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Article

Eco-Efficiency of Mealworm (*Tenebrio molitor*) Protein Extracts

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ABSTRACT: The potential of edible insects as a novel ingredient in high value-added products has been investigated to find alternatives to conventional protein sources. Low acceptability of entomophagy is the main challenge, but the development of insect protein-based food products could improve consumer perception. Nevertheless, while insect rearing has a low environmental impact, there are no studies on environmental performance related to the production of insect protein extracts. In this work, *Tenebrio molitor* (TM) protein extracts were produced by protein alkaline solubilization (one or two steps) with or without isoelectric precipitation, and the global warming potential (GWP) related to the different protein extracts was calculated. The optimal lipid extraction rate was 86.9% using a hexane: ethanol ratio of 1:2. Protein extraction and purification rates ranged from 54.7 to 94.4% and 80.0 to 49.4%, respectively. Depending on the protein purification and allocation method applied, the GWP of a TM protein extract was 3,050 to 10,871 kg CO_2 eq. per ton of the extract produced. Eco-efficiency scores associated to TM proteins extracts were between those published with plant-based and animal-based protein sources, confirming the interesting environmental performance of insect-derived protein ingredients.

KEYWORDS: *life cycle assessment, Tenebrio molitor, protein extract, environmental impact, eco-efficiency*

1. INTRODUCTION

The demand for alternative protein sources is making it more important to offer sustainable food solutions to the growing world population that decrease the environmental impact of protein production while meeting consumer demand for more affordable and nutritious food products. To address these challenges, the consumption of edible insects is being extensively studied. More specifically, it has been demonstrated that the production of edible insects has lower environmental impact compared to livestock[1−4] since insect rearing requires less energy, water, and land and generates lower amounts of greenhouse gas emissions.[5] Moreover, edible insects represent an interesting source for high-quality proteins, unsaturated fats, vitamins, and minerals.[6]

Among edible insects, the *Tenebrio molitor* (TM) mealworm is one of the most popular species as a food source.[7] Despite the environmental and nutritional benefits of including edible insects in the diet, consumer acceptance for entomophagy in the western population remains a major challenge. In trying to better understand drivers and barriers of insect consumption, studies have indicated that the acceptance of edible insects could be improved by the development of insect-based foods in which insects are not visible.[8] In this context, numerous studies have been published on the conventional and alternative food processes for the extraction, concentration, and purification of edible insect macronutrients in order to generate particular ingredients. Defatting followed by protein extraction/purification procedures are crucial steps in generating edible insect protein extracts. Lipid extraction can be carried out by mechanical pressing, aqueous and solvent-based extraction, and alternative methods such as supercritical CO_2 or a combination of high-pressure processing and solvent extraction. Overall, the use of solvents demonstrated higher lipid extraction rates compared to other strategies. From the defatted edible insect meal, the production of the edible insect protein extract was performed by alkaline solubilization with or without isoelectric precipitation. More specifically, the protein concentration recovered from edible insects varied from 61.9 to 64.7% after alkaline solubilization,[9] whereas it reached 70.1 to 78.5% after isoelectric precipitation.[10] The protein extraction rate varied from 37.7 to 58.7% and 31.0 to 38.9% after alkaline solubilization and precipitation, respectively.[9,10]

Although defatting and protein extraction/purification have been largely studied to optimize the recovery of the lipid and protein fractions, as well as to evaluate the techno-functional properties of protein-enriched fractions, the environmental impact of these two key steps has not been detailed in previous studies. Indeed, life cycle assessment (LCA) research has been conducted specifically on the production of edible insects (insect rearing with different diets) without considering the different processing steps to generate protein-enriched ingredients.[11,12] Some published studies also considered both fat and meal as end products, but by focusing only on the mechanical pressing method.[13,14] The work of Thévenot et al. on environmental impact assessment of mealworm meal for animal feed showed that 0.99 and 3.75 kg CO_2 equiv were produced to generate 1 kg of fresh TM at the farm gate and processing gate, respectively.[13] In this study, larvae meal contained 65.0% protein and was produced by sifting,

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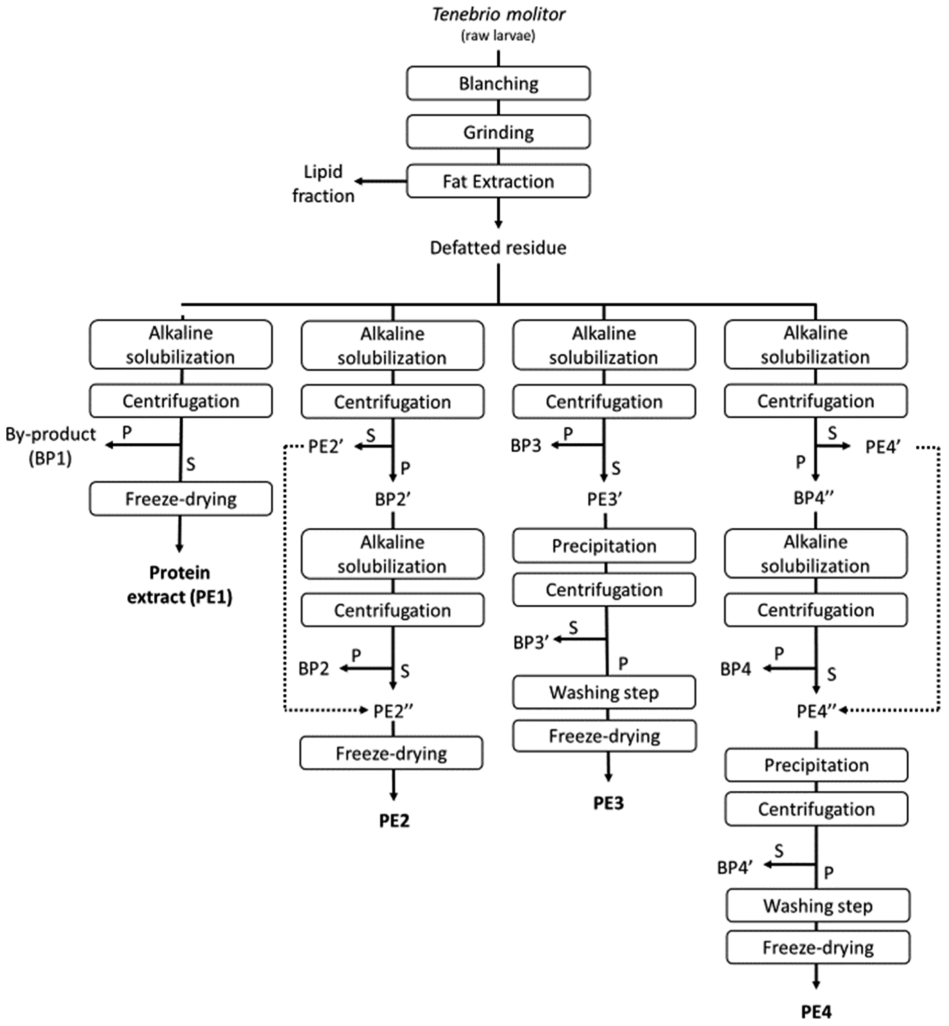

Figure 1. Experimental design of the production of protein extracts from *TM* larvae using one (PE1) or two (PE2) solubilization steps, following by a protein precipitation step (PE3 for one solubilization and PE4 for two solubilizations). P indicates the precipitate, and S indicates the supernatant.

blanching, cold pressing, and drying.[13] Similarly, the study of Smetana et al. focused on different scenarios for insect production (black soldier fly larvae) but did not describe the environmental impacts of the different processing steps for generating the insect protein extract from fresh larvae.[14] Thus, the environmental impact related to the production of edible insect protein extracts should be investigated to improve the sustainability of the insect-based food industry. Moreover, estimation of the eco-efficiency [relationship between product (or service) value and its environmental impact[15] of insect-based ingredients would help bridge their global warming potential (GWP) and value in terms of the protein content. It is worth noting that no data are currently available regarding the eco-efficiency of insect-based ingredients. Additionally, it would be relevant to compare the eco-efficiency of insect protein extracts with other protein sources to determine which is the most interesting from the protein content and GWP perspectives.

Consequently, this study focuses on determining the impact of different processing steps on the eco-efficiency of mealworm protein extracts. More specifically, the objectives of this work are (1) to study the effect of various defatting and protein extraction−purification parameters on protein and lipid extraction rates as well as protein recovery in mealworm extracts; (2) to determine the GWP associated with the production of mealworm protein extracts; and (3) to calculate the eco-efficiency scores of the protein extracts obtained.[52−54]

2. MATERIALS AND METHODS

2.1. Proximate Composition of Raw Mealworm Larvae. The proximate composition of TM provided by a local mealworm producer (Neoxis, Saint-Flavien, Québec, Canada) was determined following a freezing step. Total nitrogen was determined using the Dumas combustion method (Elementar rapid Micro N, Langenselbold, Germany). A nitrogen-to-protein conversion factor of 4.76 was used, as recommended by Janssen et al.[16] for the whole TM mealworm. The crude fat and chitin contents were obtained according to the protocols published by Laroche et al. and Spinelli, respectively.[10,17] Ash content and dry matter content were determined by the 938.08 and 950.46 AOAC methods.[18] The total solid content of frozen TM was 38.2% on a wet basis. The protein, lipid, chitin and ash contents were 43.3, 30.6, 5.5, and 4.0%, respectively. For the next processing and analytical steps, blanched mealworms (100 °C for 40 s) were used to improve their microbial quality[19] and to minimize the color change of larvae due to oxidation.[20,21]

2.2. Optimization of Lipid Extraction from Blanched Mealworm Larvae. Lipid extraction was performed on 25 g of blanched mealworm larvae. First, different ratios of hexane/ethanol (0:1, 1:0, 1:1, 2:1, and 1:2) were tested for lipid extraction. For each

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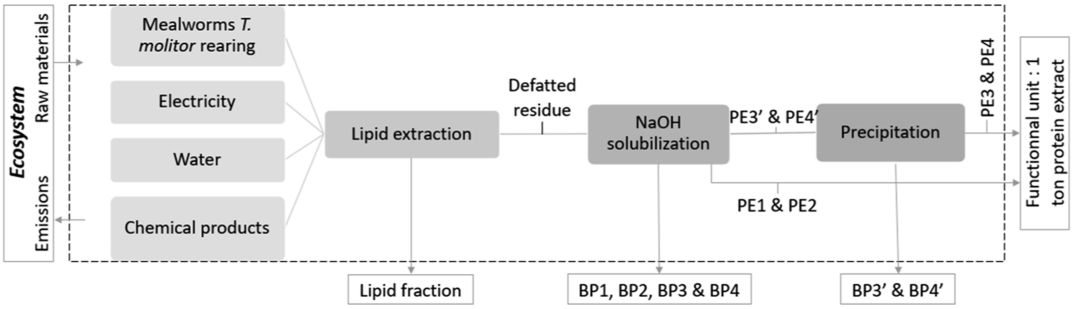

Figure 2. System boundaries for the production of the mealworm protein extract (only the steps associated with the generation of products and byproducts were considered).

ratio, the total solvent volume was 100 mL. Second, the ratio that allowed the maximum lipid extraction was selected to optimize the volume of the solvent. The volumes of the solvent tested were 50, 60, 70, 80, and 90 mL. Lipid extraction was performed using the method of Feng et al. with slight modifications.[22] Briefly, the mixture of blanched mealworm larvae and the extraction solvent was ground and homogenized at 10,000 rpm for 60 s using an Ultra-Turrax homogenizer (IKA, Staufen im Breisgau, Germany). The suspensions were centrifuged (12,000 g for 60 s), and the supernatants and pellets were recovered separately. Pellets were dried in a vacuum oven at 100 °C for 5 h to eliminate the residual solvent and water. A 6 h Soxhlet extraction with hexane was performed on the pellet to measure the final lipid content and determine the extraction rate according to eq 1:[23]

$$\text{Lipid extraction rate (\%)} = \frac{\text{pellet mass} \times \text{pellet lipid content}}{\text{initial mass} \times \text{initial lipid content}} \times 100 \quad (1)$$

Third, optimal fat extraction parameters were chosen according to the maximum lipid extraction rate and minimum volume of the solvent used. These optimal parameters were used for the following protein extractions and purifications.

2.3. Protein Extraction and Purification from Defatted Mealworm Extracts. Protein was extracted and purified from defatted mealworm extracts. Four protein extraction and purification procedures were performed as presented in Figure 1: (1) one protein solubilization step, (2) two protein solubilization steps, (3) one protein solubilization step followed by isoelectric precipitation, and (4) two protein solubilization steps followed by isoelectric precipitation. The protein solubilization step was performed under alkaline conditions (NaOH 0.25 M, 60 min at 50 °C) according to the protocol of Zhao et al.[24] After protein solubilization, the suspensions were centrifuged at 4500*g* for 10 min at 4 °C, and the supernatants consisting of the protein extracts (PE1, PE2′, PE3′, and PE4′) were recovered. A second protein solubilization procedure was performed on the pellet (BP2′ and BP4″) recovered after the first protein solubilization step. The suspensions were centrifuged at 4500*g* for 10 min at 4 °C, and the supernatants were recovered and pooled with PE2′ and PE4′. Fraction PE1, recovered from the one-step protein solubilization, and fraction PE2, recovered from the first and second solubilization steps, were adjusted to a pH of 7.0 and freeze-dried. For the isoelectric precipitation step, the pH values of supernatants recovered after protein solubilization (PE3′ and PE4′ combined with PE4″) were adjusted to 4.4, and the solutions were centrifuged (4500*g*, 10 min at 4 °C). The pellet recovered was washed with distilled water, centrifuged (4500*g*, 10 min at 4 °C) to eliminate acidic and basic residues, and freeze-dried (PE3 and PE4). Total nitrogen was determined for protein extracts recovered after solubilization and precipitation procedures using the Dumas combustion method (Elementar rapid Micro N cube, Langenselbold, Germany). A nitrogen-to-protein conversion factor of 5.60 was used, as recommended by Janssen et al. for TM protein fractions.[16] The protein extraction rate was calculated according to eq 2:[23]

$$\text{Protein extraction rate (\%)} = \frac{\text{extract mass} \times \text{extract protein content}}{\text{initial mass} \times \text{initial protein content}} \times 100 \quad (2)$$

2.4. Life Cycle Assessment. An LCA was performed on the four mealworm protein extracts obtained after protein solubilization (PE1 and PE2) and purification (PE3 and PE4) to compare the environmental impacts associated with each scenario in the context of the production of protein extracts for human consumption. The functional unit was the production of 1 ton of TM protein extract per batch at the factory gate, ready to be delivered (Figure 2). The input and output data of the TM rearing and processing phases are presented in Table S1. Sensitivity analysis was performed using two different scenarios. Scenario 1, presented in Table 1, was a mass allocation for each byproduct (protein extract; lipid fraction; chitin fractions BP1, BP2, BP3, and BP4; and supernatants BP3′ and BP4′) where valorization of all byproducts was considered. Indeed, the lipid fraction generated after defatting of mealworm larvae could be used as food and feed or for biodiesel production.[25−28] The chitin-enriched fraction recovered after protein extraction by alkaline solubilization could be used to generate pure chitin or its derivatives, such as *N*-acetyl-D-glucosamine or N,N′-diacetylchitobiose, which had already demonstrated different bioactive properties.[29] The supernatant generated after isoelectric precipitation of mealworm proteins was rich in minerals due to the use of NaOH and HCl for the protein solubilization and precipitation, respectively. Thus, this supernatant could be valuable for animal nutrition after applying a desalination step. Scenario 2 was also a mass allocation, but only the protein extract and the lipid fraction were considered valuable, whereas other byproducts (chitin and supernatant) were considered to be waste. Thus, for the protein extraction step (NaOH solubilization and precipitation), 100% GWP impacts were allocated to the protein extract and 0% for the chitin fraction and supernatant. This scenario

Table 1. Percentage of GWP Impacts of Each Protein Extraction Step Allocated to Products and Byproducts for Scenario 1[a]

protein extract	fraction	NaOH solubilization (%)	precipitation (%)
PE1	BP1	41	N/A[a]
	PE1	59	
PE2	BP2	26	
	PE2	74	
PE3	BP3	41	0
	BP3′	59	34
	PE3		66
PE4	BP4	26	0
	BP4′	74	18
	PE4		82

[a]Not applicable since no isoelectric precipitation was performed to generate PE1 and PE2.

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assumed that the lipid fraction was easy to valorize since it did not involve additional processing steps. However, chitin fraction and supernatants were identified as waste since their valorization was more difficult, especially in the small- or medium-scale factories, due to the need for different processing steps to generate valuable products out of these byproducts. Finally, for the delipidation step, since the same extraction process was performed for every extract and the lipid fraction was managed the same way in both scenarios, 73% of the GWP impact of this step was allocated to the defatted cake and 27% was allocated to the lipid fraction (mass allocation).

The LCA was carried out according to ISO 14040 and 14044[30,31] using Open-LCA software (GreenDelta, Berlin, Germany) and the Ecoinvent database (v3.6, Ecoinvent, Zurich, Switzerland). The life cycle impact assessment method was IPCC 2013. Environmental impacts were calculated from the cradle-to-processing gate and excluded cleaning-in-place operations during the processing steps, transport of raw materials, and packaging. Whenever possible, the process adopted the Quebec context, regarding, for example, environmental impact associated with electricity consumption. For the insect rearing step, the life cycle inventory data were obtained from a local mealworm producer (St-Flavien, Quebec, Canada) and from the literature.[11,13,32−36] Data for the different processing steps (blanching, grinding, centrifugation, alkaline solubilization, centrifugation, precipitation, washing, preconcentration, and drying) were collected from experiments performed in this study, from the published studies,[37−40] and from technical data sheets from manufacturer websites.[41,42] The preconcentration step was included in the process line of PE1 and PE2 since the initial total solid content in supernatants recovered from the NaOH solubilization step ranged from 3 to 5% and had to be concentrated to 20% by evaporation before final drying.[43] Although freeze-drying was applied in our experiments, it was not considered in LCA due to its high energy demand and low use at the commercial scale in the insect industry.[44] Equations used to calculate energy consumption from processing steps are available in the Supporting Information

2.5. Eco-Efficiency Calculation. The eco-efficiency of the insect protein extract and of other protein sources was calculated according to ISO 14045.[15] The protein content of the mealworm extract (or other protein sources) was considered as the product value, while the GWP represented its environmental impact

$$\text{eco-efficiency} = \frac{\text{value (g protein} \times \text{kg product)}}{\text{GWP (kg CO}_2\text{ equiv} \times \text{kg product)}} \quad (3)$$

A higher eco-efficiency score indicates a better eco-efficiency performance of the scenario.

2.6. Statistical Analysis. All experiments (lipid extraction, protein extraction, and purification) were performed in triplicate in a fully random plan. A one-way ANOVA test was performed on the lipid and protein extraction data. The least significant difference (LSD) test with a p-value of 0.05 was used to determine the significant differences.

3. RESULTS AND DISCUSSION

3.1. Optimization of the Defatting Step. The first step was to optimize the solvent ratio for the extraction. Table 2 (left side) shows the lipid extraction rates obtained after the defatting of mealworm larvae using different hexane/ethanol ratios (0:1 to 1:2). While the use of hexane (1:0) led to higher lipid extraction rates than ethanol (0:1) alone (65.6 and 34.1%, respectively), using a mixture of both solvents significantly increased the efficiency of the delipidation, regardless of the ratio. Overall, all two-solvent ratios tested (1:1, 2:1, and 1:2) produced the highest and statistically similar lipid extraction rates (89.3, 92.3, and 93.5%, respectively). This tendency was also observed by Feng et al. using black soldier fly larvae.[22] Indeed, these authors mentioned that mixing polar (such as ethanol) and nonpolar (such as hexane) solvents improved the extraction of polar lipids (glycolipids and phospholipids) and nonpolar lipids (triacylglycerols) compared to polar and nonpolar solvents used alone.[22] Although the lipid extraction rates were similar ($p > 0.05$) for hexane/ethanol ratios of 1:1, 2:1, and 1:2, a 1:2 ratio of hexane/ethanol was selected to minimize the use of hexane since it presents environmental and health issues, especially if inhaled.[37] The second step was to optimize the volume of the solvent (from 50 to 100 mL) using the optimal hexane/ethanol ratio (1:2) and lipid extraction rate for each condition. These results are presented in Table 2 (right side). As expected, and as experienced by Feng et al., the larger the volume of the solvent, the higher the rate of delipidation.[22] More specifically, the lipid extraction rate varied from 79.7 to 93.5% when the total solvent volumes used were 50 and 100 mL, respectively. However, no statistical differences were observed for solvent volumes greater than 60 mL. A solvent volume of 60 mL was selected since this represented a good compromise to limit the use of the solvent while retaining the best lipid extraction rates (Table 2). Consequently, the defatted mealworm larvae residue used for protein extraction and precipitation was obtained after delipidation using 60 mL of hexane and ethanol at a ratio of 1:2 (20 mL of hexane and 40 mL of ethanol).

Table 2. Lipid Extraction Rates (%) as a Function of Hexane/Ethanol Ratios and Solvent Volume

hexane/ethanol ratio[a]	lipid extraction rate (%)[b]	solvent volume (mL)[c]	lipid extraction rate (%)[a]
0:1	34.1 ± 2.6[a]	50	79.7 ± 6.2[a]
1:0	65.6 ± 1.9[b]	60	86.9 ± 2.4[b]
1:1	89.3 ± 10.2[c]	70	82.6 ± 5.2[ab]
2:1	92.3 ± 1.6[c]	80	87.3 ± 0.9[b]
1:2	93.5 ± 2.7[c]	90	88.4 ± 1.0[bc]

[a]For all hexane/ethanol ratios tested, the total volume of the solvent was 100 mL. [b]Means of three replicates ±standard deviation. Values in each column with different letters are significantly different (LSD, $\alpha = 0.05$). [c]Volumes tested only for a hexane/ethanol ratio of 1:2.

3.2. Protein Extraction Rates. Table 3 presents the protein and ash contents of the protein extracts (Figure 1) generated after protein solubilization alone (PE1 and PE2) or in combination with isoelectric precipitation (PE3 and PE4). The protein content of extracts generated using alkaline solubilization (PE1 and PE2) was lower after two solubilization steps compared to the content obtained after one protein solubilization step (54.7 vs 60.5%). This difference is explained by the higher ash content recovered after two protein solubilization steps (PE2—32.0%) compared to one protein

Table 3. Protein and Ash Contents and Protein Extraction Rates in Mealworm Protein Extracts Produced by Alkaline Solubilization and Precipitation, Tested Separately (PE1 and PE2) or in Combination (PE3 and PE4)

protein extract	protein content (%, dry basis)[a]	ash content (%, dry basis)[a]	protein extraction rate (%)
PE1	60.5 ± 0.7[a]	22.6 ± 0.7[a]	76.2 ± 1.8[a]
PE2	54.7 ± 1.8[b]	32.0 ± 4.2[b]	94.4 ± 5.6[b]
PE3	80.0 ± 1.2[a]	1.5 ± 1.1[a]	49.4 ± 5.7[a]
PE4	79.4 ± 0.6[a]	1.5 ± 0.4[a]	67.9 ± 5.3[b]

[a]Means of three replicates ±standard deviation. PE1 is compared to PE2 and PE3 is compared to PE4. Values in each column with different letters are significantly different (LSD, $\alpha = 0,05$).

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solubilization step (PE1—22.6%). However, the additional protein solubilization step improved the protein extraction rate by 15% (76.2% in PE1 to 94.4% in PE2), as also demonstrated by Zhao et al.[24] The protein content in PE3 obtained after one step of protein solubilization and isoelectric precipitation reached 80.0%, but the protein extraction rate (49.4%) was lower than that for alkaline solubilization alone (76.2% for PE1 and 94.4% for PE2). Similar values for the protein content and extraction rate were obtained by Laroche et al. after alkaline solubilization and isoelectric precipitation were performed on commercial mealworm meals defatted by different methods.[10] From fresh mealworms, Bußler et al. recovered a protein extract with a protein content close to 70% after the defatting step using hexane, followed by aqueous protein extraction and isoelectric precipitation.[45] As observed in the present study, Bußler et al. calculated a very low protein extraction rate of 21 to 22%.[45] This low protein extraction rate was explained by the fact that only mealworm proteins with an isoelectric point close to 4.4 could be recovered by precipitation.[46] The protein content in PE4 reached 79.4%, which was similar to the one published by Zhao et al. after two steps of alkaline solubilization followed by isoelectric precipitation applied to defatted and freeze-dried mealworm larvae.[24] However, the protein content in PE4 was similar to the value obtained for PE3 (80.0%) ($p > 0.05$), which indicates that the second alkaline solubilization step performed before isoelectric precipitation on the insoluble protein extract (BP4″) (Figure 1) did not improve the protein content in the precipitate (PE4). Nevertheless, compared to that for PE3, the protein extraction rate for PE4 increased to 67.9%, which means that the second alkaline solubilization was efficient enough to resolubilize the protein from the insoluble protein fraction PE4" (Figure 1) and to improve the protein extraction rate compared to that after the one-step alkaline solubilization. Finally, the protein contents obtained for PE3 and PE4 protein extracts were higher than protein extracts generated only after protein solubilization without precipitation (60.5% for PE1 and 54.7% for PE2). This is explained by the lower content of ash, which was mainly discarded in the supernatant fraction generated by centrifugation and washing (Figure 1), confirming that isoelectric precipitation is effective for protein recovery at a very high level of purity.[46,47]

3.3. Environmental Impact Assessment. Table 4 presents the GWP (kg CO_2 equiv) scores to produce 1 ton of the insect extract obtained by each of the four methods described previously. Results are presented for both allocation scenarios. When each byproduct is considered valuable, the GWP is lower for every production method than that for the allocation scenarios when byproducts generated during protein extraction steps are considered waste. In the first scenario, protein extracts obtained from a single alkaline solubilization, without (PE1) or with (PE3) precipitation, produce the lowest GWP, emitting 4564 kg CO_2 eq./ton and 3553 kg CO_2 eq./ton, respectively. In comparison, extracts produced using two solubilization steps (PE2 and PE4) have greater environmental impact than those produced using one solubilization for this scenario. Indeed, extracts produced by two solubilizations required more resources (water, NaOH, energy, etc), increasing the GWP associated with their production. Even though two solubilizations lead to higher extraction yields, mass allocation leads to a redistribution of the impact between every byproduct, so for scenario 1, a higher extraction yield means more impact allocated to the extract. PE2 and PE4 have GWP 58 and 37% higher than that of PE3, respectively. Overall, when considering the valorization of every byproduct, precipitation reduced the GWP associated with extracts, even if more resources were needed to precipitate the proteins (HCl, water, energy, etc.). This was due to the higher protein content in the final extracts (PE3 and PE4), having less mass compared to extracts obtained without precipitation (PE1 and PE2), which reduced the proportion of the impact allocated to these extracts. For the second scenario, where only lipid and protein extracts were valuable, it was observed that any additional step increased the GWP. Therefore, PE1 produces a GWP 42% lower than that of PE4. These differences are mainly due to fewer required resources as well as lower waste generation, such as the supernatant produced after precipitation.

Table 4. GWP Impact Category of the Four Protein Extracts Produced by Delipidation and Protein Extraction From the *TM* Mealworm

protein extract	GWP (kg CO_2 equiv/ton TM protein extract)	
	scenario 1	scenario 2
PE1	4 564	6 357
PE2	5 606	9 087
PE3	3 553	10 048
PE4	4 880	10 962

Figure 3 presents the proportional impact of different inputs of the products as a function of the production method and allocation scenario. These diagrams help identify the hot spots of protein extract production and to identify which steps to optimize to improve the environmental performance. Impacts associated with the insect breeding phase were observed to be the most environmentally harmful. Also, steam used for insect processing steps had a substantially higher GWP than other inputs. However, it is worth noting that impacts associated with steam were only present in the extracts produced from soluble protein because the supernatant containing 3−5% solid initially had to be preconcentrated to 20% solid by evaporation, which uses steam. Conversely, extracts from precipitated protein already contained 18−20% solids, so drying using electricity as an energy source was performed directly on the extract. In addition, electricity represents a small proportion of the GWP associated with the extracts. This is due to the specific context of this study since in Quebec, electricity mainly comes from hydropower.[48] The other inputs generating important quantities of greenhouse gases were the HCl and NaOH used for the extraction and isoelectric precipitation of proteins from defatted mealworm residues. It would be beneficial to find cleaner NaOH and HCl sources or to find a protocol with lower volumes required, which would reduce the emissions generated by using these chemicals. For example, a cleaner protocol could be developed, as described by Mikhaylin et al., who used electrodialysis using a bipolar membrane coupled to ultrafiltration to produce milk protein.[49] Impacts classified as " other " comprise ethanol and hexane, which make up a small proportion (<0.5%) of the total compared to other impacts. Indeed, these two solvents are mostly recovered from the lipid fraction by evaporation and are reused. The small proportion of hexane and ethanol lost in the process was calculated based on the study of Potrich et al.[37] Additionally, water, wastewater, and waste management also comprised the category "other", which represented very minor impacts in terms of CO_2 equiv

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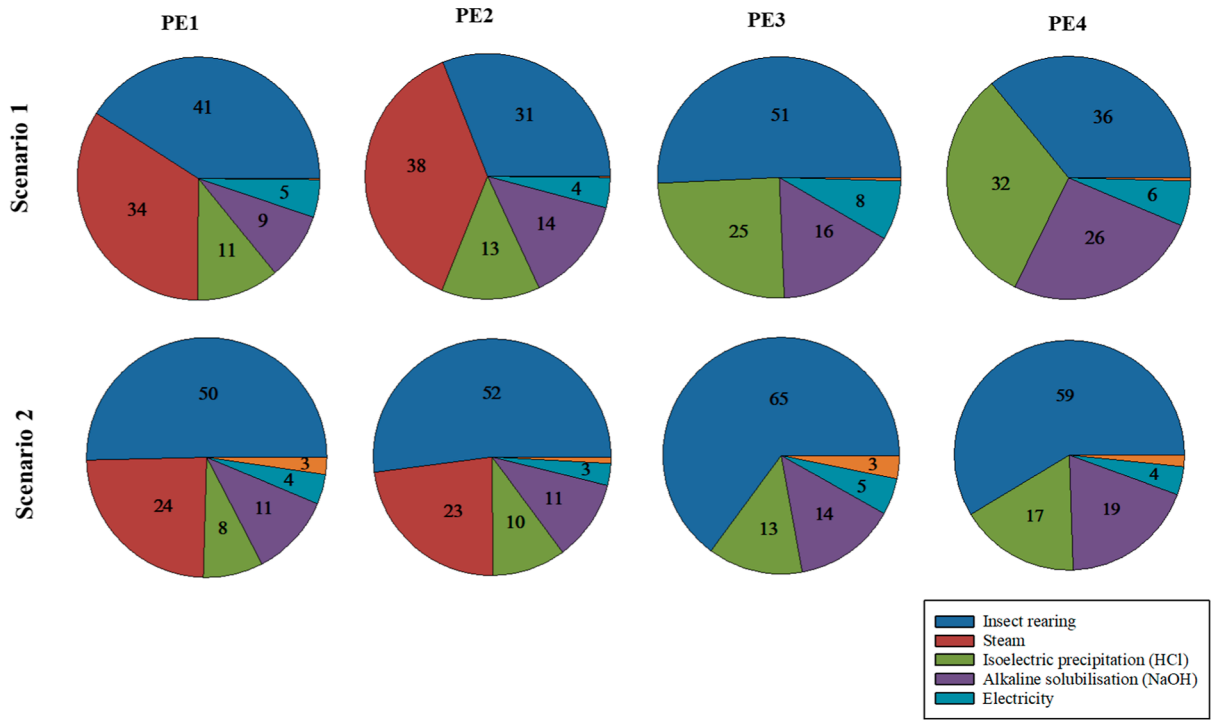

Figure 3. Proportional impact (GWP) of different inputs of TM protein extracts. Impacts from water, wastewater, hexane, ethanol, and waste were not included (<4% of GWP).

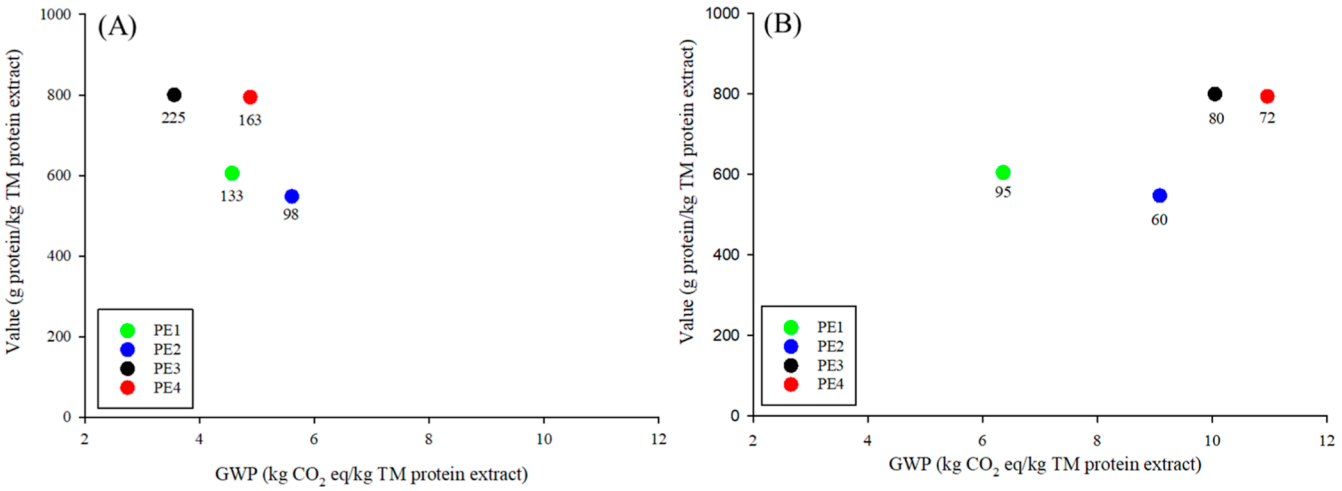

Figure 4. Eco-efficiency of insect protein extracts for scenarios 1 (A) and 2 (B).

3.4. Eco-Efficiency Assessment. To evaluate the protein extraction methods from a different perspective, the eco-efficiency of protein extracts is presented in Figure 4. Each extract's protein content is plotted as a function of GWP for both allocation scenarios with the eco-efficiency score for the protein extract indicated immediately beneath. The goal is to minimize the extract's GWP and maximize its protein content, which is indicated by a higher score. In general, when only the lipid and protein extract are valuable (scenario 2 – Figure 4), the GWP is higher when protein content increases. More processing steps and resources are needed to produce an extract rich in protein, creating more nonvaluable byproducts (waste) and causing an increased impact. Conversely, when every byproduct is valuable (scenario 1), environmental impact decreases with higher protein content (Figure 4). Moreover, increased purification of the extract is associated with the allocation of more impact to the byproducts, reducing the impact allocated to the extract. Globally, when every byproduct is considered valuable (scenario 1), it is advantageous to produce a high-purity extract. However, if byproducts cannot be valorized (scenario 2), the minimization of the processing steps is advantageous to reduce GWP. The highest eco-efficiency score in Figure 4B (scenario 2) is found for the protocol using one solubilization (PE1), while the smaller one (37% lowest) refers to the protocol using two solubilizations (PE2), confirming that when the byproducts cannot be valorized, processing should be minimized. In Figure 4A (scenario 1), the highest eco-efficiency score is obtained for the extract produced by one solubilization and one precipitation (PE3), and the smallest one (56% lower) is obtained for one solubilization (PE1). For scenarios 1 and 2, it is not advantageous to produce PE4 compared to PE3 because

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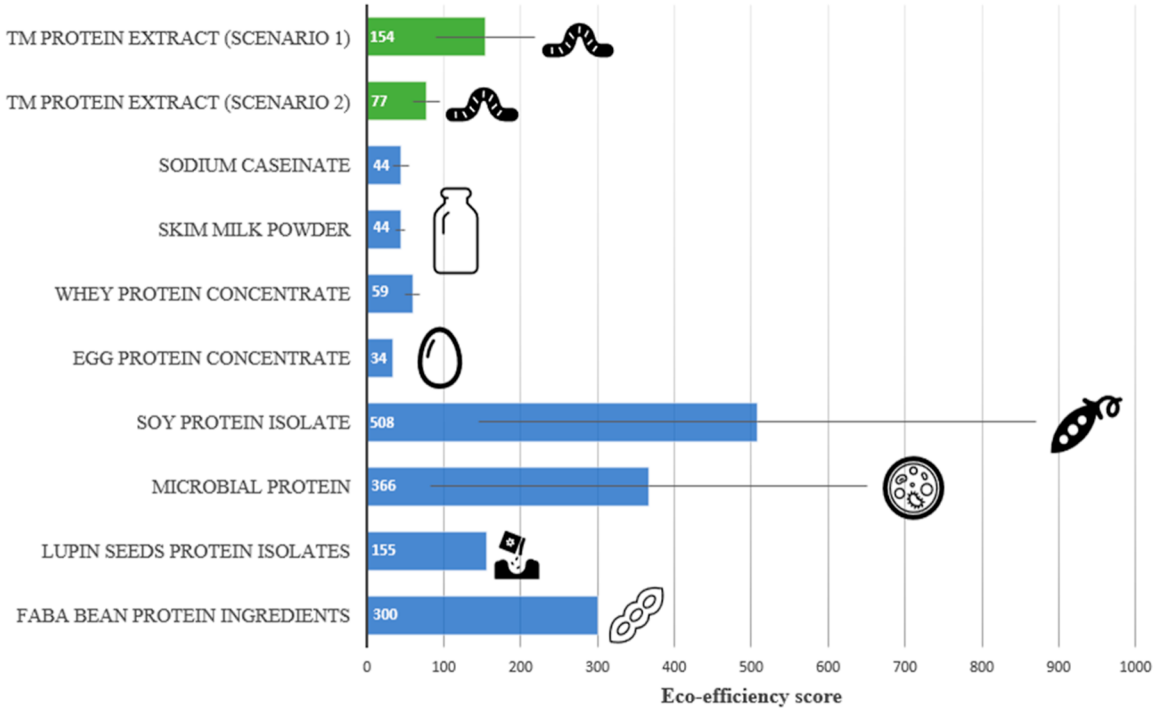

Figure 5. Eco-efficiency score of scenarios 1 and 2 for TM protein extracts (green bars) compared to other protein sources. Eco-efficient scores were calculated using eq 3 with values obtained from the literature and are summarized in Table S2.

PE4 has a lower eco-efficiency score, indicating that more resources were needed to produce an extract with the same protein concentration. Based on the eco-efficiency assessment, the protocol using one solubilization is the one to recommend in scenario 1 and the least advantageous in scenario 2, illustrating the importance of evaluating eco-efficiency considering the context of mealworm extract fabrication before recommending a particular protocol.

Figure 5 compares the eco-efficiency of different protein sources. According to results previously presented, the eco-efficiency of TM protein extracts varies between 225 and 60, depending on the method of production and the allocation scenario. There are two eco-efficiency scores for TM protein extract obtained by both allocation scenarios used in this study. Each score is the average value of the four extract production methods. When comparing with other protein-rich products, both TM protein extracts have eco-efficiency scores comparable to that of the lupin seed protein isolate and lower than that of other vegetable protein sources (Faba bean protein ingredient as well as microbial protein and soy protein isolate). The large error bars for microbial protein and soy protein isolate indicate the variations in eco-efficiency scores for these two protein sources, which are due to different production contexts.[50] Indeed, the results for the soy protein isolate were obtained from 10 different studies, resulting in different GWP scores. In addition, a sensitivity analysis was performed for microbial protein by comparing the impact of different energy sources (hydropower vs average electricity mix in Finland) to produce this protein type, which led to different GWP scores.[51] Eco-efficiency scores of TM protein extracts are comparable to those of the soy protein concentrate and microbial protein, if we consider the best scenario. As expected, considerable differences are observed between protein sources (vegetable vs animal). Plant-based proteins have higher eco-efficiency scores than animal-based proteins (sodium caseinate, skim milk powder, whey protein concentrate, and egg protein concentrate), and the TM protein extract has, in general, a score in between these two categories of proteins. The TM protein extract could be used as a replacement for the skim milk concentrate via incorporation in food preparation, and it has a higher eco-efficiency score, confirming the potential of integration of this food ingredient into the human diet. The production of the insect protein extract would be beneficial in terms of eco-efficiency if it is partially substituted for animal protein sources in human alimentation. According to Zielinska et al., the insect protein extract has better water holding capacity, oil holding capacity, foaming capacity, foam stability, emulsion activity, and emulsion stability than ground TM.[6] Globally, the TM protein extract has interesting techno-functional properties and eco-efficiency scores, making it a good substitute for other protein sources.

4. CONCLUSIONS

Depending on the number of alkaline solubilization steps and the application, or not, of an isoelectric precipitation step, protein extraction and purification rates ranged from 54.7 to 94.4% and 80.0 to 49.4%, respectively. The LCA showed that the application of one solubilization step followed by isoelectric precipitation was the best way to generate a mealworm protein extract only if all mealworm fractions (lipid, chitin, and protein extracts) could be valorized (scenario 1). Otherwise, using one alkaline solubilization to generate the protein extract was the best method. To reduce the GWP associated with the protein recovery step, eco-friendly chemicals (e.g., NaOH and HCl obtained from electrodialysis using a bipolar membrane) should be used, or a process should be developed that reduces the amounts of these chemicals. This work showed that the eco-efficiency scores of TM protein extracts occur between those of plant and animal proteins. This innovative work confirms the potential of insects as new

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sustainable foods in the Quebec context and proposes different valorization methods, considering different contexts of production. Further research should be focused on optimizing the valorization of byproducts generated from TM protein-rich extracts to explore the different allocation scenarios proposed and to promote the use of these byproducts.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsfoodscitech.2c00014.

LCA inputs and outputs; data and reference used in Figure 5; and general equations for energy consumption, including electricity for blanching, electricity for homogenization, electricity for centrifugation, electricity for stirring during protein stabilization, electricity for heating during protein stabilization, steam for preconcentration, electricity for drying, and electricity for hexane and ethanol recovery (PDF)

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Notes

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