Extracting chitin from various by-products of a BSF biowaste facility

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Introduction

Valorising organic waste with the Black Soldier Fly Larvae (BSFL) is becoming increasingly popular, especially in low- and middle-income countries. This is because the process converts organic substrates that is considered waste into valuable products that contribute significantly to the profitability of a BSFL facility. Besides the protein from the larvae, the biopolymer chitin, found in insect's skins, could be an interesting revenue stream. Especially the chemical variant chitosan (deacetylated chitin) is a promising compound with many applications as for example in pharmaceutical products or as flocculants in wastewater treatment (Hahn et al., 2020a). Some by-products of the BSFL waste process may be interesting sources for chitin. This fact sheet describes chitin extraction experiments from BSF pupae shells, BSF flies and BSF larvae skins (see Figure 1). BSF pupae shells and flies are both by-products of the rearing unit, which are currently mostly discarded at a BSF site. Pupae shells accumulate when flies emerge from the pupae. After the flies mated and laid their eggs, they die. During the molting process, larvae regularly cast off their skins, which can be collected in the rearing unit as well as the waste treatment unit. Whereas the flies can be easily collected after dismantling a fly mating cage, pupae shells and shed skins are usually mixed with remaining compost material or residue and, thus require an additional separation step prior to the chitin extraction process.

Figure 1: From left to right: Dead BSF flies, shed skinds of BSF larvae and BSF pupae shells.

Methodology

Figure 2 shows the flow diagram of chitin and chitosan extraction. The chitin extraction consists of three main steps: pre-treatment, demineralization and deproteinization. Further conversion of chitin into chitosan requires a strong alkaline treatment which results in the desired deacetylation of the chitin (Hahn et al., 2020a). The deacetylation step was not performed in this experiment. The method was developed based on the review written by Hahn et al. (2020a) and similarly to the described experiments by Hahn et al. (2020 b) as well as Wasko et al. (2016).

Pre-treatment included cleaning with water and detergent, oven drying at 105°C and grinding of the raw material into fine particles.

For demineralization, BSF samples were treated with 1.5 M HCL for 2 hours at 25°C. The 1.5 M HCL solution containing the pre-treated samples was stirred on a stirring plate. The acidic treatment decomposes minerals into water soluble salts. After two hours, the sample solution was filtered and washed with 60-120 mL deionized water per g of biomass. The washing step aimed to wash off the water-soluble mineral salts. The mineral content was determined before and after the treatment by measuring the ash content. The main component of the ash are minerals. The efficiency of the demineralization (DME) was then evaluated by the equation below:

$$DME\ [\%] = 100 - (Ash_{dm\ sample} / ash_{raw\ material}) * 100$$

In a deproteinization step, proteins are separated. This is usually achieved by a base treatment. Here, samples were stirred on a heating plate for 2 hours in a 2 M NaOH solution at 80°C. After the treatment, samples were filtered and washed with 25-55 mL of deionized water per g of biomass. The protein content was measured via Kieldahl method using the standard nitrogen conversion factor of 6.25. Similar to the DME, the efficiency of deproteinization (DPE) was evaluated by analysing the protein content before and after deproteinization and using the following equation:

$$DPE\ [\%] = 100 - (Protein_{dp\ sample} / protein_{raw\ material}) * 100$$

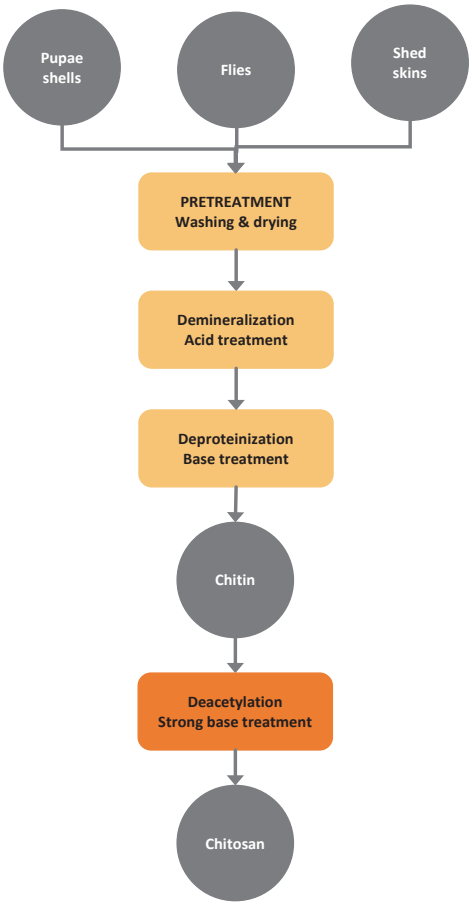

Figure 2: Flow diagram of chitin and chitiosan extraction process.

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After the demineralization as well as the deproteinization, wet sample material was dried overnight in an oven at 105°C. Samples were analysed after the treatment for ash content or for protein content respectively. During both processing steps, the amount of deionized water used and the pH of the sample after washing were noted.

The overall yield, as well as the yield after each process step was calculated by dividing process output by input dry weights. The gained chitin is expected to be the dried sample after performing the deproteinization step. It is assumed that after demineralizing and deproteinizing of the sample, only the chitin is left. The chitin yield was measured by dividing the output by the input (dry weights). This is an estimation, as the chitin purities of the samples were not tested.

Every sample type was only analysed once, as the sample volume was not enough for replicates.

Results and discussion

Table 1 summarizes measured parameters related to the demineralization step. The highest DME was achieved with pupae shells with 81% and the lowest for skins with 62%. Hahn et al. (2020b) reported higher DME's for skins (73-86%). The experiments for flies and pupae shells were within a similar range as the reported values from Hahn et al. (2020b). In the study of Hahn et al. (2020b) only the skins were analysed and a different acid was used. The acid type and concentration used, the reaction temperature as well as the washing water volume may influence the DME as well as the yield (Hahn, et al., 2020a). Even though less water was used to wash the pupae shell, the DME was highest. This indicates that other parameters such as the acid type or concentration here may be more impactful to increase the DME.

Table 1: Measurement parameters after demineralization step.

Sample	mL H2O/g	pH	Ash content before	Ash content after	DME
Flies	140	5	2.83%	0.77%	**73%**
Shed skins	137	6	6.49%	2.45%	**62%**
Pupae shells	57	5	16.6%	3.09%	**81%**

Table 2 summarises analysed parameters related to the deproteinization step. The DPE was with < 10% very low for all tested samples. As chitin also contains nitrogen, the conversion factor of 6.25 is too high and all protein contents are overestimated, which means the % DPE are underestimated. Moreover, the chitin content between the different tested samples varies, which makes the comparison between the samples difficult. Only few studies reported DPE values for insects, which were between 70 and 80% (Hahn, et al., 2020a). These values are clearly higher than the values of this study and indicate an incomplete protein removal and impure chitin. Hahn et al. (2020b) showed that an increase in reaction time, higher reaction temperature as well as higher NaOH concentration result in higher DPE. Moreover, increased water volume to wash the samples could increase the chitin purity. The pH of the flies and pupae shell samples were with 8.5 and 8 still alkali and indicates an incomplete washing step.

The chitin yield is calculated as the percentage ratio of the remaining sample after the treatments (dry weight) and the raw material (dry weight). It is assumed that only the chitin is left in the material. The highest yield was obtained from skins with 45%, second highest was from flies with 25% and pupae shells had a chitin yield of 16% (see Table 2). These values are higher than the few previously reported values for BSF and other insects, which mostly lay between 5 and 15% (Hahn, et al., 2020a). This indicates an incomplete demineralization and deproteinization. In contrast to flies, skins and pupae shells are repelled body parts, while flies are functional organisms and thus contain larger amounts of protein and fat. Therefore, chitin extraction from flies would lead to very low chitin yields and might be more difficult compared to skins and pupae shells.

Table 2: Measurement parameters after deproteinization step.

Sample	mL H2O/g	pH	Protein content before	Protein content after	DPE	Chitin yield
Flies	25	8.5	60.1%	58.4%	**2.8%**	**25%**
Shed skins	54	6.5	36.4%	33.2%	**8.8%**	**45%**
Pupae shells	40	8	44.3%	41.4%	**6.5%**	**16%**

Conclusions and outlook

This factsheet shows first results of chitin extraction from different BSF by-products. The very limited sample size of these first trials does not allow distinct judgements and conclusions. But these first trials can give some recommendations for future experiments:

- Flies are easy to collect in a BSF facility, but due to their high protein and fat content, low chitin yields are expected. Due to the low chitin content in flies, the extraction and purification process of chitin might be more difficult.

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- Larval skins and pupae shells accumulate in smaller amounts compared to flies within a BSF processing facility and require an additional separation step from residue prior to chitin extraction. However, chitin yields are expected to be between 10 and 30% (Soetemans, et al., 2020 & Hahn, et al. 2020a)

- The washing step in between the treatments is essential. The higher the water volumes used, the purer the extracted samples (Hahn, et al., 2020a).

- The method of deproteinization should be verified. Longer treatment times or stronger bases might be required to successfully remove proteins from the samples.

- For process monitoring, an alternative to the Kieldahl protein determination method is recommended, to ensure only protein is measured and the chitin bound nitrogen is not calculated as protein.

- Conversion of chitin into chitosan is recommended for application and marketing purposes, as chitosan has the desired chemical properties. However, the deacetylation process requires to work with strong bases at high temperatures for several hours, which further requires a save vessel and laboratory environment.

References

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