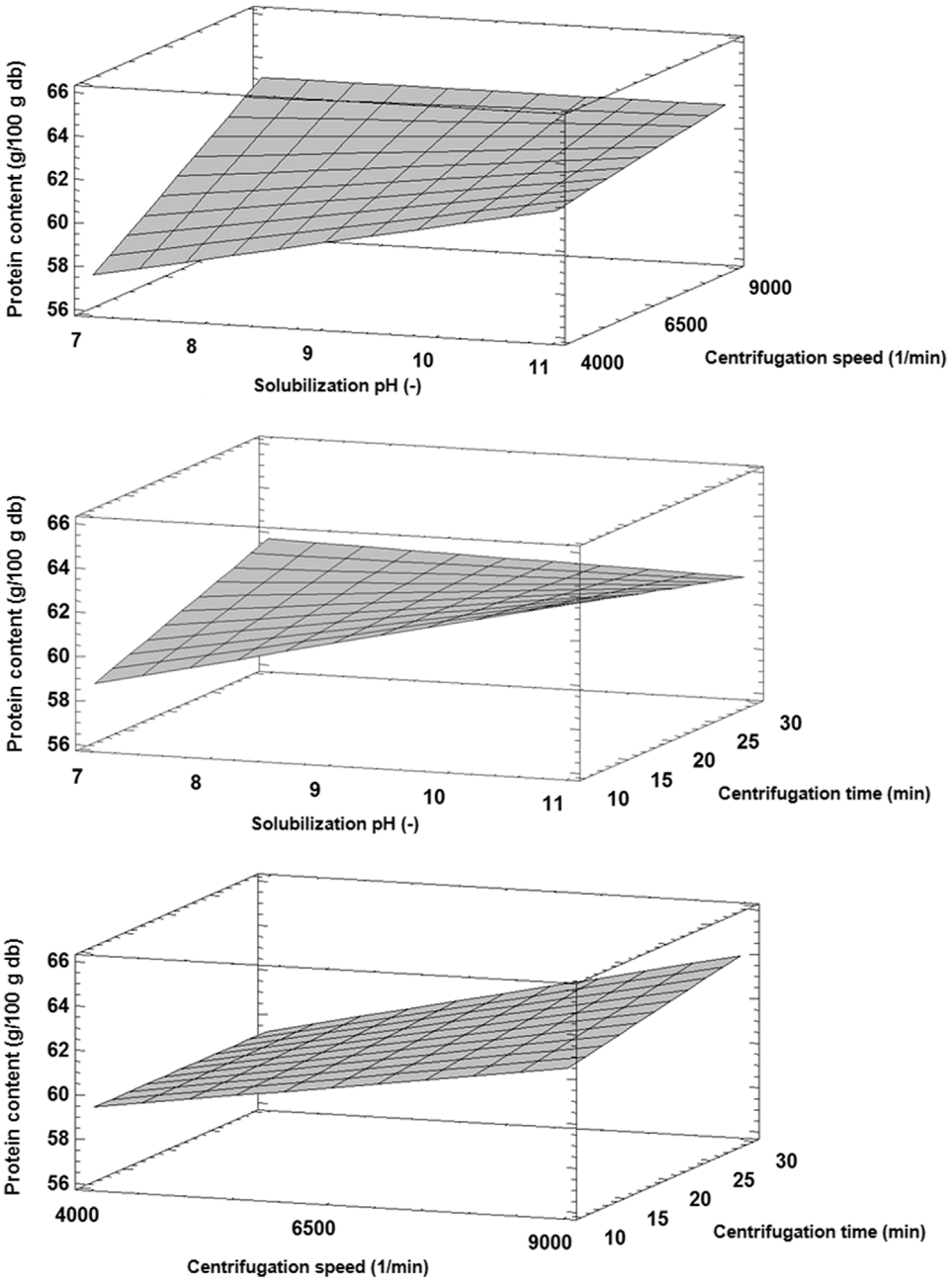

Fig. 1. - Response surface plots of the protein content (Y_C) in the supernatant fraction depending on level of the independent variables solubilization pH, centrifugation speed/force and time during centrifugal separation of mealworm larvae. Y_C is given in g/100 g dry base (db). For each plot, the third variable is set to zero level.

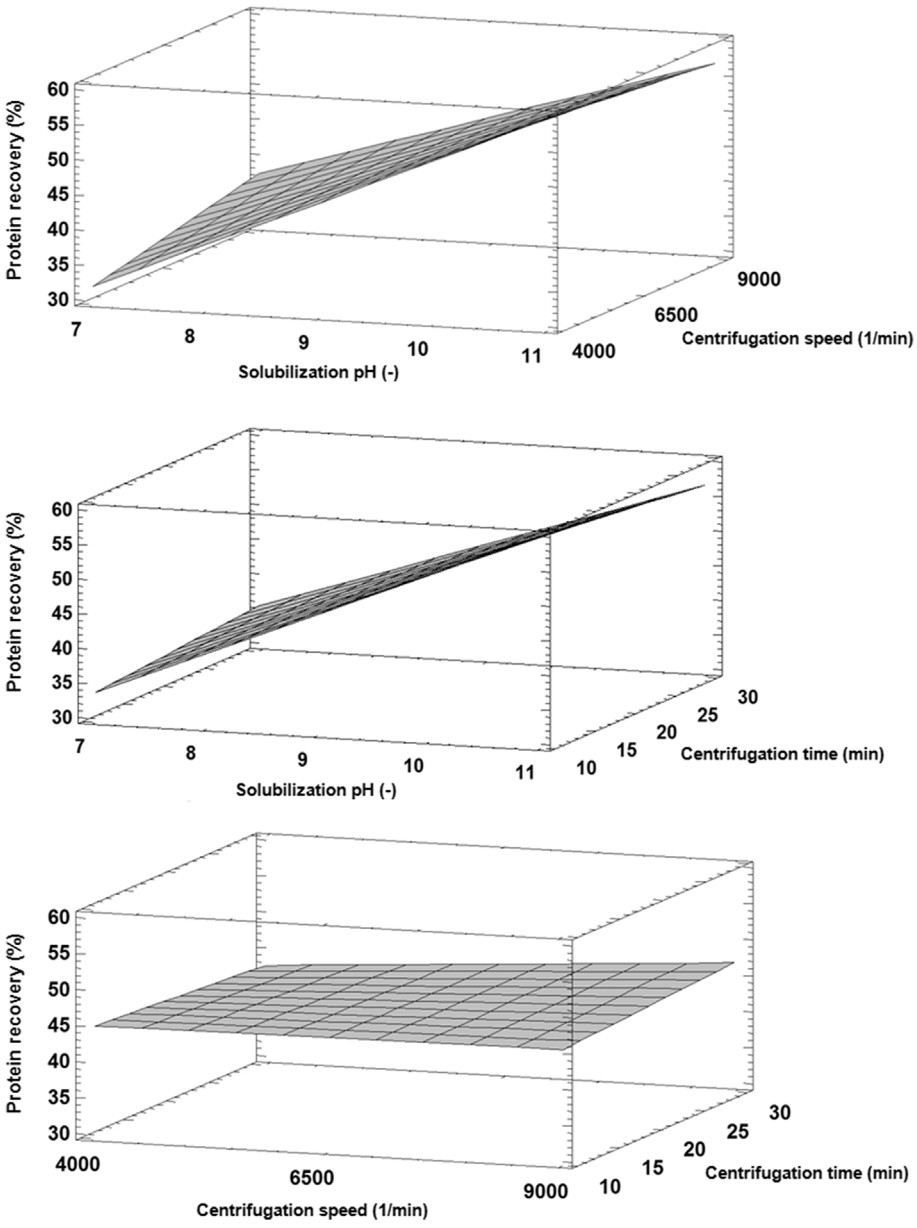

Fig. 2. - Response surface plots of the protein recovery (Y_R) in the supernatant fraction depending on level of the independent variables solubilization pH, centrifugation speed/force and time during centrifugal separation of mealworm larvae. Y_R is given in %. For each plot, the third variable is set to zero level.

content and recovery in the soluble protein fraction of mealworm larvae or even of edible insects in general. For this purpose, a 2^3 full factorial experimental design was applied in order to evaluate the main process parameters as well as their interactions.

2. Material and methods

2.1. Raw material

Mealworm larvae (TML) were purchased alive from a pet food vendor (Dragon Terraristik, Duisburg/Germany). Frass and substrate residues were separated from the larvae by sieving through 3 mm screening size. Subsequently, larvae were inactivated by freezing and stored at −30 °C until further use.

2.2. Chemical composition of larvae and protein extracts

The dry matter content was determined by oven-drying at 105 ± 3 °C based on method 950.46 (AOAC, 2002). After Soxhlet extraction with petroleum ether (b.p. 40–60 °C), the crude fat content was analysed gravimetrically according to standard No. 136 (ICC, 1984). The ash content was determined according to the method 923.03 (AOAC, 2002). The total nitrogen content was analysed using the Kjeldahl protocol according to method 928.08 (AOAC, 2002). Total protein content was calculated using the nitrogen conversion factor (N) of 4.76 and 5.60 for larvae and larval protein extracts, respectively, as recommended by Janssen, Vincken, van den Broek, Fogliano, and Lakemond (2017). All determinations were done in triplicate.

2.3. Centrifugal fractionation

Frozen TML were comminuted using a kitchen blender (MQ 300, Braun, Kronberg/Germany) for 90 s at a defined rotation speed. Deionized water was added to a concentration of 600 g/L and the slurry was mixed for additional 60 s. The desired solubilization pH was adjusted using 1 mol/L NaOH or 1 mol/L HCl. After 2 h of solubilization under continuous stirring, the slurry was centrifuged using the desired conditions according to the experimental design. The supernatant

Table 2
Analysis of variances (ANOVA, $p < 0.05$) and regression analysis (R^2) for protein content (Y_C) and recovery (Y_R) in the supernatant after centrifugal fractionation of mealworm larvae.

Variable	Sum of square		F-value		p-value	
	Y_C	Y_R	Y_C	Y_R	Y_C	Y_R
X_1 (solubilization pH)	7.16	863.20	27.92	491.68	< 0.05	< 0.05
X_2 (centrifugation speed)	27.39	9.46	106.81	5.39	< 0.05	< 0.05
X_3 (centrifugation time)	1.23	0.01	4.80	0.01	< 0.05	0.94
X_1X_2	6.51	24.15	25.37	13.76	< 0.05	< 0.05
X_1X_3	10.83	9.90	42.23	5.64	< 0.05	< 0.05
X_2X_3	1.30	6.30	5.08	3.59	< 0.05	0.06
R^2 (Y_C) = 0.991	R^2 adjusted (Y_C) = 0.935					
R^2 (Y_R) = 0.996	R^2 adjusted (Y_R) = 0.973					

fraction containing the soluble proteins was manually separated, frozen at −30 °C and freeze-dried at 0.2 mbar for 72 h (FreeZone 6, Labconco, Kansas City/USA).

2.4. Experimental design of centrifugal fractionation

A 2^3 full factorial screening design including fivefold centre point repetition was selected to investigate the influence of the process parameters (solubilization pH, centrifugation speed/force, centrifugation time) on the protein content Y_C and the total protein recovery Y_R in the supernatant fraction. The coded and un-coded levels of the independent variables used for the experimental designs are summarized in Table 1. Selected pH range were chosen according to best solubilization conditions found by Yi et al. (2017) and Zhao et al. (2016) for mealworm larvae. The experiments were performed in a randomized order to minimize the effect of external factors. The method of least squares was used to generate the regression equations for the response functions. The experimental data was fitted to a linear function as given in Eq. (1).

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i \neq j=1}^{3} b_{ij} X_i X_j \quad (1)$$

Y is the predicted response, b_0 is the constant term, b_i is the linear regression coefficient for main variable effects, b_{ij} the coefficient for two-way interaction effects, and X_i and X_j are the coded values of independent variables. Estimates of third-order interaction were used for experimental error estimation. Experimental results were evaluated by analysis of variances (ANOVA, $p \leq 0.05$) and regression analysis (R^2). Adequacy of regression models was tested comparing the results of the repeated centre points with the predicted response via one-sample *t*-test ($p \leq 0.05$).

2.5. Statistical analysis

Experimental design and statistical evaluation were carried out using Statgraphics Centurion XVI (Statpoint Technologies Inc., Warrenton/USA). Data are expressed as mean ± standard deviation (SD) of the performed replications and were subjected to one-way analysis of variances (ANOVA, $p \leq 0.05$) and Fisher's LSD post-hoc test.

3. Results and discussion

3.1. Chemical composition of larvae

The nutrient analysis of the untreated mealworm larvae revealed the following composition: 66.89 ± 0.59 g/100 g moisture, 38.64 ± 1.74 g/100 g db crude protein, 25.37 ± 1.08 g/100 g db crude fat and 7.64 ± 0.46 g/100 g db ash. The considerably low protein content in comparison to values between 49 and 60 g/100 g db reported in previous studies (Buβler et al., 2016; Finke, 2002, 2015) can be ascribed to the different conversion factor used (6.25 vs. 4.76). The composition might be further compromised by the gut content, as larvae were not starved prior to inactivation.

3.2. Evaluation of the experimental design

Experimental results are represented in Table 1 and illustrated in the response surface plots (Fig. 1 and Fig. 2). The protein content and the total protein recovery in the supernatant varied from 56 to 65 g/100 g db and 31–58%, respectively, depending on the applied process parameters. Table 2 summarizes the results of the ANOVA. Regression models for the relation between the responses and the independent variables solubilization pH (X_1), centrifugation speed/force (X_2), centrifugation time (X_3), and their interaction (X_1X_2, X_1X_3, X_2X_3) are given in Eq. (2) and Eq. (3). Insignificant interactions ($p > 0.05$) have already been eliminated.

$$Y_C = 29.261 + 3.236X_1 + 0.002X_2 + 0.514X_3 - 2{\cdot}10^{-4}X_1X_2 - 0.066X_1X_3 + 1.6{\cdot}10^{-5}X_2X_3 \quad (2)$$

$$Y_R = -39.221 + 9.838X_1 + 0.003X_2 + 4{\cdot}10^{-4}X_1X_2 + 0.064X_1X_3 \quad (3)$$

ANOVA demonstrated that all factors have a significant influence on the response Y_C while the centrifugation speed/force (X_2) was found to be the most important variable due to the better separation of insoluble solids and fat at higher centrifugal force as shown in Table 3. The effect of the solubilization pH (X_1) on Y_C is more pronounced at low centrifugal force and time (see Fig. 1). Higher concentration of protein can be achieved via precipitation as shown by Zhao et al. (2016). In contrast, only four out of six experimental factors significantly affected the protein recovery (Y_R). The centrifugation time (X_3) and the interaction between centrifugation speed/force and time (X_2X_3) were not

Table 3
Composition of the supernatant fractions on dry base (db) after centrifugal fractionation of mealworm larvae including the independent variables solubilization pH (X_1), centrifugation speed/force (X_2) and time (X_3).

Uncoded variables			Composition[a]		
X_1 [-]	X_2 [min^{-1}]/[g]	X_3 [min]	Fat [g/100 g db]	Ash [g/100 g db]	Other[b] [g/100 g db]
7.00	4000/1860	10	6.84 ± 1.02	11.31 ± 0.10	25.73
7.00	4000/1860	30	2.77 ± 0.19	10.95 ± 0.03	27.35
7.00	8850/9107	10	2.66 ± 0.08	11.62 ± 0.15	24.40
7.00	8850/9107	30	2.00 ± 0.07	11.26 ± 0.06	22.01
10.50	4000/1860	10	4.54 ± 0.07	10.68 ± 0.12	22.13
10.50	4000/1860	30	2.95 ± 0.16	9.60 ± 0.03	26.66
10.50	8850/9107	10	2.84 ± 0.07	10.19 ± 0.39	23.73
10.50	8850/9107	30	1.46 ± 0.29	10.62 ± 1.39	24.91

[a] Results are means ± standard deviation of triplicate determinations.
[b] Residual components were expressed as "Others" calculated by subtracting the analysed components (protein, fat and ash) from 100.

significant. ANOVA revealed that the solubilization pH (X_1) is by far the most important factor with regard to protein recovery (see Fig. 2). This can be ascribed to the better solubilization of the mealworm proteins at alkaline pH similarly shown by Zhao et al. (2016), Yi et al. (2017) and Buβler et al. (2016). The influence of the centrifugation speed on Y_R is negligible as indicated in the response surface plots. As reported by Buβler et al. (2016), re-extraction of the insoluble pellet will increase the total protein recovery.

4. Conclusion

For the first time, the influence of centrifugation parameters on the concentration and yield of soluble proteins from mealworm larvae was investigated. The centrifugation speed and the solubilization pH were found to be the key parameters affecting the protein content and the total protein recovery, respectively. The obtained results demonstrated promising process windows for centrifugal separation processes of edible insects and will help to design effective and sustainable process conditions for insect protein recovery. Further research is needed with regard to the influence of the chosen process conditions on the protein composition (SDS-PAGE). Furthermore, the characterisation of the techno-functional properties of the derived protein-rich fraction will be of crucial importance for their future utilization in food formulations.

Conflict of interest

The authors have declared no conflicts of interest.

References

Adámková, A., Kouřimská, L., Borkovcová, M., Kulma, M., & Mlček, J. (2016). Nutritional values of edible Coleoptera (*Tenebrio molitor*, *Zophobas morio* and *Alphitobius diaperinus*) reared in the Czech Republic. *Potravinárstvo - Slovak Journal of Food Sciences, 10*, 663–671.

AOAC. (2002). Official methods of analysis (W. Horwitz Ed. 17 ed. Vol. 2). Gaithersburg: Association of Official Analytical Chemists.

Azagoh, C., Ducept, F., Garcia, R., Rakotozafy, L., Cuvelier, M. E., Keller, S., ... Mezdour, S. (2016). Extraction and physicochemical characterization of *Tenebrio molitor* proteins. *Food Research International, 88*, 24–31. https://doi.org/10.1016/j.foodres.2016.06.010.

Balzan, S., Fasolato, L., Maniero, S., & Novelli, E. (2016). Edible insects and young adults in a north-east Italian city an exploratory study. *British Food Journal, 118*, 318–326. http://dx.doi.org/10.1108/BFJ-04-2015-0156.

Bußler, S., Rumpold, B. A., Jander, E., Rawel, H. M., & Schlüter, O. K. (2016). 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, 2*, e00218. http://dx.doi.org/10.1016/j.heliyon.2016.e00218.

Cortez Ortiz, J., Torres Ruiz, A., Morales-Ramos, J. A., Thomas, M., Rojas, M., & Tomerlin, J. (2016). Insect mass production technologies. In A. T. Dossey, J. A. Morales-Ramos, & M. Guadalupe Rojas (Eds.). *Insects as sustainable food ingredients - production, processing and food applications* (pp. 153–200). London: Academic Press.

FAO (2013). *Edible insects - future prospects for food and feed security.* (0258–6150). Retrieved from Rome.

Finke, M. D. (2002). Complete nutrient composition of commercially raised invertebrates used as food for insectivores. *Zoo Biology, 21*, 269–285. http://dx.doi.org/10.1002/zoo.10031.

Finke, M. D. (2015). Complete nutrient content of four species of commercially available feeder insects fed enhanced diets during growth. *Zoo Biology, 34*, 554–564. http://dx.doi.org/10.1002/zoo.21246.

Gere, A., Székely, G., Kovács, S., Kókai, Z., & Sipos, L. (2017). Readiness to adopt insects in Hungary: A case study. *Food Quality and Preference, 59*, 81–86. https://doi.org/10.1016/j.foodqual.2017.02.005.

Hartmann, C., Shi, J., Giusto, A., & Siegrist, M. (2015). The psychology of eating insects: A cross-cultural comparison between Germany and China. *Food Quality and Preference, 44*, 148–156. https://doi.org/10.1016/j.foodqual.2015.04.013.

van Huis, A. (2013). Potential of insects as food and feed in assuring food security. *Annual Review of Entomology, 58*, 563–583.

ICC (1984). *Cereals and cereal products - determination of total fat content.* ICC Recomendation No. 136 *ICC Standard methods*.

Janssen, R. H., Vincken, J.-P., van den Broek, L. A. M., Fogliano, V., & Lakemond, C. M. M. (2017). Nitrogen-to-Protein conversion factors for three edible insects: *Tenebrio molitor, Alphitobius diaperinus*, and hermetia illucens. *Journal of Agricultural and Food Chemistry, 65*, 2275–2278. http://dx.doi.org/10.1021/acs.jafc.7b00471.

Osimani, A., Garofalo, C., Milanović, V., Taccari, M., Cardinali, F., Aquilanti, L., ... Clementi, F. (2016). Insight into the proximate composition and microbial diversity of edible insects marketed in the European Union. *European Food Research and Technology, 243*, 1157–1171. http://dx.doi.org/10.1007/s00217-016-2828-4.

Purschke, B., Stegmann, T., Schreiner, M., & Jäger, H. (2017). Pilot-scale supercritical CO2 extraction of edible insect oil from *Tenebrio molitor* L. larvae – influence of extraction conditions on kinetics, defatting performance and compositional properties. *European Journal of Lipid Science and Technology, 119*, 1–12. http://dx.doi.org/10.1002/ejlt.201600134.

Rumpold, B. A., & Schlüter, O. K. (2013). Nutritional composition and safety aspects of edible insects. *Molecular Nutrition & Food Research, 57*, 802–823. http://dx.doi.org/10.1002/mnfr.201200735.

Xing-Qian, Y., Dong-Hong, L., & Cui, H. (2001). Some factors' effects on the solubility of protein from yellow mealworm (*Tenebrio molitor* L) larvae. *Journal of Zhejiang University Science, 2*, 436–438. http://dx.doi.org/10.1007/bf02840562.

Yi, L., Lakemond, C. M. M., Sagis, L. M. C., Eisner-Schadler, V., van Huis, A., & van Boekel, M. A. J. S. (2013). Extraction and characterisation of protein fractions from five insect species. *Food Chemistry, 141*, 3341–3348. https://doi.org/10.1016/j.foodchem.2013.05.115.

Yi, L., Van Boekel, M. A. J. S., & Lakemond, C. M. M. (2017). Extracting *Tenebrio molitor* protein while preventing browning: Effect of pH and NaCl on protein yield. *Journal of Insects As Food and Feed, 3*, 21–31. http://dx.doi.org/10.3920/jiff2016.0015.

Zhao, X., Vázquez-Gutiérrez, J. L., Johansson, D. P., Landberg, R., & Langton, M. (2016). Yellow mealworm protein for food purposes - extraction and functional properties. *PLoS One, 11*.