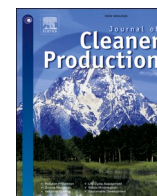


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# Co-composting of banana peel and orange peel waste with fish waste to improve conversion by black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) larvae

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## ABSTRACT

Black soldier fly (BSF) larvae composting is a promising waste treatment that can add value to available biodegradable waste. However, substrates that have low protein content and contain complex molecules (e.g. fruit peels) are not easily degraded by the larvae. This study evaluated the impact on the BSF larvae composting efficiency of co-composting different mixtures of banana and orange peels with incremental increase of fish waste. Mixtures (in total 50 distinct mixtures) of varying proportions of banana peels, orange peels and fish waste were evaluated. BSF fed on orange peel and banana peel mixtures, containing no fish waste, resulted in a lower biomass conversion efficiency ( $4.5\% \pm 1.3$ ) on a volatile solids (VS) basis ( $BCE_{VS}$ ). Co-composting the fruit peels with fish waste increased the biomass conversion efficiency and the highest  $BCE_{VS}$  (25%) was attained when 75% fish waste was included. However, the  $BCE_{VS}$  varied greatly ( $18.0\% \pm 5.8$ ), likely due to varying fish waste composition. A 25% fish waste inclusion resulted in more than twice as high  $BCE_{VS}$  ( $12.3\% \pm 2.1$ ) compared to when no fish waste was included. As the conversion efficiency variance increased with increasing fish waste inclusion, it was recommended to keep the inclusions of the fish waste to around 25% of the total mixture, in order to increase the reliability of the BSF larvae composting efficiency.

## 1. Introduction

Globally, municipal solid waste generation was estimated to be 2 billion tons in 2016 and has been projected to increase to 3.4 billion tons by 2050 if the current waste generation rate continues (Kaza et al. 2018). This is a particular problem for low-, and middle-income countries with poor waste management, where waste generation is increasing faster than income changes, and is expected to more than double by 2050 (Kaza et al. 2018). Municipal authorities responsible for waste management logistics in these countries are not likely to have the capacity and resources to handle these increasing amounts of waste (Agamuthu Pariatamby et al. 2019). Currently, 93% of waste generated in most low-income countries (mostly containing > 50% biodegradable waste) end up being burned or dumped on roadsides or open land, or in waterways (Kaza et al. 2018). Other fractions, such as plastics and

metals, are collected by the informal sector for recycling (Linzner and Lange, 2013). Inadequate biodegradable waste management can have detrimental impact on the environment, by emitting greenhouse gas emissions contributing to climate change, and leaching plant nutrients into water bodies which contribute to eutrophication (Ferronato and Torretta, 2019). The informal sector does not collect the biodegradable waste and there are a number of reasons that could explain why: for one thing, there is no source segregation, making collection more difficult (Hettiarachchi et al., 2018); furthermore, a reported problem in waste management is that treatment cost exceed the value of the generated products (Lohri et al., 2014). Composting, small-scale anaerobic digestion and use of insects and worms have been suggested as potential treatment technologies for converting the biodegradable waste into more valuable products (Lohri et al., 2017). One specific technology that has attracted great interest in the past decade is waste conversion using

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the black soldier fly (BSF) larvae (Surendra et al., 2020).

The larvae of BSF (*Hermetia illucens* L.; Diptera: Stratiomyidae) can process a wide variety of biodegradable wastes, even up to 80% on a wet weight basis for some substrates (Lalander et al. 2019). The larvae biomass have crude protein content of around 40% on a dry matter (DM) basis, but the content varies somewhat (by  $\pm 5$  percentage points), depending on the substrate the larvae have been reared on, larval size and age (Wang and Shelomi, 2017; Do et al., 2020). The fat content of the larvae is around 30% but varies greatly (by  $\pm 15$  percentage points) depending on the substrate (Ewald et al. 2020). The larvae can be used in animal feed or in production of biodiesel or other industrial products (Surendra et al., 2016). The treatment residues can be used as an organic fertiliser (Kawasaki et al., 2020) or as a raw material in other processes, such as feedstock in anaerobic digestion (Lalander et al. 2018).

Achieving high biomass conversion efficiency is important in BSF larvae composting, since the larvae biomass has higher economic value than the treatment residue (Lalander et al. 2018). Although the larvae can consume a wide variety of biodegradable substrates, low treatment efficiencies have been reported for substrates with imbalanced carbon:nitrogen ratio and/or high fibre content (Nyakeri et al., 2017) and substrates containing substances that could be toxic to the larvae, e.g. phenols (Isibika et al., 2019). Moreover, protein:carbohydrate ratios have been found to play a role and ratios between 1:2 (Lalander et al. 2019) and 1:1 (Cammack and Tomberlin, 2017; Gold et al., 2020) have been demonstrated to favour the conversion of waste into larval biomass. Homogenous substrates from a single source, such as food industry waste, are generally nutritionally imbalanced in some way, e.g. high content of fibre or carbohydrates. Different forms of pre-treatments have been demonstrated to improve the BSF larvae composting efficiency of these substrates. For example, different types of microbial inoculation was found to aid the breakdown of complex compounds into forms more easily available to the larvae and thereby increase the biomass conversion efficiency (Yu et al., 2011; Isibika et al. 2019; Somroo et al., 2019).

Co-composting fibre or carbohydrate-rich fractions with fractions that have e.g. higher protein content is another way of improving the BSF larvae composting efficiency of these substrates. Rehman et al. (2017) reported increased degradation of cellulose, hemicellulose and lignin, and increased final larval weight, reduction efficiency and biomass conversion efficiency when 40% dairy manure (fibre-rich) was co-composted with 60% chicken manure (nitrogen-rich). Lalander et al. (2019) demonstrated that supplementing low-protein substrates (fruit & vegetable waste) with a protein-rich substrate (abattoir waste) improved the biomass conversion efficiency from 4% DM (pure fruit & vegetable waste) to 14% DM (1:1 fruit & vegetable waste: abattoir waste). Nyakeri et al. (2019) demonstrated that co-composting 30% faecal sludge with other organic wastes, such as banana peels, increased the biomass conversion efficiency and waste reduction in BSF larvae composting compared with composting faecal sludge only. Lopes et al. (2020) found that a small inclusion (10–15%) of a protein-rich substrate (aquaculture waste) could improve the biomass conversion efficiency and protein content of generated larvae when BSF larvae composting a carbohydrate-rich waste (reclaimed bread).

Lopes et al. (2020) determined the impact of BSF larvae co-composting of two rather high-quality waste fractions, that is, whole fish carcasses of rainbow trout (*Oncorhynchus mykiss*) and reclaimed bread. However, the composition of many food industry waste fractions is not of such a high quality as the ones studied in Lopes et al. (2020). Therefore, the aim of this study was to assess the impact of co-composting a protein-rich low-quality waste stream (fish waste, comprising fins and all internal contents of the fish) with a fiber-rich low-quality waste stream (peels of orange and banana) on BSF larvae composting efficiency in term of biomass conversion, waste reduction and larvae survival. To understand the impact of inclusion of the protein-rich waste stream, incremental increase/decrease (from 0 to 100%) of orange peel, banana peel and fish waste were evaluated,

totalling 50 distinct mixtures.

## 2. Materials and methods

### 2.1. Material and preparation

#### 2.1.1. BSF larvae

BSF larvae (5–7 d old) were obtained from our BSF colony that has been in operation since 2015 (Swedish University of Agricultural Sciences, Uppsala, Sweden). The BSF larvae were reared for around 7 d on chicken feed (Granngården Hönsfoder Start, metabolisable energy content of 11.2 MJ kg<sup>-1</sup>, 80% moisture) at a feed rate of 0.83 g 100 larvae<sup>-1</sup>.

#### 2.1.2. Waste substrates

**2.1.2.1. Fish waste.** Different fish species of wild-caught fish containing mainly perch and roach, but also some bleak, rudd, smelt, ruffe and herring were fished using multimesh gillnets in the Sea of Åland, collected and supplied by the Department of Aquatic Resources (Kustlaboratoriet, Öregrund, Sweden). Batches of approximately 100 kg fish were supplied on four different occasions and the batches differed in terms of both species and fish sizes, depending on availability at the source. Once received, the fins and all internal contents of the fish, including gills, river, kidney, intestines, heart, stomach and swim bladder, were collected and mixed. This fish waste fraction represented available fish waste in Tanzania.

**2.1.2.2. Banana peels and orange peels.** Banana and orange peels were provided in several different batches by the fruit and vegetable wholesaler Grönsakshallen Sorunda (Stockholm, Sweden). Only orange peels were provided, while the bananas were provided whole and peeled upon arrival.

**2.1.2.3. Processing of the waste substrates.** All three substrates were homogenised separately to mimic the pre-treatments used in BSF larvae treatment facilities (Dortmans et al., 2017), using a blender (Robot Coupe Blixer 4 V, France), divided into feeding portions and stored at  $-20$  °C until use. The mixture substrates aimed at supplying 0.25 g VS larva<sup>-1</sup> in total over the entire treatment, however a range of 0.26–0.4 g VS larva<sup>-1</sup> was supplied (Supplementary information, Table S2).

### 2.2. Physico-chemical and nutritional analysis

#### 2.2.1. Dry matter and total volatile solids

Dry matter content (DM) of the waste materials were determined by heating samples at 70 °C for 48 h. The drying was done at lower temperatures to prevent losses of volatile organic substances (Vahlberg C et al., 2013). After drying, the dried materials were heated in a furnace (LH30/12, Nabertherm GmbH, Germany) first at 200 °C for 2 h (to prevent sample losses due to rapid heating at high temperatures) then heated again to 550 °C for 4 h (ISO, 18122:2015) for determination of VS.

The percentage dry matter (DM) and total volatile solids on a dry matter basis (VS) were calculated as:

$$DM = \left( \frac{m_{dry.sample}}{m_{wet.sample}} \right) \times 100 \quad \text{Equation 1}$$

$$VS = \left( \frac{m_{dry.sample} - m_{ash.sample}}{m_{dry.sample}} \right) \times 100 \quad \text{Equation 2}$$

where,  $m_{wet.sample}$ ,  $m_{dry.sample}$  and  $m_{ash.sample}$  are the sample weights before and after, the drying and after the combustion, respectively.

### 2.2.2. pH

The pH of the substrate mixtures and the treatment residues was determined using an InoLab Laboratory pH meter. For this, a 10 g sample was placed in a 50-mL centrifuge tube, diluted to 50 mL with deionised water and left to stand for 1h at room temperature before the pH readings.

### 2.2.3. Proximate analysis

Proximate analyses of protein, fat, fibre and total phenols (Table 1) were performed in triplicate on the first batch of the pure waste streams (fish waste, banana peel, orange peel) at Eurofins Food & Agro Testing Sweden AB (Swedac-accredited laboratory). Modified EU method 2009/152 was used to analyse protein and fat, while ISO 5498 modified method was used to analyse fibre. Total phenol content was analysed using the laboratory in-house Folin-Ciocalteu method, while carbohydrate content was estimated by subtracting the weight (g) of protein, fat, water and ash from the total weight (g) of the sample. The composition of these substances in the mixed substrates was calculated based on their concentration in each fraction (Supplementary information, Table S1) and the total amount of each fraction in respective mixture.

### 2.2.4. C/N ratio

Carbon:nitrogen (C/N) ratio was calculated by dividing percentage of organic carbon (calculated as percentage VS divided by 1.8; Haug 1980) by percentage of total nitrogen (DM basis). The organic carbon and total nitrogen were calculated based on the resulting total amounts of protein and volatile solids contents established from the individual substrates in the substrate mixtures.

## 2.3. Experimental set up

All treatments were performed at 28 °C in individual plastic containers (Smartstore classic 2, with dimensions L21xW17xH11 cm<sup>3</sup>), each covered with a plastic lid with a rectangular fabric mesh-covered opening (L9xW5 cm<sup>2</sup>) to allow air circulation. The portioned and frozen fish waste, banana peel and orange peel substrates were thawed at room temperature (28 °C) for 24 h and thoroughly mixed according to the required ratio in 50 different combinations (Table S1). Fish waste inclusion rate in the mixture was fixed at 0%, 10%, 25% 50%, 75% or 100%, while the banana peel and orange peel inclusion rates varied from 100% to 0%, at either 5% or 10% increments. Each treatment mixture received 700 larvae (>0.2 cm in size, 7 d old), resulting in a density of 2 larvae cm<sup>-2</sup>. The larvae were fed on days 0, 4 and 7. After the last feeding event, the boxes were monitored until around 10% of the larvae had become pre-pupae, at which point the larvae were harvested and the treatment was terminated. The treatment time varied between 2 and 3 weeks.

One sample (~5 g) of each treatment mixture from any part of the treatment box was collected once a week for DM, VS and pH determination. After termination of treatment, larvae/pre-pupae were picked manually from the residues. The DM and VS content of all harvested biomass of larvae and of treatment residues were determined. For the treatment residues, the pH was also measured.

## 2.4. Calculations

Percentage material reduction on a VS basis (RED<sub>VS</sub>) was calculated

**Table 1**

Physico-chemical and nutritional properties of fish waste, banana peel and orange peel used for black soldier fly larvae composting.

	Dry matter (%)	Total volatile solids (%DM)	Protein g 100g <sup>-1</sup>	Fat g 100g <sup>-1</sup>	Fibre g 100g <sup>-1</sup>	Carbohydrate g 100g <sup>-1</sup>	Total phenols (%)
Fish waste	28.2 ± 0.1	86.3 ± 0.9	15.9 ± 0.4	5.8 ± 5.3	0.2 ± 0.01	2.1 ± 4.8	0.3 ± 0.04
Banana peel	11.3 ± 0.01	86.3 ± 0.1	0.9 ± 0.1	1.1 ± 0.5	1.9 ± 0.4	6.6 ± 0.3	0.1 ± 0.08
Orange peel	18.8 ± 0.04	96.6 ± 0.6	1.1 ± 0.04	0.3 ± 0.01	2.6 ± 0.3	14.1 ± 0.01	0.4 ± 0.03

as (Diener et al., 2009):

$$RED_{VS} = \left( 1 - \frac{m_{res} * DM_{res} * VS_{res}}{m_{mix} * DM_{mix} * VS_{mix}} \right) \times 100 \quad \text{Equation 3}$$

where,  $m_{res}$  and  $m_{mix}$  is the mass,  $DM_{res}$  and  $DM_{mix}$  is the percentage dry matter, and  $VS_{res}$  and  $VS_{mix}$  is the percentage total volatile solids in treatment residues and substrate mixture, respectively.

Percentage biomass conversion efficiency on a VS basis (BCE<sub>VS</sub>) was calculated as (Lalander et al. 2019; Gold et al. 2020):

$$BCE_{VS} = \frac{m_{lv} * DM_{lv} * VS_{lv}}{m_{mix} * DM_{mix} * VS_{mix}} \times 100 \quad \text{Equation 4}$$

where,  $m_{lv}$  and  $m_{mix}$  is the mass,  $DM_{larvae}$ ,  $DM_{mixture}$  is the percentage dry matter, and  $VS_{larvae}$  and  $VS_{mixture}$  is the percentage total volatile solids in the larvae and substrate mixture, respectively.

Percentage survival rate (SR) of the larvae was calculated as (Gold et al. 2020):

$$SR = \left( \frac{lv_{end}}{lv_{start}} \right) \times 100 \quad \text{Equation 5}$$

where,  $lv_{end}$  is the number of larvae that survived to the end of the treatment and  $lv_{start}$  was the initial number of larvae used in the treatment (n = 700).

The respired VS (Resp<sub>VS</sub>) was calculated as (Lundgren, 2019):

$$Resp_{VS} = \frac{mVS_{mix} - mVS_{lv} - mVS_{res}}{mVS_{mix}} \times 100 \quad \text{Equation 6}$$

where,  $mVS_{mix}$ ,  $mVS_{lv}$  and  $mVS_{res}$  are the mass of VS in the substrate mixture, the larval biomass and the treatment residues, respectively.

### 2.4.1. Statistical analysis

Principal component analysis (PCA) was performed to find the variables that contributed most to variation in the data, while multi-linear regression was used to verify correlations of selected variables. Normality with 95% confidence was verified in the model residuals with Shapiro-Wilk test. R statistical software (R Core Team, 2016) was used for statistical analysis and for graphical presentation of the data.

## 3. Results

### 3.1. Physico-chemical parameters

Dry matter content was below 28% in all substrate mixtures, while VS content ranged between 83 and 95% on a dry matter basis (Table S2). The pH of the substrate mixtures varied between 4 and 7. The protein content increased, while the carbohydrate content decreased, with increasing amount of fish waste in the mixture. The C/N ratio increased with orange peel inclusion and generally decreased with fish waste inclusion in the substrate mixture. The treatment residues of all mixtures had pH between 5 and 10, while the moisture content ranged between 52 and 90%.

### 3.2. BSF larvae composting efficiency

The larval survival in the substrate mixtures with 0% fish waste was

more than 97%, except for mixture 8 (F0%B70%O30%; 62% survival rate) (Table 2). All the substrate mixtures containing fish waste had a survival rate of more than 50%, with the exception of mixtures 46 and 48 (75% fish waste inclusion, survival rate 34% and 36%, respectively). The larvae from these two treatments had the highest final larval weight, 269 mg larva<sup>-1</sup> for mixture 46 (F75%B10%O15%) and 255 mg larva<sup>-1</sup> for mixture 48 (F75%B20%O5%). All 700 larvae died within two days when fed 100% fish waste.

Material reduction on a VS basis was generally greater for the substrate mixtures than for the homogenous peel substrates (mixtures 1 and 11, around 50%) (Table 2). Biomass conversion efficiency increased gradually with increasing inclusion of fish waste in the substrate mixtures. Substrate mixture 8, with 0% fish waste, had the lowest BCE<sub>VS</sub> (2%), while substrate mixtures 45, 47 and 49, all with 75% fish waste, had the highest BCE<sub>VS</sub>, 25%, 23% and 20%, respectively. VS respiration generally decreased with increasing inclusion of fish waste in the substrate mixtures.

**Table 2**

Process efficiency in BSF larvae composting. Larvae survival rate, final larval weight, biomass conversion efficiency, material reduction rate and respired volatile solids (VS) for substrate mixtures 1–49. All values are based on single samples.

Mix. no.	Substrate	Survival rate (%)	Final larval weight (mg larva <sup>-1</sup> )	Biomass conversion efficiency (%VS)	Material reduction (%VS)	Respired VS (%)
1	F0%B0%O100%	100.0	50.9	3.9	49.7	45.8
2	F0%B10%O90%	100.0	48.8	4.0	62.5	58.5
3	F0%B20%O80%	100.0	54.4	3.8	62.8	59.1
4	F0%B30%O70%	100.0	62.6	4.7	66.1	61.4
5	F0%B40%O60%	100.0	65.9	3.9	73.1	69.3
6	F0%B50%O50%	100.0	71.8	4.4	66.5	62.1
7	F0%B60%O40%	100.0	69.3	4.0	71.7	67.7
8	F0%B70%O30%	62.3	84.9	2.3	67.7	65.4
9	F0%B80%O20%	100.0	93.9	6.0	59.3	53.3
10	F0%B90%O10%	97.9	88.3	6.8	51.0	44.2
11	F0%B100%O0%	100.0	82.7	5.8	49.5	43.8
12	F10%B0%O90%	72.0	61.0	3.4	56.0	52.6
13	F10%B10%O80%	57.1	137.3	6.1	47.8	41.8
14	F10%B20%O70%	69.4	138.7	7.2	55.6	48.4
15	F10%B30%O60%	72.5	154.0	7.9	65.8	57.8
16	F10%B40%O50%	72.4	139.0	9.4	62.8	53.4
17	F10%B50%O40%	75.0	144.3	9.1	47.9	38.9
18	F10%B60%O30%	74.0	144.3	9.7	57.4	47.7
19	F10%B70%O20%	73.5	146.0	9.2	63.1	53.9
20	F10%B80%O10%	63.0	162.0	8.3	63.4	55.2
21	F10%B90%O0%	72.5	140.3	7.8	55.1	47.3
22	F25%B0%O75%	96.9	137.7	13.5	68.1	45.8
23	F25%B5%O70%	96.0	141.0	13.4	66.0	58.5
24	F25%B10%O65%	85.8	161.0	13.6	74.5	59.1
25	F25%B15%O60%	71.2	179.9	12.3	75.8	61.4
26	F25%B20%O55%	78.9	169.7	11.7	72.0	69.3
27	F25%B25%O50%	86.7	143.7	12.9	61.9	62.1
28	F25%B30%O45%	66.0	219.7	13.8	72.2	67.7
29	F25%B35%O40%	68.0	184.0	12.0	72.5	65.4
30	F25%B40%O35%	70.4	172.7	10.1	68.4	54.6
31	F25%B45%O30%	86.3	161.3	11.5	73.6	52.6
32	F25%B50%O25%	79.3	166.0	11.7	71.2	60.9
33	F25%B55%O20%	91.1	185.0	14.7	68.7	63.5
34	F25%B60%O15%	92.2	174.7	13.9	70.8	60.2
35	F25%B65%O10%	75.5	152.7	9.4	68.3	49.0
36	F25%B70%O5%	99.7	169.0	14.8	67.5	58.4
37	F25%B75%O0%	83.5	169.0	6.9	60.7	60.5
38	F50%B0%O50%	79.5	168.7	17.0	59.7	58.3
39	F50%B10%O40%	55.7	189.0	11.5	63.4	62.2
40	F50%B20%O30%	63.2	182.3	13.5	72.7	59.5
41	F50%B30%O20%	50.0	216.3	13.3	59.2	54.0
42	F50%B40%O10%	49.7	207.7	11.2	55.0	57.0
43	F50%B50%O0%	95.4	131.3	12.8	52.0	58.9
44	F75%B0%O25%	58.6	181.7	15.8	49.4	52.7
45	F75%B5%O20%	97.2	198.3	25.3	62.6	53.9
46	F75%B10%O15%	33.8	268.7	11.7	45.7	42.7
47	F75%B15%O10%	91.1	215.3	23.1	65.7	51.9
48	F75%B20%O5%	35.6	255.0	11.8	50.0	59.1
49	F75%B25%O0%	79.7	198.3	20.4	64.8	45.9

### 3.3. Principal component analysis results and model strength

Principal component analysis was conducted to identify the most influential parameters contributing to BSF larvae composting efficiency. The first two principal components explained 73.3% of the variation in the dataset (PC1 47.3%, PC2 26.0%) (Fig. 1a), with phenol, protein, fat, fibre and carbohydrate content making the greatest contributions (Fig. 1b). Content of phenols correlated positively with orange peel inclusion and protein content correlated positively with fish waste inclusion, while no specific nutritional parameter appeared to correlate with banana peel inclusion. BCE<sub>VS</sub> correlated positively with protein content, and negatively with carbohydrate content.

In the multilinear regression analysis, carbohydrate content was the only substrate property that contributed (66%) to the variation in final larval weight (Model 1.0, Table 3) and the found correlation was negative. Biomass conversion efficiency correlated positively with protein content in the substrate mixtures (Model 2.0, Table 3). The BCE<sub>VS</sub> increased with increasing protein content in the substrate mixtures

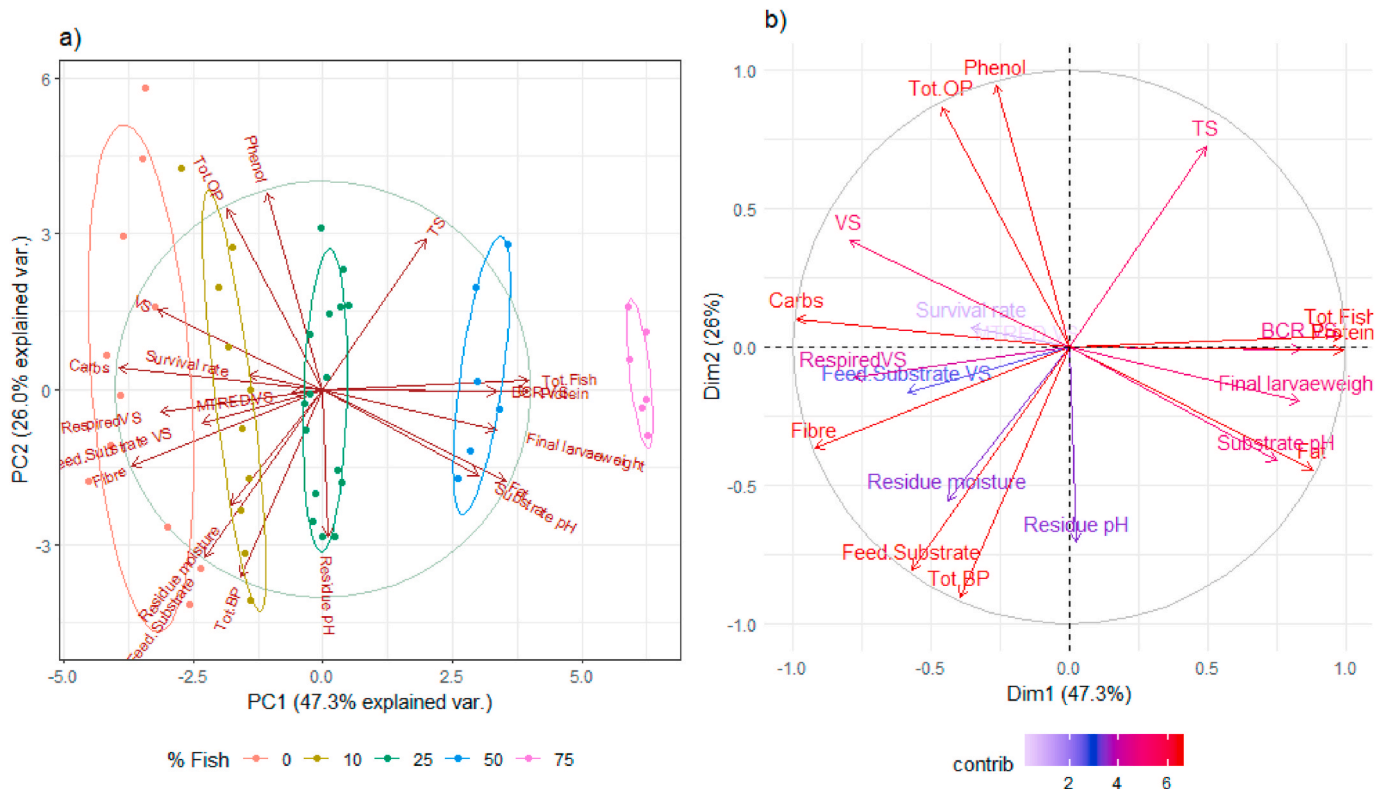


Fig. 1. Principal component (PC) plots obtained for fish waste, banana peel and orange peel substrate mixtures 1–49. Percentage of variation in the data explained by a) PC1 and PC2 and b) contribution of different variables (red indicates large contribution).

Table 3

Model strength (coefficient of determination adjusted  $R^2$ ) and F-test significance value (p) of two models of dependent variables and predictors in the co-composting process. Negative correlations are denoted by the minus (-) sign. B represents coefficient slope values for the regression equation for predicting the dependent variable from the independent variable.

Model No.	Dependent variable	Model	Coefficients		Adjusted $R^2$	p- value
			B	Std. Error		
1.0	Final larval weight	Carbohydrates	-0.016	0.002	0.664	0.000
2.0	Biomass conversion efficiency	Protein	0.166	0.017	0.656	0.000

(Table 2, Fig. 1a).

#### 4. Discussion

##### 4.1. BSF larvae composting efficiency of pure fish waste, banana peel and orange peel

When the larvae were fed 100% fish waste, no observable larvae growth occurred, and all the larvae died within the first two days of treatment (Table 2, Fig. 1). This high mortality rate could have been caused by the high fat content in the fish waste. Nguyen et al. (2013) observed that high-fat restaurant and fish wastes decreased larvae growth and survival rates, with no survival being observed of larvae reared on fish waste, and attributed this to high amounts of fat and heavy metal contamination in the fish waste. Difficulties for the BSFL to metabolize and utilize the high fat contents (>6 g/100 g) in fish waste and bioaccumulation of heavy metals in the BSFL biomass were the factors that were associated with the observed inhibition in metabolism, health, and immunity of the BSF larvae that resulted in 100% larval mortality (Nguyen et al. 2013). Diener et al. (2015) observed no negative effects on larval survival when larvae were fed chicken feed spiked with heavy metals (cadmium, lead, zinc). The heavy metal content in the fish waste used in this study was not measured, but fish from the source

area (Baltic sea north of Stockholm) are associated with high levels of heavy metals (Manzetti, 2020). Perch from the Baltic sea are known to have high levels of mercury (~209  $\mu\text{g kg}^{-1}$  wet weight) of which depends on the mobility ability of perch along associated aquatic systems, that is, rivers, lakes and oceans (Suhareva et al., 2021). Accumulation of mercury by BSF larvae negatively impacted the size and development rate of the BSF larvae to pupae stage due to inhibition that also slowed the rate of food consumption when fed on food waste mixed with mercury (Attigbo et al., 2019). Hence, presence of mercury could have similarly inhibited the BSF larvae growth when fed 100% fish waste in this study. Lopes et al. (2020) observed increasing larvae mortality with increasing aquaculture waste inclusion and it was speculated that the oily film formed on top of substrates with a high aquaculture waste inclusion prevented the larvae from breathing. The lack of structure and sticky nature of the fish waste substrate could also have led to suffocation of the larvae in this study, as the small larvae could not aerate, move and process this waste.

Waste substrates characterised by high fibre content (Rehman et al. 2017) and low nitrogen content (Lalander et al. 2019) have been reported to be challenging in terms of nutrient utilisation and conversion by larvae. The pure banana peel was low in protein and high in fibre (Table S2), which likely caused the observed low final larval weight and low biomass conversion efficiency (Table 2), and this is in accordance

with earlier findings for banana peel (Isibika et al. 2019).

Pure orange peel had the lowest protein content and highest amount of carbohydrates and phenols (Table S2). The final larval weight and BCEVs was also observed to increase with decreasing carbohydrate content in the mixtures (Fig. 1, Model 1.0 and Table 3). The high protein:carbohydrate ratio (1:14) in the orange peels could have contributed to the observed low material reduction, BCEVs and final larval weight (Gold et al., 2020). Citrus peel is known to be difficult to treat biologically (Mizuki et al., 1990; Ruiz and Flotats, 2014). Calabrò et al. (2016) showed that anaerobic digestion of orange peel waste was inhibited by an increasing concentration of citrus essential oils (mainly D-limonene). In fact, citrus essential oils are used in insecticides as they have strong insecticidal activity, while they can also serve as antimicrobials, minimising the growth of several fungi and bacteria strains (Bora et al., 2020). Kumar et al. (2012) observed insecticidal activity against larvae and pupae of housefly (*Musa domestica*) from direct contact with essential oil from orange peel (*Citrus sinensis* L.). Presence of phenols in banana peel was also found to negatively impact the BCEVs (Isibika et al. 2019). Toxicity effects of essential oils (Kumar et al. 2012) and anti-nutritional effects (Isibika et al. 2019) from the high phenol content in orange peel could have also affected the conversion efficiencies of the 100% orange peels substrate in this study (Table 2).

#### 4.2. BSF larvae composting efficiency with treatment mixtures of fish waste, banana peel and orange peel

Substrate mixtures of banana peel, orange peel and fish waste generally resulted in improved BFS larvae composting efficiency, compared with the individual substrates (Table 2). However, although all the peel-containing mixtures gave a very high larval survival rate, the BCEVs (<7% VS) and final larval weight (<95 mg larva<sup>-1</sup>) were still low. Pure fruits and vegetable wastes have previously been shown to be poor substrates for larval development, due to low protein and high carbohydrate content (Jucker et al., 2017). It was noticed that banana peel generated a slightly higher BCEVs than orange peel: 5.8% compared to 3.9% for pure orange peel. Increasing the orange peel concentration generally reduced the BCEVs, likely due to the orange peel toxicity

discussed above. When diluting orange peel with other waste fractions, the toxicity of the orange impacted less on the overall efficiency, as the BCEVs increased.

Adding fish waste as a protein source generally resulted in increased final larvae weight and biomass conversion efficiency (Table 2). The increase in BCEVs and final larval weight was most likely due to the observed increase in protein content from the fish waste balancing the high carbohydrate and fibre content in the fruit peels.

The highest BCEVs (25%) was achieved on substrate mixture 45, with 75% fish waste (F75%B5%O20%). However, large variations in BCEVs (18 ± 6%) were observed for the six substrate mixtures containing 75% fish waste, indicating an unstable process (Fig. 2). In fact, supplementation with 25% fish waste more than doubled the BCEVs compared with 0% fish waste, and thus could be considered sufficient.

Gold et al. (2020) found a 1:1 protein:carbohydrate ratio in substrate to be ideal for BSF larvae. However, the BCEVs increased 2.7-fold (from 2% to 12%) on increasing the protein: carbohydrate ratio from 0.1 (0% fish) to 0.4 (25% fish) (Fig. 2b), while no significant increase in BCEVs (13%) was found on doubling the protein: carbohydrate ratio to 0.9 (50% fish inclusion). Lopes et al. (2020) concluded that mixing bread with small quantities (<15%) of aquaculture waste was sufficient to maximise the positive impact of nitrogen supplementation on larval development. In the present study, a higher level of fish waste (inclusion of 25%) was needed to achieve larger positive responses in conversion efficiency. This was likely due to the fish waste in this study having a lower protein content (15.9% of DM) than the aquaculture waste (60.3% of DM) used by Lopes et al. (2020). The total protein addition in Lopes et al. (2020) for 15% addition of aquaculture waste was 10%, similar to the addition in this study for the 75% addition, which yielded a 12% protein addition. However, the bread had a somewhat higher protein content than the fruit peels in this study. The lower fat content of the fish waste, as well as the higher fibre content in banana and orange peel compared to bread, could explain why more fish waste could be added in this study without increasing the mortality of larvae (Table 2).

The observed variations in BCEVs and final larval weight with higher fish waste inclusion could have been caused by differences in nutritional composition between the different batches of fish waste, which

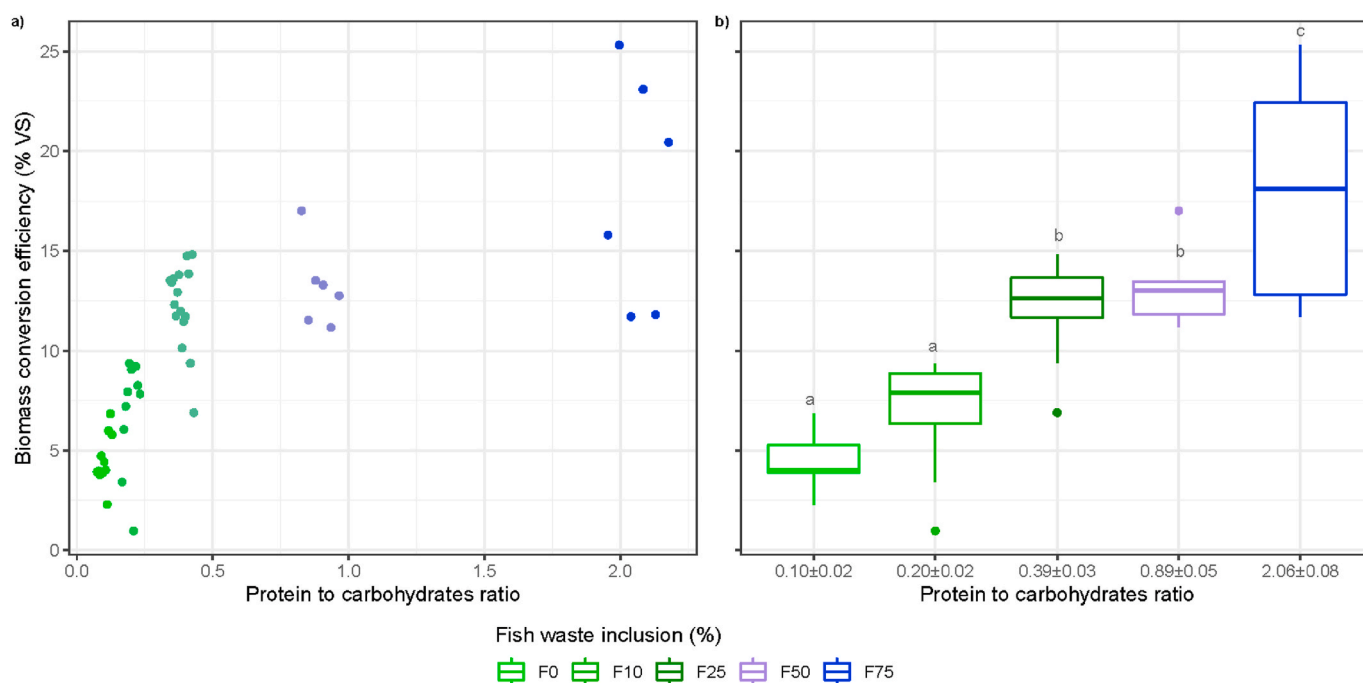


Fig. 2. a) Scatterplot and b) boxplot showing the impact of protein to carbohydrate ratio with increasing fish (F) inclusion level from 0% (green) to 75% (blue) on biomass conversion efficiency on a volatile solid basis (BCEVs). Different letters on boxes in (b) indicate significant difference (95% confidence interval) in mean BCEVs.

contained different types of fish species depending on availability at the source at the time of delivery. Furthermore, during processing of small and large fish species to obtain fish waste, it was found that the stomach contents differed between batches. In some batches great amounts of fat and/or eggs were found in the fish, while the fish in other batches had no or low amounts of fats and/or eggs in the stomach. Thus, the nutritional composition, including fat content and hormone composition of the fish waste, may have caused the large variations observed, particularly for the mixtures with 75% fish waste. Variations in process efficiency when treating substrates with similar and different nutritional composition have been reported to impair the reliability and sustainability of this treatment, especially in industrial-scale operations (Gold et al. 2018). Maintaining lower levels of fat (<40% of DM) and fibre (<50% of DM) in substrate mixtures has been suggested to increase fly larvae treatment performance and reduce variations (Gold et al. 2020). This may be difficult to achieve in a waste treatment facility, as all waste that arrives must be treated, however, keeping the inclusion of the more variable sources smaller (here around 25–50%) can minimise the variations.

Relatively high material reduction (50–75%) was achieved for most substrate mixtures in this study (Table 2). VS respiration correlated negatively with biomass conversion efficiency and positively with material reduction, indicating that the material reductions achieved probably resulted from both BSF larvae and microbial degradation. Microbial activity in the substrates could have partly contributed to the variable treatment conversion efficiencies (material reduction rates) seen for the different substrate mixtures.

Overall, this study demonstrated that it is possible for BSF larvae to degrade challenging low-quality substrates such as orange peel and banana peel, with almost doubled  $BCE_{VS}$ , with a relatively small inclusion of a low-quality protein-rich waste stream. The protein content of the fish waste was relatively low compared that reported for e.g. aquaculture waste by Lopes et al. (2020), who found that 15% inclusion was sufficient in co-composting with bread. This suggests that a smaller inclusion rate of the protein-rich fraction may be required with a higher-protein substrate. Many organic wastes are currently not fully utilised and end up polluting the environment. Composting by BSF larvae can add value to these wastes, by converting them into insect products potentially suitable for various applications, for example in animal feed.

## 5. Conclusions

The aim of this study was to see the potential of improving BSF larvae composting efficiency of low-quality food industry wastes by means of co-composting. A fibre-rich, hard to degrade waste stream such as fruit peels, was co-composted with a low-quality protein-rich waste stream (fish waste). BSF larvae did not survive in sticky, fat-rich fish waste and BSF larvae composting of pure and mixed banana and orange peel mixtures resulted in lower final larval weights and  $BCE_{VS}$ . Final larval weight and  $BCE_{VS}$  generally increased with increasing protein content in the substrate mixtures. Combining fish waste with fruit waste increased  $BCE_{VS}$ , to up to 25% with 75% fish waste (12% protein addition) in the substrate mixture. In other words, around 4-fold increase in  $BCE_{VS}$  was achieved for the 75% fish waste inclusion compared to what was achieved for pure banana and orange peels. However, large variation in  $BCE_{VS}$  ( $18.0\% \pm 5.8$ ) and final larval weight ( $219 \text{ mg larva}^{-1} \pm 35$ ) was found when 75% fish waste was included. Lower inclusion rates of fish waste were thus suggested, as a 2.7-fold increase in  $BCE_{VS}$  was found when with 25% fish waste (4% protein addition) as compared to no fish waste inclusion, while the variance in efficiency was kept lower ( $BCE_{VS}$   $12.3\% \pm 2.1$ ). Lower variations in process efficiency renders higher reliability of the treatment process. This study demonstrated the potential of using low-quality protein-rich waste to improve the BSFL composting of low-quality fibre-rich wastes. The present study provides a scientific basis for future studies that should investigate whether these small-scale results are transferrable to industry-scale in order to advance

the industrialization of BSFL composting treatment of biodegradable waste fractions.

## CRediT authorship contribution statement

**A. Isibika:** Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **B. Vinnerås:** Conceptualization, Methodology, Supervision, Writing – review & editing. **O. Kibazohi:** Funding acquisition, Supervision, Writing – review & editing. **C. Zurbrügg:** Conceptualization, Supervision, Writing – review & editing. **C. Lalander:** Conceptualization, Methodology, Formal analysis, Supervision, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jclepro.2021.128570>.

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