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Bioconversion of Organic Wastes by Black Soldier Fly for Chitin, Lipid and Protein Production

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ABSTRACT

The bioconversion of organic waste into black soldier fly (BSF) larvae, which is a valuable source of lipid, and protein, is part on an ongoing creative effort in valorising waste. Besides lipid and protein, BSF larvae also contain significant amounts of chitin, a polymer of N-acetylglucosamine that make up the backbone of the polysaccharide structure normally found in insects. In this study, the effects of organic waste type and moisture content on biomass conversion ratio were investigated. BSF larval development (larvae to prepupae to pupae) was found to occur to the highest extent (98.90 ± 0.71 % biomass converted) when kitchen waste (KW) was used compared to when vegetables waste (VW) (84.6 ± 1.41 % biomass converted) or fruit waste (FW) was used (87.5± 0.71 % biomass converted). The optimal moisture content of the kitchen waste was found to be 80 %, which results in the highest waste biomass conversion of 99.8 % ± 0.58. The BSF larvae were found to have approximately 38 – 42 % crude lipid, 34 – 41 % crude protein, and 8 – 9 % ash content. Chitin was isolated through a three-step process of deproteinization, demineralization, and decolorization. A yellowish-grey powder was obtained at the end of the isolation process and the chitin yield was determined to be 4 – 6% (g/g). Fourier transform infra-red (FTIR) analyses confirmed the chitin yield by identifying the -OH, -CH_3CONH, and -CO stretching of its polysaccharides and glucosamine rings; and the lipid yield by noting the disappearance of the hydroxyl stretching that had been present in the spectra of unprocessed BSF larvae, and the appearance of X–H stretching which indicates the presence of triglyceride functional groups.

Keywords: Black Soldier Fly, Insect Biomass, Lipid, Crude Protein, Chitin, Bioconversion.

1. INTRODUCTION

Rapid globalization has led to demands of better standards of living and the resulting increased anthropogenic activity have directly or indirectly caused a large generation of waste. The annual global waste generation is 600 million tons and this is projected to reach one billion tons by 2025 and 3.4 billion tons by 2050 (Abdel-Shafy & Mansour, 2018). The current prevailing approach to dispose organic waste is through landfills, which can contribute to global warming and groundwater pollution. Increasing awareness of the importance of environmental protection have led researchers around the globe to find and develop sustainable ways to valorise waste so that the waste can be kept out of landfills and instead contribute to the economy.

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Bioconversion of organic waste by insects into insect biomass is an alternative and creative method to add value to waste. Fly larvae can be farmed on various streams of organic waste and its versatility makes it attractive for use in sustainable waste management. One species that have recently gained considerable attention is the black soldier fly (BSF) *Hermetia illucens* larvae. It is known to feed on a wide range of organic material such as food waste and animal manure (Barragan-Fonseca *et al.,* 2017) and can consume a relatively large amount of them to become valuable high-nutrient animal feed. BSF larvae can significantly reduce sludge biomass, abattoir waste, both human and animal faecal sludge, and coffee pulp; but it must be pointed out that the dietary composition greatly affects the bioconversion efficiency and development of the larvae (Singh & Kumari, 2019). BSF larvae is so appealing because it is not a vector of any known disease and have no negative effects on the environment. The BSF life cycle is divided into four stages: egg, larva, prepupae/pupae, and adult. The development of the black soldier fly takes an average of 22–24 days from egg to pupae, or an average of 40 – 43 days from egg to adult and can take up to 4 months under less favourable growth conditions (Čičková *et al.*, 2015). The growth of the BSF larvae is not only affected by the substrate used, but also various other factors such as the temperature, relative humidity, pH of medium, ventilation volume, growth space density, and light source (Feng *et al*., 2020).

The extent of the waste decomposition by BSF larvae is closely related to the content of nutrition available in the waste. The BSF larvae can accumulate a significant amount of protein (32–58 % dry wt. biomass), lipid (40 - 45% dry wt. biomass) as well as minerals and other macro- and micro-nutrients (Gold *et al.*, 2018; Sheppard *et al*., 2002; Spranghers *et al.*, 2017). BSF larvae are capable of transforming low-value organic wastes into a protein and fat source for use in meat meal, poultry, and aquaculture (Gold *et al.,* 2018). Previous studies have shown that BSF larvae-based meal can replace 25 to 50% of fish meal and it has significant amounts of saturated fatty acid (SFA), eicosapentaenoic acid (EPA), and docosahexanoic acid (DHA), making it suitable for encouraging the growth of fish and shrimps (Ewald *et al.*, 2020). Recently, BSF larvae have also found use as a feedstock for biofuel production. Leong *et al.,* (2016) performed a feasibility study on biodiesel production using *Hermetia illucens* larvae fed with coconut endosperm waste. Wang *et al.,* (2017) explored the eco-process of microwave assisted lipid extraction from BSF larvae for biodiesel production. This low-cost and high larvae biomass can also be potentially used to produce bio-lubricant.

Beside these, research on chitin isolation from BSF larvae is a new and exciting venture which is attractive for the biopolymer industry (Caligiani *et al.,* 2018; Zhu *et al.*, 2016). Chitin is the second most abundant polysaccharide in nature after cellulose and it has several desirable properties such as biocompatibility, biodegradability, the ability to scavenge heavy metal and cholesterol or other fats, and antimicrobial and antioxidative behaviour (Berezina, 2016). Previous studies have reported that chitin isolated from BSF have a similar chemical structure and physicochemical properties as commercially available chitin derived from the exoskeleton of crustaceans (Smets *et al.,* 2020). The type of organic waste and its moisture content play a vital role in BSF growth and effective bioconversion of waste. Therefore, the aim of this study is to investigate these effects. The approximate composition analysis and characterization of crude lipids, crude proteins, and chitin from the three stages of the BSF life cycle is also provided.

2. MATERIALS AND METHODS

2.1 Chemical and Raw Materials

Analytical grade hydrochloric acid, sodium hydroxide, potassium permanganate and tris hydrochloride were purchased from Sigma-Aldrich and Merck Chemicals. The Black soldier fly eggs were purchased from Buggy Farm Enterprise (Bukit Mertajam, Penang, Malaysia) and stored at −20 °C in zip lock bags until further use. Organic wastes i.e., kitchen waste (KW), vegetable waste (VW) and fruit waste (FW) were collected from a local market in Kangar, Perlis, Malaysia.

2.2 BSF Eggs Cultivation

About 200 BSF eggs were cultivated in a plastic container (0.21 m x 0.17 m x 0.11 m) at 28 ^{0}C and 70 – 90% moisture content. The eggs were allowed to transform into the larvae, prepupae, and pupae stages. Kitchen waste (KW), vegetable waste (VW), or fruit waste (FW) was fed to the larvae at a feeding rate of 150 mg/day/larvae. Samples were collected in triplicates.

2.3 BSF Pupae Powder Preparation Cultivation

The BSF pupae were collected and washed with distilled water, dried at 105 °C until constant weight, then crushed into a fine powder using a food processor and finally stored at 4 °C until further use.

2.3.1 Crude Lipid and Protein Extraction

First, 10 g of BSF powder was placed in a vessel with petroleum ether as the solvent for lipid extraction. The extraction process was performed based on the method described by Hao *et al.,* (2021). Crude lipid was obtained after evaporating the solvent using a rotary evaporator, and the yield was calculated using Equation 1:

$$\text{Lipid Yield (\%)} = \frac{\text{Weight of extracted lipid(g)}}{\text{Weight of dried BSF powder (g)}} x\ 100 \qquad (1)$$

The crude protein content was measured using the Kjeldahl method (AOAC International, 2002). About 1 g of ground BSF powder was digested in concentrated H_2SO_4 solution (20 ml) in a Kjeldahl unit (Buchi Kjeldahl K370, Germany) for 4 hours. The mixture was distilled and then titrated to obtain the crude protein. Crude protein content was determined using Equation 2 and a nitrogen-to-protein conversion factor of 6.25 (Shumo *et al.,* 2019) was used for the calculation of crude protein:

$$\text{Crude Protein (\%)} = \text{\% Kjeldahl nitrogen } x\ 6.25 \qquad (2)$$

2.3.2 Chitin Isolation

Ten g of sample BSF powder was demineralized with 1 M HCl solution (200 ml) for 1 hour. The demineralized powder was then heated in a water bath at 55 °C for 1 h at 200 rpm/min. After that, the demineralized pupae sample was added to 1 M NaOH (200 ml) for deproteinization. The process was carried out by heating in a water bath at 80 °C for 24 h at 200 rpm/min. The sample was then filtered and washed repeatedly using the same method. The filtered sample was then decolorized using 15 ml of 1% (w/v) of $KMnO_4$. The greyish white final product was filtered and washed with distilled water and dehydrated in an oven at 50 °C for 12 h. The chitin was stored in a refrigerator until further use. The isolated chitin yield was determined using Equation 3 (Złotko *et*

al., 2021):

$$\text{Chitin Yield (\%)} = \frac{m_1}{m\,(1-\text{MC})} \text{x } 100 \qquad (3)$$

where m is the mass (g) of the original BSF powder sample, m_1 is the mass (g) of the obtained chitin, and MC is the moisture content of the BSF powder sample.

2.4 Analytical Model

The moisture and ash content of BSF sample was determined based on the method described by Ljubojević *et al.,* (2015). The ash content was measured after thermal degradation at 550 ± 25 ℃ for 4 hours in a furnace. The moisture content was measured after drying at 105 ℃ to constant weight (about 24 hours). The ash and moisture content are reported as a percentage calculated using Equation 4:

$$\text{Ash/Moisture content (\%)} = \frac{m-m_1}{100} \text{x } 100 \qquad (4)$$

2.5 Fourier-Transform Infrared Spectroscopy

The functional groups of the BSF-isolated lipid and chitin were identified using Fourier transform infrared spectroscopy (FTIR) (Perkin Elmer, USA). The samples were scanned at room temperature and the absorbance values were measured from 4000 to 450 cm^{-1}. About 0.5 g of the sample was dehydrated, pulverized, mixed methodically with KBr, and formed into a pellet for use in FTIR analysis.

2.6 Statistical Analysis

The functional groups data was collected in triplicate and reported as a mean with standard deviation. Results were analyzed using Microsoft Excel to a significance level of 5%. The results obtained for larvae, prepupae and pupae were statistically evaluated using one-way ANOVA.

3. RESULT AND DISCUSSION

3.1 Effect of organic wastes substrate on waste-to-BSF biomass conversion

Figure 1 shows the effect of substrate type on larval growth. The highest waste-to-BSF biomass was achieved when kitchen waste (KW) (98.9 ± 0.71%) was used followed by fruits and vegetable waste. On day 7 to 12 (Phase I) of the experiment, the larvae emerged slightly from their eggs and after consuming the substrates they achieved the second stage of adult larvae between day 12 to 20 (Phase II). Approximately more than 50% of the larvae had emerged exponentially and were actively consuming the substrates during this phase. The larvae fed with kitchen waste (58.5 ± 0.71%) were significantly higher in numbers compared to larvae fed with the other two. This is because kitchen waste is rich in carbohydrates, proteins, and fats. Between day 20 to 30 (Phase III), the percentage of larvae turning to prepupae is highest when kitchen waste (KW) is used (98.50 ± 0.71%), followed by fruit waste (FW) (87.5 ± 0.71%) and vegetable waste (VW) (84.6 ± 1.41%). Waste with high organic content such as kitchen waste is a suitable feed for BSF larvae to grow.

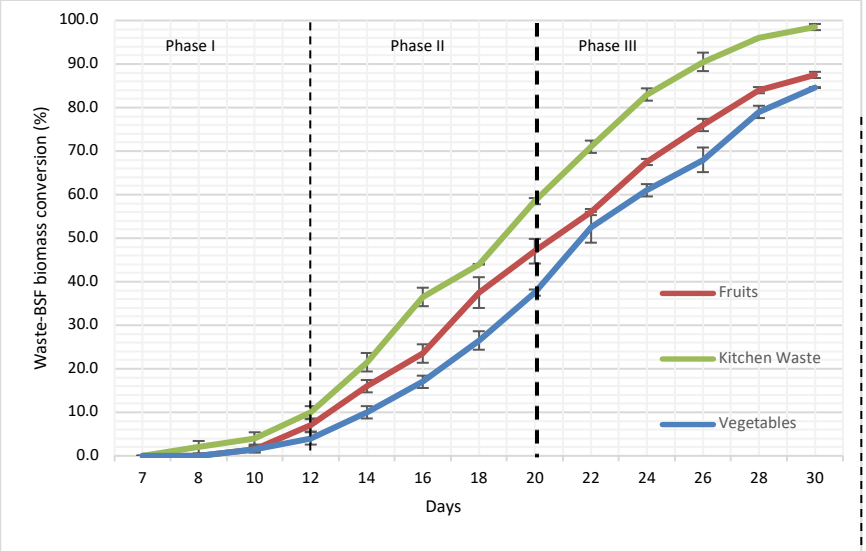

Figure 1. The effect of different types of organic waste- kitchen waste (KW), fruits waste (FW), vegetables waste (VW)

As can be interpreted from Figure 1, BSF larvae start to rapidly consume waste at the early-stage larvae and exponentially increase its consumption until they reach the BSF pupate stage. Kitchen waste is a desirable feed stock as it contains high amounts of carbohydrates, proteins, and fats. A lack of particular nutrients and an imbalanced diet retards larval development and extends its growth period (Kinasih *et al.,* 2020). Cheng *et al.*, (2017) reported that the high content of protein in kitchen waste is beneficial for improving the weight of larvae biomass and its development.

3.2 Effect of substrate moisture content on waste-to- BSF biomass conversion

The growth and survival of BSF larvae is highly influenced by the moisture content of the organic waste substrate. Figure 2 shows the effect of moisture content on waste-BSF biomass conversion. Kitchen waste with five different levels of moisture content (70, 75, 80, 85 and 90%) was used for the cultivation of BSF. The highest percentage of waste to BSF-biomass conversion was observed at 80% of moisture content (99.8 ± 0.58%). A similar trend was observed by Cheng *et al.*, (2017) who found the preferable moisture content on waste to be in the range of 70–80%. A high moisture content is desirable for the BSF larvae to grow, and this can shorten the BSF production time. Further increasing the moisture content does not significantly improve waste-to-BSF biomass conversion as at least 95.7% ± 4.61 was always attained above this level.

In fact, a moisture content of more than 85 % led to a difficulty in producing a fine residue and it is generally less preferable for larval development (Cheng *et al.,* 2017). Excessive moisture content results in the formation of granules and a sticky slurry which complicates the post-harvest process (Singh & Kumari, 2019).

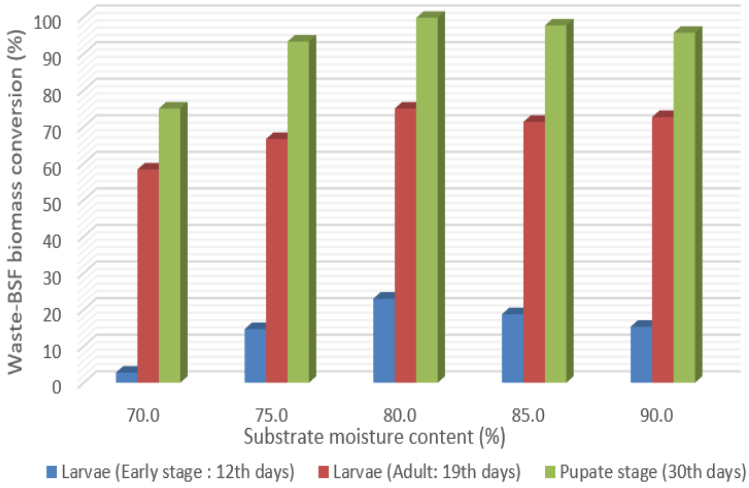

Figure 2. Percentage of waste-to-BSF biomass conversion grown on kitchen waste with different moisture content

3.3 BSF biomass composition fed in kitchen waste

Table 1 shows the approximate composition of BSF biomass (larvae, prepupae and pupae) fed with kitchen waste and the comparison with BSF larvae fed with other organic waste. A considerable amount of crude lipid, crude protein, chitin, ash and dry matter was found in the BSF larvae, prepupae and pupae fed with kitchen waste. The crude fat of BSF larvae is slightly higher (42.88 ± 0.23% (g/g)) compared to prepupae (40 ± 1.61% (g/g)) and pupae (38.8 ± 0.26% (g/g)). This result is in line with a previous study that reported a high content of crude fat in BSFL, being in the range of 21 – 35% (Oonincx *et al.,* 2019).

The crude protein content of the BSF pupate stage (40 - 41% (g/g)), is slightly higher than for the larvae stage (34.2 ± 1.0% (g/g)). The high crude protein content for the BSF pupate stage originated from the BSF pupae shells or exuviae (Bhavsar *et al.,* 2021). The BSFL crude protein values obtained in this study is 34.2 ± 1.0 % (g/g) and it is slightly lower than that reported by Spranghers *et al.,* (2017) who reported their crude protein content to range between 39 to 43% (g/g) depending on the substrate (chicken feed, vegetable waste, biogas digestate and restaurant waste). In this study, the crude protein was determined using the Kjeldahl method, which first measures the total nitrogen content, then finding its equivalent protein content by multiplying with a standard nitrogen-to-protein conversion factor of 6.25. The total nitrogen content in BSF can however include nitrogen that originate from non-protein sources which in our study, is the chitin (Caligiani *et al.,* 2018).

Table 1. Proximate analysis BSF (larvae, prepupae, pupae) and comparison with BSF larvae in different feeding sources

	BSF Larvae	**BSF Prepupae**	**BSF Pupae**	**BSF larvae**	**BSF larvae**	**BSF larvae**
Feeding sources	Kitchen waste			Animal manure	Fresh fruit waste	Food manufacturing by-product
Crude Lipid (%)	42.88±0.23	40.01±1.61	38.8 ± 0.26	31-35	41.7	21-35
Crude Protein (%)	34.2 ± 1.0	40.2 ± 0.4	41.8 ± 1.87	42-44	37.8	38-46
Chitin Yield (%)	4.21± 0.29	6.14± 0.14	6.52 ± 0.49	NA	NA	NA
Ash Content (%)	9.13 ±0.25	9.12 ±1.5	8.47 ± 0.12	-	-	-
Ref.	This study	This study	This study	[27]	[20]	[26]

As for chitin isolation, three different processes were performed: deproteinization, demineralization, and decolorization. Figure 3 shows the isolation process of chitin and the yellowish-grey chitin powder obtained in this study.

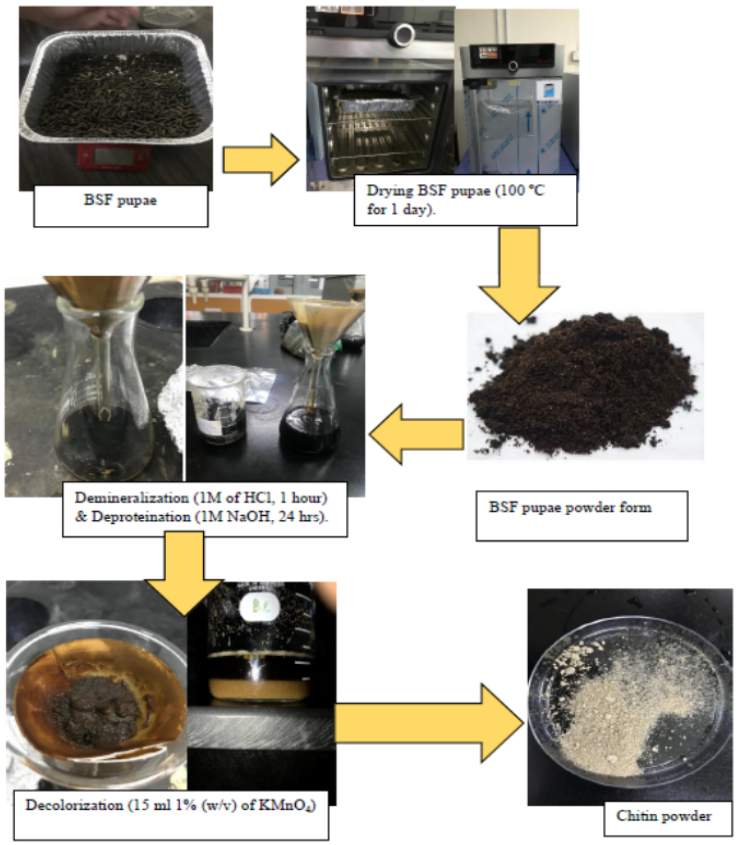

Figure 3. Steps of isolation process (deproteinization, demineralization, and decolorization) and obtained yellowish grey BSF-based chitin powder

Table 1 shows that the yield of chitin obtained from BSF pupate stage (6%) is slightly higher than that from the BSF larvae stage (4.21 ± 0.29 % (g/g)). These values are in line with data reported in literature. Caligiani *et al*., (2018) showed that the chitin yield can vary between 5 to 7% (g/g) depending on the parameters used during the isolation process. Also, Spranghers *et al.,* (2017) reported that different isolation techniques and amount of chemicals used during deproteinization and demineralization will affect the yield of BSF chitin.

3.4 Functional Group Analysis of BSF-based Chitin and Lipid

Figure 4 shows the FTIR spectra that elucidates the functional groups of the chitin and lipid content in BSF. The analyses were performed in the frequency range of 4000 to 450 cm^{-1}. For the spectrum of the chitin obtained, the bands at 3448.2 and 3266 cm^{-1} are attributed to the OH and NH stretching respectively. The shape and intensity of these peaks will change if the hydrogen bonding network in chitin is altered. The bands in the range from 2886 to 2961 cm^{-1} represent the CH, CH_3 symmetric stretching and the CH_2 asymmetric stretching. The CH bending, symmetric CH_3 deformation, and CH_2 wagging bands are found at 1380 and 1312 cm^{-1}. The peaks at 1629 and 1662 cm^{-1} are assigned to the Amide I band (two types of hydrogen bonds in a C=O group with the NH group of the adjacent chain and the OH group of the inter-chain). Amide II band (in-plane N-H bending and C-N stretching mode) and Amide III band (in-plane mode of CONH group) are observed at 1540 and 1317 cm^{-1} respectively. The bands at 1028.4 to 1163 cm^{-1} are attributed to the asymmetric bridge oxygen and C-O stretching (Wang *et al.,* 2020).

As for the lipid obtained (Figure 4b), the FTIR spectra shows a very close agreement with the work done by Wang *et al*., (2017). A strong absorbance between 2854.0 cm^{-1} and 2926.4 cm^{-1} indicate the presence of a large amount of methyl and methylene groups which causes the aliphatic C–H stretching vibration. The absorption peak of 1745.7 cm^{-1} is assigned to the stretching vibration of the C=O group in ketones and carboxylic acids, which was consistent with the high content of ketones in the lipid. The peak at 1616.6 cm^{-1} indicates the presence of alkenes (-C=C- stretching). The X–H stretching vibration (X=C, N) is indicated by the absorbance peak between 1377.3 cm^{-1} and 1465.5 cm^{-1}. This confirms the presence of the triglyceride functional group in BSF larvae. The presence of aromatic amine (C–N stretching) was indicated by an absorption peak at 1160 cm^{-1}. The C–O stretching or C–H bend of ester group is confirmed by the absorbance peak at 1112.8 cm^{-1}.

When comparing the FTIR spectra of raw biomass (Figure 4c) and extracted BSF lipid, a significant difference can be seen as the raw biomass has an absorption peak at 3438.2 cm^{-1} which might indicate the hydroxyl group (O–H) stretching while it is absent in the spectra of the extracted lipid (Afolabi *et al.,* 2018). This might be due to the removal of the hydroxyl group during the lipid extraction process.

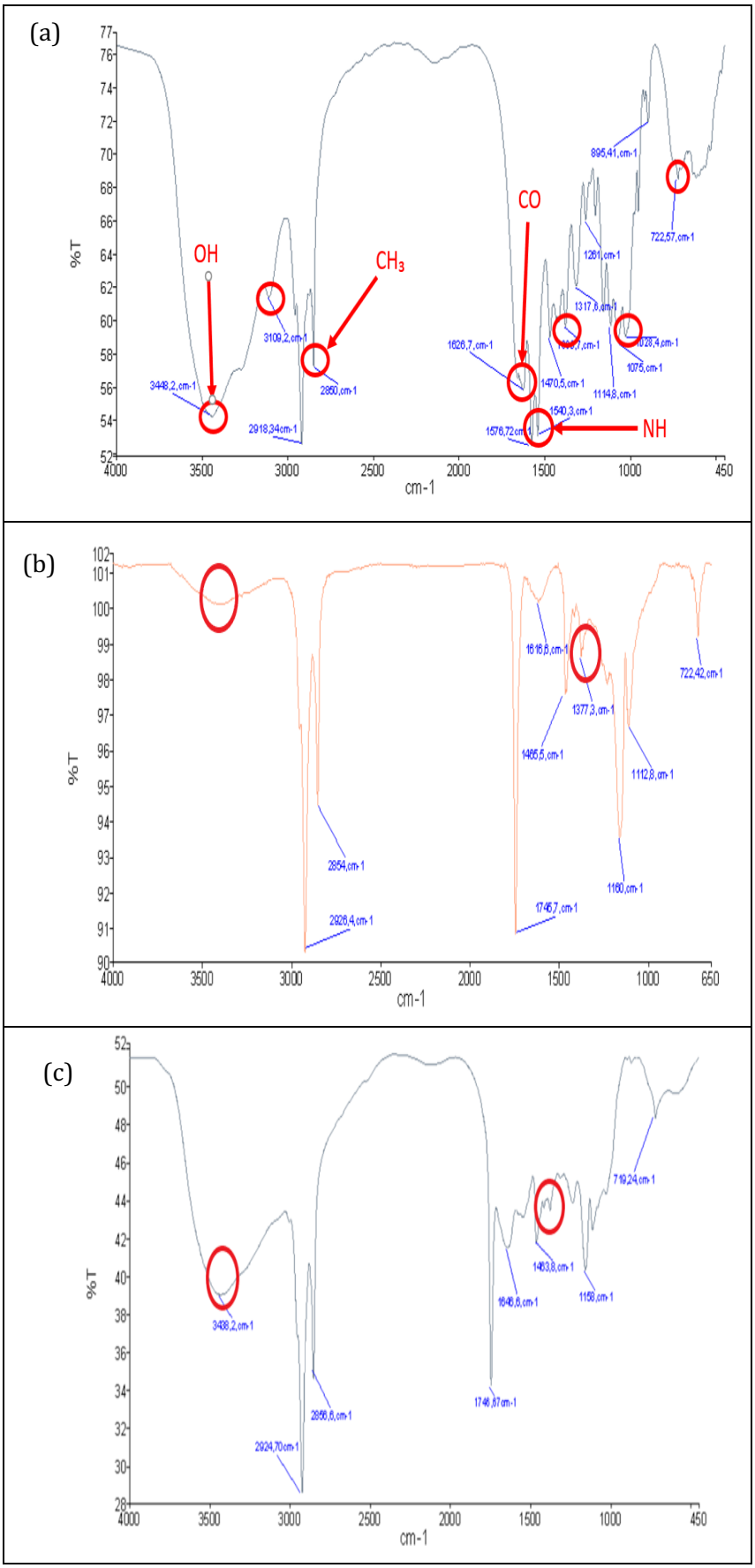

Figure 4. FTIR spectra of (a) BSF-based chitin (b) BSF-based lipid (c) raw BSF powder

4. CONCLUSION

In this study, BSF were fed with three different diets of either kitchen waste, fruits waste or vegetable waste. Kitchen waste was found to result in the highest waste-to-BSF biomass conversion. BSF larval development was also found to be more pronounced at 98.90 % ± 0.71. A low moisture content in kitchen waste (70%) was found to have a negative impact on bioconversion. Increasing the moisture content to 80% improves waste-to-BSF biomass conversion with a highest value of 99.8% ± 0.58 being obtained. Further increasing moisture content to more than 85% was found to have no significant impact on waste-to-BSF biomass conversion. The BSF larvae, prepupae and pupae were also analysed for an approximate composition of three major products (crude lipid, crude protein and chitin). A considerable amount of crude lipid, crude protein and ash was observed in BSF larvae, prepupae and pupae which agrees with previous reports. A yellowish-grey chitin powder and a considerable amount of chitin yield of 6% was obtained after performing the three isolation steps (deproteinization, demineralization and decolorization). FTIR analyses confirms the presence of chitin and lipid in BSF. BSF chitin is indicated by the presence of OH, CH_3CONH, and CO which are present in polysaccharides and glucosamine rings. BSF lipid is indicated by the disappearing of the O–H stretching of the hydroxyl group that was present in the spectra of raw BSF. This suggests the removal of the hydroxyl groups during the extraction process. Also, the appearance of X–H stretching in the spectra for extracted lipid indicates the presence of triglyceride functional groups.

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