ALTERNATIVE DEFATTING METHODS TO OBTAIN PROTEIN ISOLATE FROM THE EDIBLE INSECT *TENEBRIO MOLITOR*

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ABSTRACT

The edible insect *Tenebrio molitor* is a promising alternative food source. Its nutritional composition is related, mainly, by proteins and lipids. In this work, Soxhlet and other alternative extraction methods, such as supercritical fluid and ultrasound-assisted extraction, were employed for oil efficient extraction to guarantee enriched fractions of both lipids and defatted proteins. The alternative methods minimally altered the protein structure of *T. molitor*, in all methods of oil extraction, defatted proteins showed increased solubility in alkaline pH, data similar to the behavior of the control method Soxhlet. A change was observed in relation to the protein denaturation temperature, determined by DSC. Non-conventional methods reached lower denaturing temperatures (SFE= 43.17 °C, UB=52.96 °C, UP = 59.19 °C) than temperatures from Soxhlet extraction (66.86 °C). Although in terms of protein thermostability, the methods showed lower temperatures, it is worth mentioning that they are sustainable methods, in addition they did not significantly affect protein quality.

Keywords Edible insect. *T. molitor*. Protein. Alternative methods.

1 INTRODUCTION

Edible insects are a promising food source in the food industry. The United Nations Food and Agriculture Organization (FAO) through the document "Edible Insects: Future Perspectives for Food and Nutritional Security" highlighted the valuable role that insects play, motivating the search for alternatives that optimize processing and increase quality of products based on these edible insects [1]. Among the variety of consumed species, the *Tenebrio molitor* L. has promising properties for large-scale industrial and commercial use [2]. The *T. molitor* larvae have a high nutritional value [3]. The protein content can vary from 45.7 to 52.99% (% in dry matter) [4] . The *T. molitor* also has good lipid bioavailability, with efficient extraction of this oil it is possible to ensure enriched fractions of lipids and defatted proteins, possibly with improved functionalities and characteristics [5].

Alternative methods of extraction, such as supercritical fluid, pressurized liquid extraction and ultrasound-assisted extraction, are being employed for the extraction of lipids, mainly to replace conventional methods with the use of organic solvent, high temperatures and/or longer process time [6].

Therefore, the present study aimed to analyze the alternative methods: Supercritical fluid (SFE), Ultrasound bath (UB) and Ultrasound probe (UP) to obtaining defatted *T. molitor* proteins and compare with the traditional Soxhlet method (SOX), in relation to the content of proteins, solubility of proteins after defatting and their thermal stability as well as the morphology of proteins by different methods.

2 MATERIAL & METHODS

Lipids were extracted from *T. molitor* meal using hexane as solvent, with a mass/solvent ratio: 3 g of *T. molitor* biomass/200 mL of solvent [7]. The oil was extracted in a Soxhlet apparatus for 6 h. The defatted meal was left to dry for 24 h in an oven at 40 °C and then stored in a desiccator for future analysis.

SFE extraction of lipids was performed in a laboratory-scale unit [8], 15 g of the meal of *T. molitor* was placed inside the extraction column. The experiments were performed at a constant solvent flow rate of 0.8 kg CO_2/h. The extraction time was set at 74 min and the experimental condition used was 300 bar and 70 °C. Samples were collected in amber flasks previously weighted on an analytical balance and stored in amber flasks at -20 °C prior to analysis. UB and UP extraction of lipids was performed using 3 g of insect meal and 200 mL of solvent hexane and ethanol, respectively. The system UB was placed in an ultrasound bath (SSBu-3.8 L, SolidSteel), operating at 40 kHz, at 45 °C, and the system UP in an ultrasonic probe (model DES500, Unique) for 15 min at room temperature and sonication output amplitude about of 20% in continuous pulse by direct sonication at 20 kHz. Both methods were performed at 15 min. After the extraction step, the solid-liquid solutions were filtered on filter paper to recover the defatted meal [9].

The total protein content was analyzed by the Kjeldahl protocol according to method 928.08 (AOAC, 2000). Total protein content was calculated using a nitrogen conversion factor (N) of 6.25. Protein solubility was determined with Borremans [10] method with some modifications. 0.1 g of meal from each extraction process was mixed with 10 mL of distilled water, and the pH of the mixture was adjusted to 5, 7, and 9 with 1 M NaOH or 1 M HCl. The protein concentration of the supernatant was assessed by the Lowry method. The thermal stability was analyzed through the denaturation profile of the defatted meal [11] through DSC analysis (Jade-DSC Model, Perkin Elmer). The DSC equipment was set to a temperature setting of 20 at 180 °C with a heating rate of 2 °C/min

The denaturation process was quantified by the midpoint of the thermal transition (Tm). For morphological analysis, samples were lyophilized (Liotop, L110) using critical point. The morphology of the samples was observed with a scanning electron microscope (model JSM 6390 LV, Jeol, Japan). Samples were analyzed at an accelerating voltage of 10 kV and micrographs at magnifications between 500 and 2500X.

3 RESULTS & DISCUSSION

The crude protein content was 44.2 (g/100 g) ± 0.29, this value is in line with the values already obtained in other studies with *T. molitor* larvae, different authors obtained a protein content value of 55.83%[12] and of 46.44%[13].

In relation to the protein content of defatted *T. molitor* larvae, this value reached 71.80% for samples defatted with the UP. Other authors[14] obtained a protein content value of 76.5% for *T. molitor* samples defatted with hexane by SOX. The protein contents of the meal defatted by these processes reported in this study, ranging from 65.54% ± 3.36 (SFE method), 70.66 ± 1.55 (SOX method), 71.80% (UP method) and 68.68 ± 1.62 (UB method), were all higher than the protein content of the defatted soybean flour of 53.11 ± 0.95%[15], for example, which indicates potential applications as a source of protein in the development of new foods. Comparing the values between the alternative methods, the values were in the same range of protein content, including the error values.

Solubility is a functional property that depends on the physicochemical properties of proteins and influences their application characteristics [16]. The pH is one of the factors that interfere with the solubility of proteins, the isoelectric point of food proteins, including edible insects, are in the range of 3 to 6, that is, a greater tendency to obtain a lower solubility at pH close to these values [17]. Figure 1A shows the protein solubility values of defatted meals from all extraction processes in terms of pH conditions (5, 7, and 9). The treatment method minimally altered the protein structure. As expected, pH had a significant impact on protein solubility, an increase in solubility was observed as pH increased, indicating that *T. molitor* protein can be better solubilized at neutral to pH alkaline values. This result was consistent with results reported in the literature, [18], observed the same behavior for *T. molitor* proteins defatted with hexane using SOX, which was justified by the authors that the degreasing process can cause a change in the isoelectric point value, which can also be applied in this study. All alternative extraction methods showed good solubility results and mainly that there was little protein denaturation during the degreasing processes. With an emphasis on the SFE extraction, which showed good solubility results even superior to SOX, which may be related to the extraction temperature (70 °C), as the protein solubility is increased at higher temperatures[19], which justifies the lower solubility values of the UP extraction, as it was performed at room temperature.

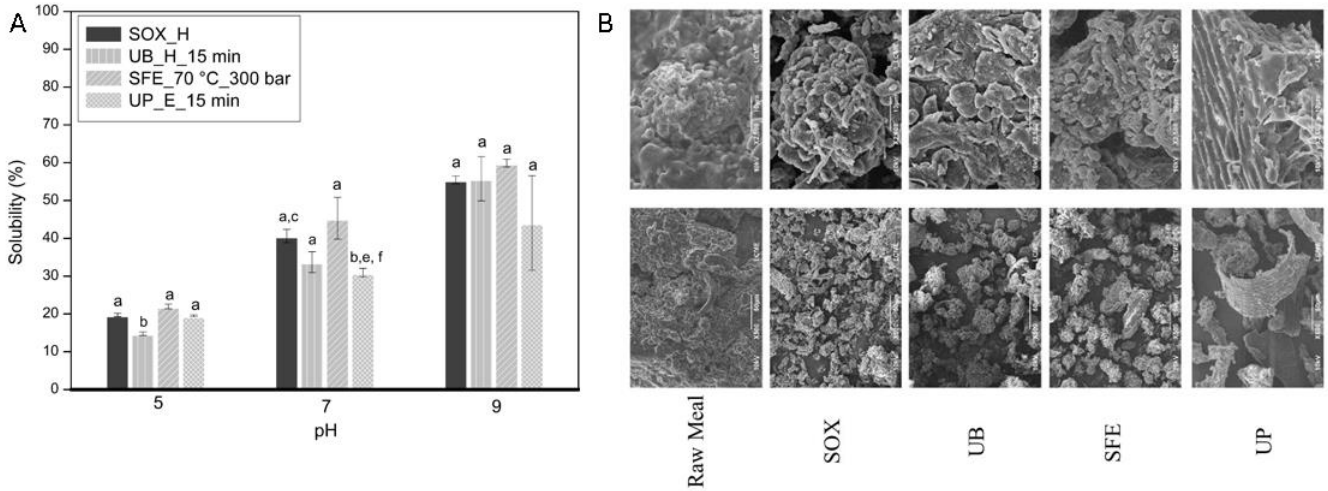

Figure 1 A) Solubility test of defatted *T. molitor* flour protein at pH 5, 7 and 9 and B) Scanning electron micrographs from *T. molitor* meal, where SOX: Soxhlet; UB: ultrasonic bath; H: hexane; SFE: supercritical; UP: ultrasound tip and E: ethanol.

To further investigate the impact of defatting methods on the protein structure of *T. molitor* meal, the surface morphology of raw meal and protein concentrates are shown in Figure 1B. The raw meal showed a denser and globular surface, while the meals from the SOX, SFE, UB methods showed a similar surface in relation to the formed globules but less dense. The images revealed differences only in the UP process sample. In the UP method, irregularly shaped particles were formed, exhibiting large particles with surface morphology similar to a scaly plaque.

The DSC analysis method has been used to detect heat-induced protein denaturation or protein structure unfolding, where the denaturation temperature (Td) indicates the thermostability of the protein [20]. For peak identification, the Pyris software was used. During the heating of the samples enthalpic peaks were observed, the results are shown in Table 1.

Table 1 Effect of different extraction methods on the thermal properties of proteins present in the defatted meal of the insect *T. molitor* obtained through the analysis of differential scanning calorimetry (DSC), where Td: denaturation temperature.

Extraction method	Td (°C)	ΔH (J/g)
SOX	66.86	16.820
SFE	52.96	46.250
UB	59.19	59.649
UP	43.17	25.554

Lower denaturation temperatures were found by alternative methods (SFE= 43.17 °C, UB= 52.96 °C, UP= 59.19 °C) compared to SOX (66.86 °C). The method that most impacted the denaturation temperature was SFE, this may be related to the depressurization suffered during the defatting process, which somehow may have affected this result. However, higher enthalpy values were observed in the UP (59.65 J/g), UB (46.25 J/g) and SFE (25.55 J/g) methods in relation to the SOX (16.2 J/g). According to the study [11], probably during heating greater hydrogen bonds were broken along the unfolding of proteins in the alternative methods than in the SOX method.

4 CONCLUSION

It is important to evaluate the influences of each method on the process of concentrating protein in *T. molitor* meal. The SFE alternative method showed better solubility (59,88 ± 1,03) compared to the SOX traditional method (55,46 ± 1,02). The studied methods presented lower protein thermostability temperatures (SFE= 43.17 °C, UB= 52.96 °C, UP= 59.19 °C) compared to the SOX method (66.86 °C), and despite the lower temperatures, the quality of the protein was not significantly affected. Furthermore, alternative methods are cleaner and more sustainable, making these methods promising alternatives.

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