

**Presentation of Project**

**“Sequential extraction and quantification of fat, protein and carbohydrate from black soldier fly larvae cultivated on organic waste for enhanced waste management”**

**Submitted to**  
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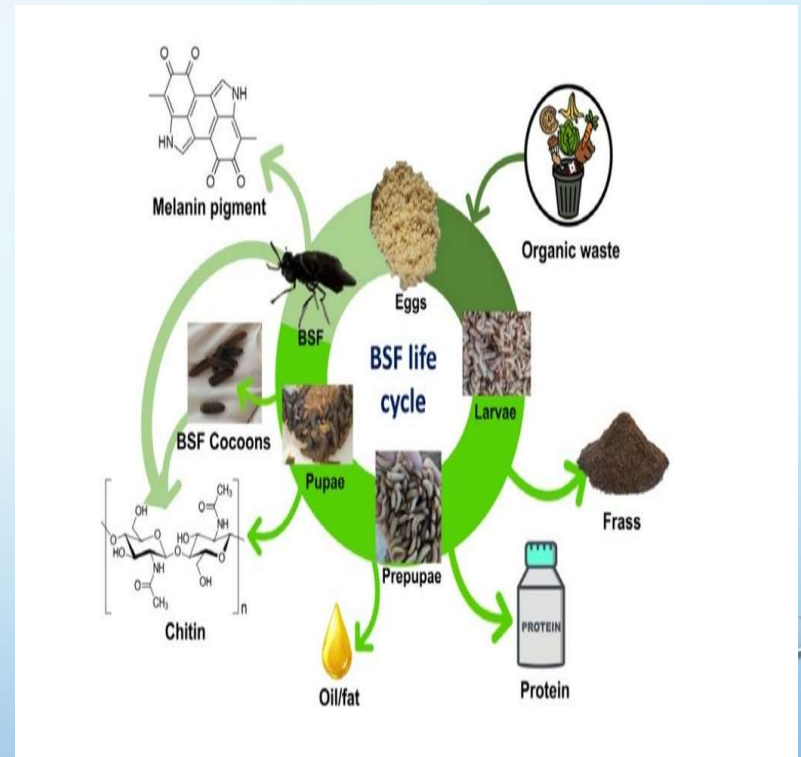
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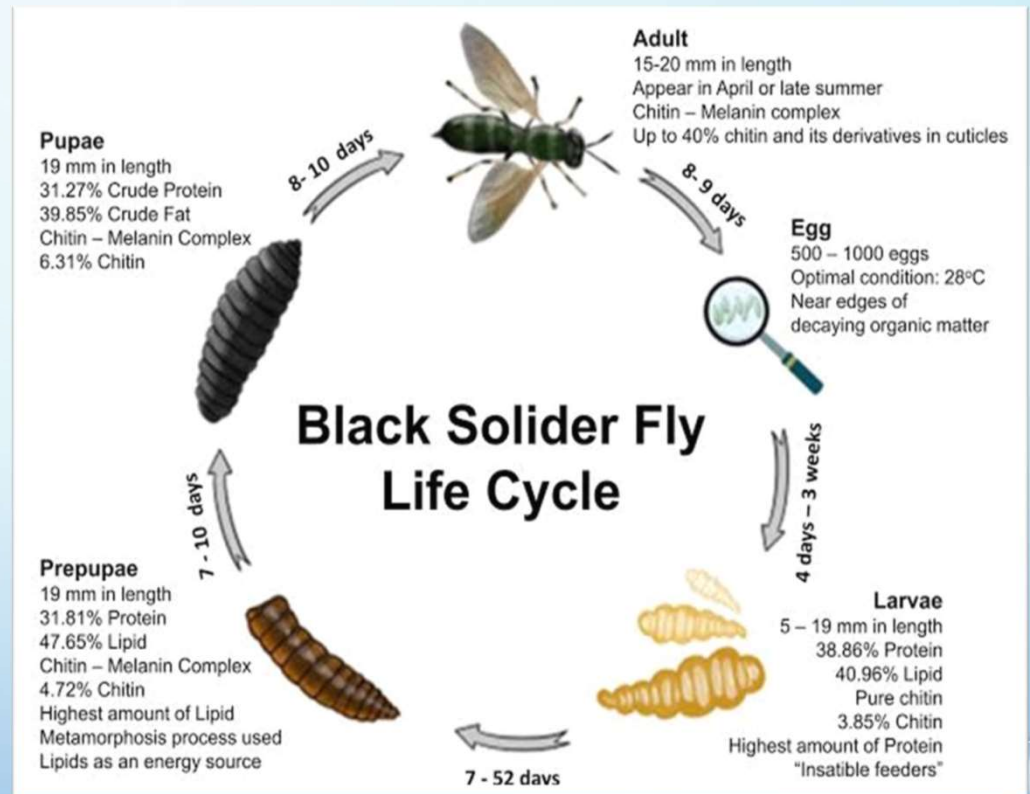
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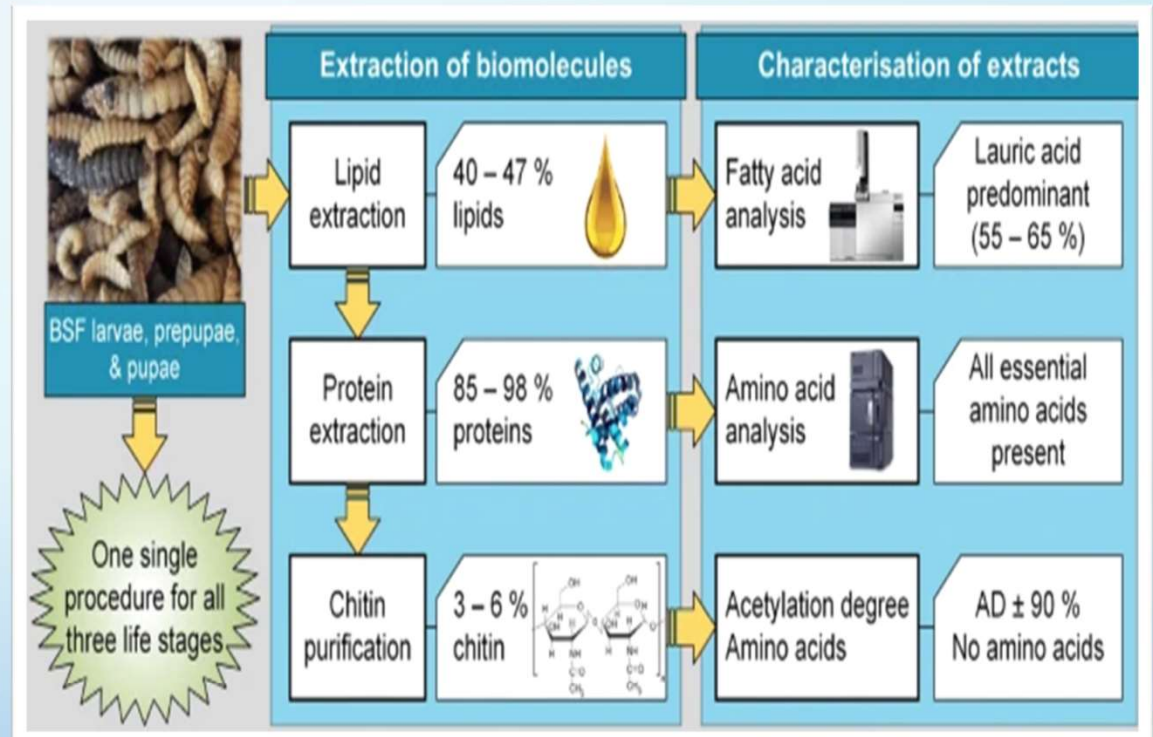


# INTRODUCTION

- The increase in waste production because of population growth is among the major concerns in many areas around the world.
- One of the most innovative technology for waste management is the bioconversion of side streams by insects.
- Many insects naturally feed in organic wastes, converting biomass nutrients into their own biomass and reducing the amount of waste material.
- *Hermetia illucens*, better known as black soldier fly (BSF), is one of the most important species proposed as a converter of organic waste.



- For their high protein content, BSF larvae/prepupae have been proposed to be used as feed for different species as fish, chicken and pigs and as a pet food.
- Moreover, due to the large amount of fat in the prepupae, another application exploited for BSF biomass is the production of biodiesel.
- Thus, from an economic point of view, a sequential extraction delivering products with high purity is preferred.



# MATERIALS AND METHODOLOGY

## 1. Proximate Composition of Fat and Analysis:

### Extraction of oil by Soxhlet Extraction Method:

10 g of BSF larvae taken in Soxhlet apparatus

Added 120 ml of Petroleum Ether

Extracted for 6 h at 70 to 80°C on heating mantle

solvent removed by a rotary evaporator

weight of oil measured

Degree of Unsaturation was determined by Iodine value





## 2. Proximate Composition of Protein:

### A) Extraction and Estimation of Protein by Lowry Method:

BSF powder mixed with 150 ml 0.25 N NaOH

Heated to 40 °C for one hour with constant agitation at 400 rpm for 15 min.

mixture was centrifuged at 4500 rpm for 15 min

pH of the supernatant was adjusted to 4.0–4.3 by adding 37% HCl and 1N HCl

centrifugation (4000 rpm, 15 min) to obtain the precipitated proteins

Precipitated protein redissolved in 0.1 M NaOH

Lowry assay was performed for protein solution to estimate the protein

## B) Protein Determination by Kjeldahl Method:

### Digestion

- 0.5 g BSFL powder digested with conc.  $\text{H}_2\text{SO}_4$  using Potassium Sulphate to raise boiling point with catalyst Copper Sulphate.

### Distillation

- pH of solution is raised by 45% NaOH and solution transferred to Kjeldahl flask. 40 ml trapping solution (0.5 N HCl) placed in conical flask with end of condenser dipped in it. Collected distillate for 20 min.

### Titration

- After 20 min. trapping solution titrated with 0.5 N NaOH using phenolphthalein indicator till appearance of pink colour.



## **Determination of total carbohydrate Anthrone method :**

- Weighed 100mg of the defatted. Hydrolyzed it by keeping it in a boiling water bath for three hours with 5mL of 2.5 N HCl.
- Neutralized it with solid sodium carbonate until the effervescence ceased and made the volume to 100mL and centrifuged it.
- Collected the supernatant and took 0.2 and 0.5mL aliquots for analysis.
- Made up the volume to 1mL in all the tubes by adding distilled water.
- Added 4mL of Anthrone reagent to each tube.
- Heated them for eight minutes in a boiling water bath.
- Read the green to dark green colour at 630nm.



## RESULTS AND DISCUSSION

### Fat composition:

The lipid percentage was calculated by the following formula:

$$\text{Fat \%} = \frac{\text{weight of oil}}{\text{weight of sample}} \times 100$$

Table 1: Crude fat composition in BSF larvae:

Sample No.	Empty weight of round bottom flask (gm)	Weight of round bottom flask with oil (gm)	Weight of oil (gm)	% of oil
1.	123.520	129.105	5.585	55.85
2.	122.513	128.113	5.6	56
3.	148.792	154.196	5.404	54.04

Crude fat content in BSFL, which is determined to be  $55 \pm 1\%$  in the experiment.

The degree of saturation or unsaturation was determined by checking the Iodine value:

Table 2: Blank Titration

Oil (gm)	Chloroform (ml)	Iodine solution (ml)	Stopper the bottle and keep it in dark for 1 hr.	Starch (ml)	Reading (ml)
0	10	20		0.5	24.3

Table 3: Back Titration

Oil (gm)	Chloroform (ml)	Iodine solution (ml)	Stopper the bottle and keep it in dark for 1 hr.	Starch (ml)	Reading (ml)
10	10	20		0.5	21.5

Iodine value was calculated by following formula: 
$$\text{Iodine value of oil} = \frac{(\text{blank} - \text{back}) \times N \text{ of } Na_2SO_4 \times 0.02538 \times 100}{\text{Weight of oil}}$$

Iodine value of BSFL oil was found to have 7.1064 g I<sub>2</sub>/100g fat. Which indicates the moderately low level of unsaturation in the oil and high level of saturated fat in the oil.

## Protein Estimation by Lowry Method:

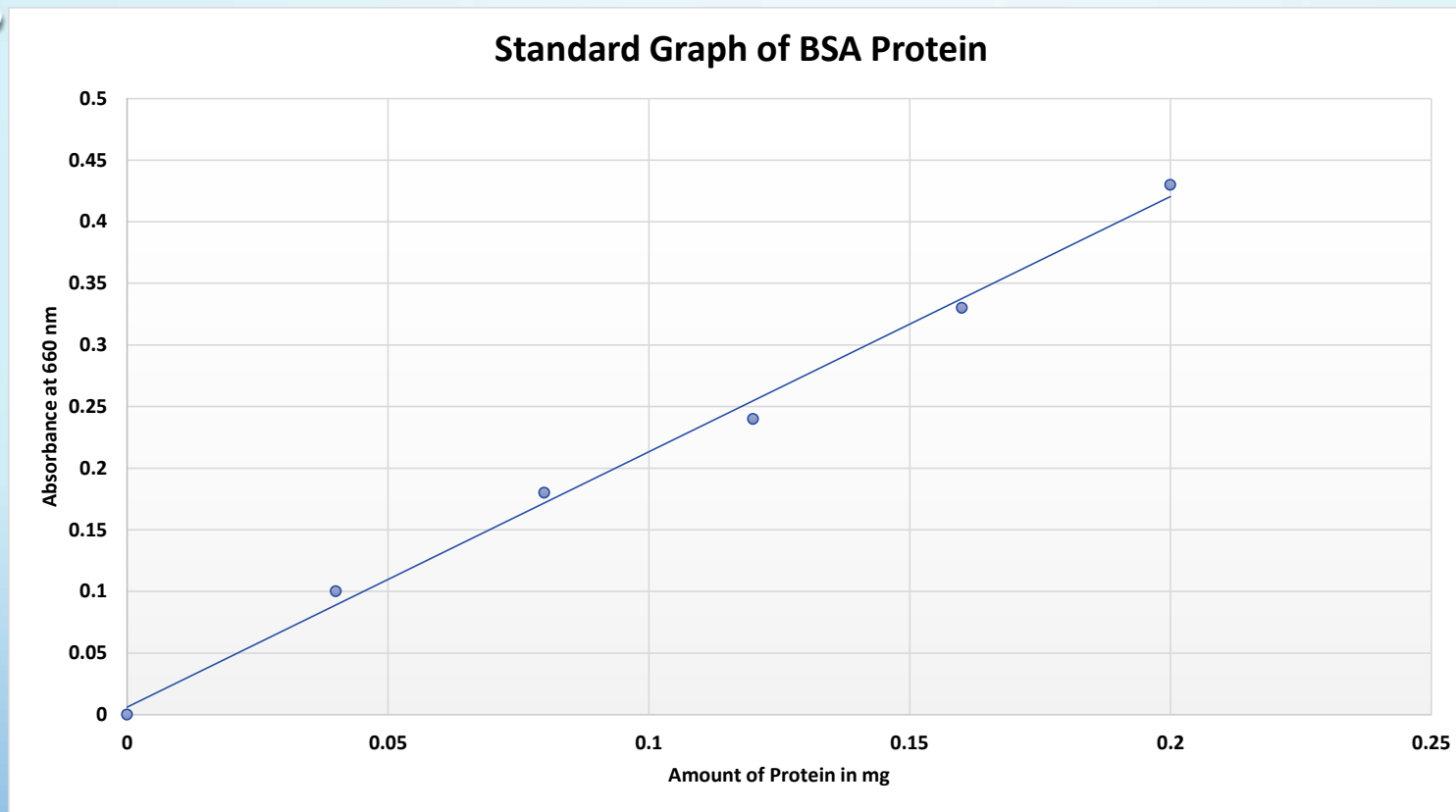


Table 4: protein estimation of sample 1 by Lowry method.

<b>Protein solution (ml)</b>	<b>DW (ml)</b>	<b>Lowry C (ml)</b>	<b>Mix and wait for 15 mins.</b>	<b>Follin Phenol reagent (ml)</b>	<b>Mix and wait for 30 mins. in dark.</b>	<b>Absorbance at 660 nm</b>	<b>Dilution factor</b>
<b>0.2</b>	0.8	3.0		<b>0.5</b>			
<b>0.2</b>	0.8	3.0		<b>0.5</b>			

From calculation done using standard graph of BSA, the amount of protein found in 10 g of BSF larvae is 2.5758 g.

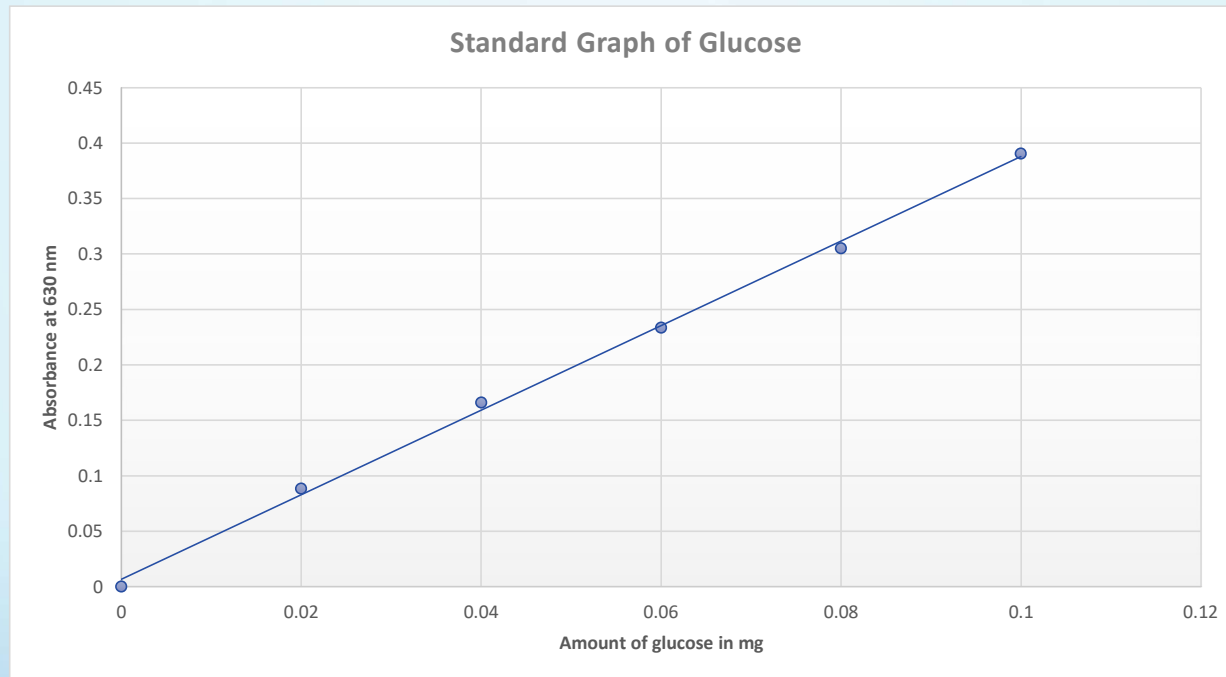
i.e. 25.75% of protein found in 10 g of BSF larvae.

## Protein Determination by Kjeldahl Method:

- Percent Nitrogen was calculated by formula,  
$$\% \text{ Nitrogen} = 1.4007 \times (V_b - V_s) \times N/W$$
- Percent Protein was calculated by multiplying % nitrogen with nitrogen to protein conversion factor 6.25.  
$$\% \text{ Protein} = \% \text{ Nitrogen} \times 6.25$$
- The percentage of nitrogen obtained in sample- 1 and sample-2 was 5.32% and 5.04% respectively.
- Thus, percentage of protein came out to be 33.25% and 31.5% for sample-1 and sample- 2 respectively.



## Determination of Total Carbohydrates:



Amount of carbohydrate present in 100mg of the sample was calculated by following formula,

$$\text{Total carbohydrate} = \frac{\text{mg of glucose} \times 100}{\text{Volume of the test sample}}$$

Table 5: Sample 1

<b>Volume of solution (ml)</b>	<b>D.W (ml)</b>	<b>Anthrone (ml)</b>	<b>Boil for 8 mins and cool</b>	<b>Absorbance At 630nm</b>
<b>0.2</b>	<b>0.8</b>	<b>4.0</b>		<b>0.2019</b>
<b>0.5</b>	<b>0.5</b>	<b>4.0</b>		<b>0.5270</b>

Total amount of carbohydrate found in sample-1 was 1.1751 g in 10 g BSF larvae which was 11.75 % of 10 g larvae.

Table 6: Sample 2

<b>Volume of solution (ml)</b>	<b>D.W (ml)</b>	<b>Anthrone (ml)</b>	<b>Boil for 8 mins and cool</b>	<b>Absorbance At 630nm</b>
<b>0.2</b>	<b>0.8</b>	<b>4.0</b>		<b>0.2121</b>
<b>0.5</b>	<b>0.5</b>	<b>4.0</b>		<b>0.5440</b>

Total amount of carbohydrate found in sample-2 was 1.1922 g in 10 g BSF larvae which was 11.92 % of 10 g larvae.

## CONCLUSIONS

- By following extraction and estimation procedure successfully found composition lipids, proteins, and carbohydrate from Black Soldier Fly (*Hermetia illucens*) larvae.
- Fat content of black soldier fly larvae (BSFL) was determined to be  $55 \pm 1.0$  %.
- Iodine value of BSFL oil was found to have 7.1064 g I<sub>2</sub>/100g fat. Which indicates the moderately low level of unsaturation in the oil and high level of saturated fat in the oil.
- Protein estimated from the larvae was found to be 25.75% by Lowry method and 32.37% by Kjeldahl method
- Carbohydrate content of BSF larvae was determined to be 11.84%.

## FUTURE SCOPE

- Exploration of Novel Extraction Methods
- Enhanced Protein Recovery
- Application Development
- Sustainability and Circular Economy
- Enhanced Waste Management

## REFERENCES

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**THANK YOU**

