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Almond by-product composition impacts the rearing of black soldier fly larvae and quality of the spent substrate as a soil amendment

Lydia Palma,[a†] Jesus Fernández-Bayo,[a†] Ferisca Putri[a] and Jean S VanderGheynst[a,b*]

Abstract

BACKGROUND: Insect biomass is a sustainable alternative to traditional animal feeds, particularly when insects are produced on low-value high-volume agricultural by-products. Seven samples of almond by-product (hulls and shells) were obtained from processors in California and investigated for larvae production. Experiments were completed with and without larvae and spent substrate samples were assessed for their potential as soil amendments based on standard compost quality indicators.

RESULTS: On average, specific larvae growth and average larval harvest weight were 158% and 109% higher, respectively, when larvae were reared on Monterey and pollinator hulls compared to nonpareil hulls and mixed shells. Larvae methionine and cystine contents were highest when larvae were reared on Monterey hulls and mixed shells, respectively. Available phytonutrients in spent substrate were affected by feedstock sample and larvae rearing. Spent nonpareil substrate without larvae had the highest NH_4-N levels and spent pollinator substrate incubated without larvae had the highest PO_4-P levels. Spent mixed shell substrate had the lowest availability of phytonutrients.

CONCLUSION: The findings demonstrate that by-product composition has a significant impact on larvae growth and the properties of the spent substrate, and that spent substrate from larvae rearing requires further stabilization before application as a soil amendment.

Supporting information may be found in the online version of this article.

Keywords: insect rearing; insect protein; amino acids; compost quality; almond by-product; soil amendment; frass

INTRODUCTION

Food production and agriculture create an abundance of by-products and residues, which have been estimated at 1.3 billion metric tons annually in the USA alone.[1] Almonds represent one of many agricultural commodities that generate significant amounts of lignocellulosic by-products. California is the global leader in almond production, producing over 80% of the global supply.[2] Almond hulls and shells are by-products of the industry and are abundant low-value residue streams. In 2018, California produced 2.8 million metric tons of hulls and shells combined, a 19% increase since 2015.[2]

Historically, livestock feed and bedding have been the main end uses for almond hulls and shells. However, the demand for hulls and shells for feed has decreased in California while their production has increased.[3] The almond industry has been investigating alternative uses for almond by-products along with domestic and export outlets including China, the EU, Korea and India.[4] Valorizing almond hulls and shells through insect rearing systems would provide an alternative end-use for by-products and could help meet increasing demands for food and feed. Our research group demonstrated that almond hulls can be used to rear *Hermetia illucens* L., or black soldier fly larvae (BSFL).[5] Black soldier fly larvae have a high nutritional value for inclusion in feed for poultry, swine, and fish, and are a promising replacement for soymeal and fishmeal.[6-9] However, rearing larvae to yield a consistent feed ingredient has been a challenge because the BSFL nutritional profile depends on the growth substrate and rearing environment.

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Spranghers *et al*. found the larvae composition varied for BSFL reared on chicken feed, digestate, vegetable waste and restaurant waste.[10] For example, crude protein varied from 86 to 246 g kg^{-1}, ash content varied from 45 to 299 g kg^{-1}, and methionine content varied from 7.1 to 8.7 g kg^{-1} (dry weight basis).[10] Almond by-product composition has been shown to vary based on variety and location grown. Offeman *et al*. (2014) reported that total sugars ranged from 340 to 500 g kg^{-1} of the dry weight in 36 nonpareil hull samples collected from seven counties in California.[11] This study also reported that total sugars in Monterey hulls ranged from 268 to 366 g kg^{-1} on a dry weight basis. Given the potential for variation in almond by-product composition, it is important to understand the impact of feedstock on larvae growth and composition.

The BSFL-rearing process produces both insect biomass and spent substrate. Furthermore, studies have shown that the process can reduce and stabilize organic wastes and accelerate substrate bioconversion.[12, 13] The BSFL consume between 25 and 500 mg day^{-1} of substrate, depending on properties such as particle size, fiber content, larval instar, and moisture level.[6] Lalander *et al*. observed the composition of feedstock affected material reduction during BSFL bioconversion.[14] Black soldier fly larvae reared on chicken feed had an average of 84.8% dry-matter reduction compared to 46.7% dry-matter reduction when reared on fruits and vegetables.[14] Larvae reared on a mixture of dairy and chicken manure at different ratios resulted in a reduction of total nitrogen ranging from 69% to 53%, a reduction in phosphorus from 60% to 57%, a reduction in carbon from 68% to 57%, and a 2–4 unit decrease in the carbon to nitrogen ratio (C/N) in the spent substrate.[15] A separate study examining BSFL reared on chicken manure noted substrate maturity could be achieved in a period of 24 days.[13] These prior studies demonstrate that the larvae-rearing process could enhance decomposition but also generate a variable stream of spent substrate. This variability, and the impact of BSFL rearing on spent substrate maturity and quality, need to be understood in order for spent by-products to be used as an organic fertilizer or soil amendment.[16, 17]

There are reports of BSFL production on a variety of organic residues however few have considered the role of feedstock composition on both larvae production and spent substrate quality. The main objectives of this research were to determine the impacts of feedstock composition, through the variation in almond by-product composition, on larvae growth, larvae composition, and quality of spent substrate as a soil amendment.

MATERIALS AND METHODS

Acquisition and processing of almond hull feedstocks

Almond orchards include at least two almond varieties because almond trees cannot self-pollinate. The main almond tree variety used for nut production is Nonpareil and common pollinator varieties include Monterey, Padre, Butte, Carmel, and Fritz.[18] Seven samples of almond hulls and shells were obtained from processors in California, USA for use in the experiment (Table 1). The samples were ground using a hammer mill with a 6.35 mm screen and then stored in airtight plastic bags. The calcium, total sugar, total starch, total nitrogen, total carbon, acid detergent fiber, neutral detergent fiber, and acid detergent lignin were measured by JL Analytical Services Inc (Modesto, CA, USA). Calcium was measured using EPA Method 6010C through inductive coupled plasma-atomic spectrometry.[19] Total sugar was measured using AOAC 980.13.[20] Total starch was determined using an enzymatic-colorimetric method.[21] Total nitrogen was measured using AOAC 990.03 through sample combustion, which converts all organic and inorganic substances into combustion gases and detected using gas chromatography.[20] Total carbon was measured using AOAC 993.13.[22] Neutral detergent fiber was measured using National Forage Testing Association (NFTA) 5.1 where a neutral detergent solution is used to dissolve pectin, protein, sugars, and lipids separating the fibers of cellulose, hemicellulose, and lignin.[23-25] The acid detergent fiber and acid detergent lignin were measured using AOAC 973.18 through acid and alkaline titration methods.[26] Compositions of samples are listed in Table 2.

BSF larvae rearing

Larvae rearing methods are described in detail in Palma *et al*.[5] Larvae were reared from eggs purchased from Symtom Black Soldier Fly (College Station, TX, USA) on chicken feed (Purina Premium Poultry Feed Layena Crumbles, Purina Animal Nutrition LLC, Shoreview, MN, USA) at a moisture content of 500 g kg^{-1} wet basis with incubation at 28 °C. Seven-day old larvae were used for the experiment. Prior to inoculation onto samples, larvae were separated from feed using 1 and 2-mm sieves and weighed. Samples of larvae were collected for moisture content measurement and average dry weight per larva. The average initial weights of larvae were 0.003 g dry weight per larva and approximately 100 larvae were added to each bioreactor.

Feedstock preparation and incubations

Feedstock samples were amended with distilled water and urea (Fisher Scientific Company LLC, Hampton, NH, USA) to achieve a target C/N ratio of 26.[5] Urea was used as a model nitrogen source because urea could be obtained consistently and distributed uniformly throughout the feedstocks. Prior to incubation, three random samples from each mixture were collected to measure pH and moisture content as previously described.[5]

The experiment was designed as a randomized block design. There were four replicates for each treatment inoculated with larvae and three replicates for non-inoculated treatments. Incubations were completed over 14 days in bioreactors as previously described.[5]

Larvae harvest and analysis

At the end of the experiment the contents of bioreactors were frozen at −20 °C. Larvae were separated and counted at a later date; total larvae weight and numbers of larvae recovered per bioreactor were recorded. Larvae moisture content was measured gravimetrically for each treatment as previously described. Separated larvae were stored at −20 °C and homogenized with an oscillating ball mill (MM400, Retsch Inc., Newtown, PA, USA). The homogenized larvae were freeze dried (VirTis 50-SRC-5, SP Scientific, Warminster, PA, USA) for 4 days. The methionine and cystine contents were measured at the VetMed Amino Acid Testing Laboratories (Davis, CA, USA) using performic oxidation with hydrolysis.[27] The calcium, dry matter, crude fat, total crude protein, total glucose, total non-structural carbohydrates (TNC) and ash were measured at the UC Davis Analytical Laboratories (Davis, CA, USA). Calcium was measured using nitric acid digestion and determined by Inductively Coupled Atomic emission spectrometry.[28] Dry matter was measured based on the gravimetric loss of free water associated with heating to 105 °C for 3 h.[29] Crude fat was measured using AOAC 2003.05 through a Randall modification of the standard Soxhlet extraction method.[30] Total crude protein was

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Table 1 Description of almond by-product feedstocks

Almond by-product feedstock sample	Description	Harvest Year	Region
1	Pollinator hulls	2016	Chico, CA, USA
2	Nonpareil hulls	2017	Chico, CA, USA
3	Pollinator hulls	2017	Chico, CA, USA
4	Nonpareil hulls	2017	Buttonwillow, CA, USA
5	Monterey hulls	2017	Buttonwillow, CA, USA
6	Pollinator hulls	2017	Buttonwillow, CA, USA
7	Mixed almond shells	2017	Buttonwillow, CA, USA

measured using AOAC 990.03 and calculated from a protein factor of the nitrogen content.[31] Total glucose and TNC were measured using enzymatic hydrolysis where the TNC is the sum of the total glucose, free fructose and free sucrose.[32] The ash content was measured using AOAC 942.05 through the gravimetric loss by heating the samples to 550 °C for at least 3 h.[33] Average larva harvest weight was calculated by dividing the total dry weight of larvae by the number of larvae harvested. Specific larvae growth was calculated by dividing the change in larvae dry weight by the initial larvae dry weight. This value represents the accumulation of larvae biomass within a bioreactor normalized by the initial larvae inoculation weight. Hull consumption was calculated by dividing the change in hull dry mass by the initial hull dry mass.

Spent substrate analysis

At the end of the incubations, samples of spent substrate were analyzed for moisture content and pH using methods described previously.[5] For each sample, a fraction was frozen, and the rest was air-dried under ambient conditions to perform fertility and stability analyses. The analyzed parameters related to fertility of spent substrate were total content of carbon (C), nitrogen (N), ammonium-nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N), phosphate-phosphorus (PO_4-P), potassium (K), and calcium (Ca). The nutrient content analyses were conducted at the UC Davis Analytical Laboratories (Davis, CA, USA). Total C and N were measured according to AOAC 972.43, through the flash combustion method using thermal conductivity/IR detection (LECO FP-528 and TruSpec CN Analyzers).[34] Soluble NH_4-N, NO_3-N, PO_4-P and K were extracted using 2% acetic acid solution.[35,36] PO_4-P was measured spectrophotometrically at 880 nm using a flow injection analyzer by reacting with ammonium molybdate and antimony potassium tartrate under acidic conditions and reducing the complex with ascorbic acid.[37] Soluble K and Ca were measured by coupled plasma atomic emission spectrometry (ICP-AES).[38]

Volatile fatty acid (VFA) content, cumulative CO_2 evolution (cCER) and phytotoxicity were measured to assess spent substrate stability. For VFA analysis, 2 g of spent substrate samples was mixed with 10 mL of deionized water in a 15 mL tube and equilibrated for 30 min at 25 °C. After mixing, samples were centrifuged at 10 000 *g* for 10 min at 20 °C and supernatants filtered through a 0.2 μm filter (Titan-3, 17 μm filter blue 0.2 μm polytetrafluoroethylene, membrane, Thermo Fisher Scientific Inc. San Diego, CA, USA) into a high-performance liquid chromatography (HPLC) vial. Acetic, propionic, formic, butyric, and isobutyric acids were measured using an HPLC-UFLC-10Ai (Shimadzu, Columbia, MD, USA) equipped with an Aminex HPX-87H (300 7.8 mm) column (Life Science Research, Education, Process Separations, Food Science, Hercules, CA, USA) and a SPD-M20A diode array detector set at 210 nm. The HPLC conditions are described in Simmons *et al.*[39] Of the monitored VFAs, only the acetic acid peak had a purity level that allowed quantification. The cCER was measured using 250 mL bioreactors operated under aerobic conditions[40] and following guidelines from the California Compost Quality Council (Table S1).[41] Briefly, prior to loading the bioreactors, the spent substrate samples were moistened to 50% moisture content (wet basis) with distilled water, thoroughly mixed, and equilibrated overnight at 4 °C. The bioreactors were loaded with 7 g (dry weight) of moist spent substrate. Bioreactors were kept in an incubator at 37 °C and supplied with air at a rate of 20 mL min^{-1}. Carbon dioxide concentrations in reactor influents and effluents were measured using an infrared CO_2 sensor (Vaisala, Suffolk, UK). Air flow rate was measured using a mass flow meter (Aalborg, Orangeburg, NY, USA). Samples were incubated for 10 days. The CO_2 evolution rate (CER) was calculated for each bioreactor based on mass balances of CO_2 at each time point and normalized to the dry mass of spent substrate in each bioreactor. The cumulative respiration (cCER) for each bioreactor was then calculated for the 10 days of incubation ($cCER_{10}$, mg CO2/g dw). The phytotoxicity of the spent substrate was determined using an adapted method involving radish (*Raphanus sativus* var. Sparkler) seed germination.[42-44] Briefly, extract solutions were

Table 2 Composition of almond by-product feedstocks prior to amendment with urea

	Composition of feedstock (g kg^{-1} dry matter)[a]								
Almond by-product feedstock sample	Fat	Protein	Ca	ADF	NDF	ADL	Starch	Sugar	C/N ratio
1	31.0	40.1	2.4	258.5	358.0	67.1	5.53	152.7	72.68
2	20.5	46.3	2.1	176.7	264.4	44.2	3.57	243.5	60.42
3	24.8	41.0	2.3	220.6	318.8	57.2	5.03	178.1	69.71
4	22.3	55.3	2.2	174.6	252.5	34.5	4.23	291.3	50.43
5	26.5	67.7	2.8	285.6	403.8	74.7	4.33	119.2	42.23
6	22.9	40.6	2.6	255.6	359.3	64.2	4.93	202.1	70.58
7	14.6	42.6	1.9	527.5	749.7	158.5	3.65	53.2	69.50

[a]Composition analysis: fat, protein, calcium (Ca), acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), starch, sugar and carbon to nitrogen (C/N) ratio.

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Table 3 Comparative means (and standard deviation) of average harvest weight, specific larvae growth and feedstock consumption

Almond by-product feedstock sample	Harvest average weight (g dry larva)$^{-1}$ [a, b]	Specific larvae growth[c] (g g^{-1} dry)[a, b]	Hull consumption[d] with larvae (g g^{-1} dry)[a, b]	Hull consumption[d] without larvae (g g^{-1} dry)[a, b]
1	0.029 (0.002) A	8.83 (0.55) A	0.224 (0.021) C	0.221 (0.033) BC
2	0.011 (0.001) C	2.73 (0.26) C	0.297 (0.033) AB	0.264 (0.022) AB
3	0.023 (0.004) AB	6.37 (0.54) B	0.294 (0.018) AB	0.282 (0.011) AB
4	0.012 (0.001) C	2.91 (0.18) C	0.344 (0.024) A	0.313 (0.039) A
5	0.027 (0.003) AB	7.93 (0.53) A	0.258 (0.024) BC	0.268 (0.044) AB
6	0.024 (0.001) AB	6.13 (1.14) B	0.287 (0.017) B	0.251 (0.0081) AB
7	0.014 (0.004) C	2.87 (0.54)	0.131 (0.029) D	0.144 (0.016) C

[a]Means and standard deviations in parentheses. Four replicates for all treatments containing larvae and three for replicates without larvae.
[b]Means followed by the same letter within columns are not statistically different at $\alpha = 0.05$ based on the Tukey–Kramer HSD test.
[c]Specific larvae growth was calculated by dividing the change in larvae dry weight by the initial larvae dry weight.
[d]There were no significant differences in hull consumption between treatments with and without larvae ($P > 0.05$).

prepared by horizontally shaking 2 g of spent substrate samples with 20 mL of deionized water in a 50 mL tube for 6 h at 25 °C. After shaking, samples were centrifuged at 7100×*g* for 20 min at 20 °C.[44] The supernatant was tested at two strengths: 100% and 50% dilution. For the germination test, petri dishes (10 cm diameter) were lined with filter paper (Whatman Grade 1.90 mm) sterilized under UV light for 20 min for each side. Each dish received 10 radish seeds and 5 mL of the extract supernatant solution or deionized water (control). After 72 h of incubation in the dark at 25 °C, germinated seeds were counted (G) and the root length (L) was measured. Seeds were considered germinated when at least 5 mm of primary root was visible.[42] The germination index (GI) was calculated according to the following equation:

$$GI = \left(\frac{G}{G_o}\right) \times \left(\frac{L}{L_o}\right) \times 100 \quad (1)$$

where G is the average number of seeds germinated in the treatment, L is the average root length in the treatment, G_o is the average number of seeds germinated in the control, and L_o is the average root length in the control.

Quality score estimation

A quality score was determined to assess the quality of the spent substrate as soil amendment. This score was estimated using the mean values of the parameters included in the compost maturity index developed by the California Compost Quality Council:[41] carbon to nitrogen ratio, CO_2 evolution rate, ammonia, volatile organic acids (estimated as total acetic acid), and germination index. Each parameter was assigned a maturity / stability score (2 – high; 1 – medium; 0 – low) given the range of values provided in Table S1. The final score was determined by the sum of all the scores of each parameter where a higher score indicates a higher quality of spent substrate.

Data analysis

Responses for average larval harvest weight, specific larvae growth, hull consumption, and final larvae composition were analyzed using Tukey's honestly significant difference (HSD) test. A two-way ANOVA was used to assess significant effects of sample and / or incubation with larvae on parameters related to biological stability and soil fertility of spent substrate samples. Tukey's HSD and Student's *t*-tests were used to define significant differences within varieties or between incubations with or without larvae, respectively. All statistical tests were performed using JMP-IN software (version Pro 12, SAS, Cary, NC, USA). The significance level was set at 0.05.

RESULTS

Impact of feedstock composition on larvae growth and feedstock consumption

The carbohydrate, protein, and fat content varied with feedstock variety and source (Table 2). The largest differences were

Table 4 Composition of larvae grown on different almond by-products

Almond by-product feedstock sample	Composition of larvae (g kg^{-1} dry matter)[a]					
	Fat	Protein	Ash	Ca	Total glucose	TNC
1	68.3	403.0	111.2	26.4	28.4	29.4
2	31.0	458.5	109.1	25.4	51.3	51.3
3	51.4	419.5	117.9	29.6	32.4	32.4
4	56.4	474.0	101.2	20.9	40.2	41.2
5	44.3	490.4	135.6	36.8	39.3	39.3
6	40.5	482.7	126.9	30.7	30.5	30.5
7	31.5	511.7	120.7	25.9	32.6	32.6

[a]Replicates of larvae combined for analysis: fat, protein, ash, calcium (Ca), total glucose, total non-structural carbohydrates (TNC).

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Table 5 Comparative means (and standard deviation) of amino acid content in harvested larvae

Almond by-product feedstock sample	Methionine (g kg^{-1} dry matter)[a,b]	Cystine (g kg^{-1} dry matter)[a,b]
1	4.69 (0.37) C	2.86 (0.20) B
2	4.49 (0.19) C	3.21 (0.15) B
3	4.63 (0.19) C	3.41 (0.17) B
4	5.24 (0.29) C	3.55 (0.11) AB
5	7.84 (0.14) A	3.72 (0.11) AB
6	6.13 (1.11) BC	3.76 (0.58) AB
7	7.20 (1.60) AB	4.37 (0.91) A

[a]Means and standard deviations in parentheses. Four replicates for all treatments.
[b]Means followed by the same letter within columns are not statistically different at $\alpha = 0.05$ based on the Tukey–Kramer HSD test.

observed in the neutral detergent fiber and sugar content of samples. Mixed shells (sample 7) had the highest neutral detergent fiber content of 749.7 g kg^{-1} and nonpareil sources (samples 2 and 4) had the lowest content, averaging 258.5 g kg^{-1}. On average, pollinator hulls had 34% higher neutral detergent fiber compared to nonpareil hulls. Average sugar content was highest in nonpareil hulls (samples 2 and 4) at 267.4 g kg^{-1}; an average of 1.5 times higher than pollinator hulls, 2.24 times higher than Monterey hulls and over five times higher than mixed shells.

Average larval harvest weight, specific larvae growth, and hull consumption were statistically different between the samples tested (Table 3, $P < 0.05$). Average larval harvest weight ranged from 0.011 g dry larva^{-1} for sample 2 (nonpareil hulls) to 0.029 g dry larva^{-1} for sample 1 (pollinator hulls). Specific larvae growth was the highest for sample 1 at 8.83 g g^{-1} dry weight and statistically similar to sample 5 (Monterey hulls). Specific larvae growth for sample 2 was the lowest at 2.73 g g^{-1} dry weight and statistically similar to samples 4 and 7. Hull consumption ranged between 0.131 g g^{-1} dry weight for mixed shells (sample 7) to 0.344 g g^{-1} dry weight for sample 4. There was significantly greater hull consumption observed for sample 4 than samples 1, 5, 6 and 7 for treatments reared with larvae ($P < 0.05$). There were no significant differences in hull consumption between treatments incubated with and without larvae ($P > 0.05$).

Impact of feedstock on larvae composition

Fat, protein, ash, calcium, total glucose, and total non-structural carbohydrates of harvested larvae varied with feedstock sample (Table 4). Fat content in harvested larvae ranged between 31.0 g kg^{-1} dry matter for larvae reared on sample 2 (nonpareil hulls) to 68.3 g kg^{-1} dry matter for growth on sample 1 (pollinator hulls). Protein content in harvested larvae varied between 403.0 g kg^{-1} dry matter for growth on sample 1 (pollinator hulls) to 511.7 g kg^{-1} dry matter for growth on sample 7 (mixed shells). Ash and calcium content in harvested larvae was highest at 135.6 g kg^{-1} dry matter and 36.8 g kg^{-1} dry matter, respectively, for sample 5 (Monterey hulls) and lowest at 101.2 g kg^{-1} dry matter and 20.9 g kg^{-1} dry matter, respectively, for sample 4 (nonpareil hulls). Total glucose and non-structural carbohydrates in harvested larvae were highest at 51.3 g kg^{-1} dry matter and 51.3 g kg^{-1} dry matter for sample 2 (nonpareil hulls) and lowest at 28.4 g kg^{-1} dry matter and 29.4 g kg^{-1} dry matter for sample 1 (pollinator hulls), respectively.

Methionine and cystine content in harvested larvae was statistically different between the feedstock samples tested (Table 5, $P < 0.05$). Methionine content ranged between 4.49 g kg^{-1} dry matter for sample 2 (nonpareil hulls) to 7.84 g kg^{-1} dry matter for sample 5 (Monterey hulls). Cystine content ranged between 2.86 g kg^{-1} dry matter for pollinator hulls (sample 1) to 4.37 g kg^{-1} dry matter for mixed shells (sample 7).

Impact of feedstock sample and larvae rearing on soil amendment properties of spent substrate

Parameters related to biological stability of the spent substrate are summarized in Table 6. In the treatments without larvae, the pH was significantly lower for samples 2 and 4 and the C/N ratio was significantly higher for sample 7 compared to the other

Table 6 Characteristics of spent substrates based on parameters related to biological stability in treatments incubated without (w/o) and with (w) larvae

	pH[a, b]		C/N[a, b]		cCER10d[c] (mg CO_2 g^{-1} biomass)[a, b]		GI (%)[a, b]		Glx2 (%)[a, b]		Acetic acid[c] (mg g^{-1} biomass)[a, b]	
Sample	w/o larvae	w/ larvae	w/o larvae	w/ larvae	w/o larvae	w/ larvae	w/o larvae	w/ larvae	w/o larvae	w/ larvae	w/o larvae	w/ larvae
1	8.19A	8.60A*	25.86B	26.97B*	127.33A	62.19A*	5A	17A*	49A	53A	7.45C	6.45CD*
2	5.63C	7.22B*	21.70B	22.01C	93.15AB	65.30A	1A	0B	1BC	6CD	12.80AB	13.39A
3	8.22A	8.48A*	25.74B	26.55B*	95.46AB	76.18A	5A	6B	43A	19BC	6.60C	9.03BC
4	7.05B	7.66B*	20.42B	20.45C	122.65A	47.24A*	0A	0B	1C	0D	15.24A	11.87AB
5	8.16A	8.80A*	25.99B	27.54B*	95.40AB	53.43A*	0A	2B	12BC	9BCD	8.83BC	3.85D*
6	8.01A	8.46A*	25.76B	26.66B	110.35A	75.35A	0A	4B	20BC	17BC	9.28BC	8.79BC
7	8.41A	8.54A	34.76A	38.29A	59.83B	49.47A	6A	6B	30AB	24B	4.33C	4.25D

[a]Means followed by the same letter within columns are not statistically different at $\alpha = 0.05$ based on the Tukey–Kramer HSD test. Four replicates for all treatments containing larvae and three for replicates without larvae.
[b]Stars denote significant differences between treatments without and with larvae incubation based on Student's *t*-test ($P < 0.05$).
[c]Characteristics of spent feedstocks: pH, carbon to nitrogen (C/N) ratio, cumulative respiration at 10 days (cCER10d), germination index of non-diluted (GI) and diluted 1:1 (v:v, Glx2) extracts, and concentration of acetic acid.

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Table 7 Characteristics of spent substrates based on parameters related to soil fertility in treatments incubated without (w/o) and with (w) larvae

	Total N[a] (g kg^{-1} dry matter)[b,c]		Total C[a] (g kg^{-1} dry matter)[b,c]		NH4-N[a] (ppm)[b,c]		K[a] (g kg^{-1} dry matter)[b,c]		PO4-P[a] (ppm)[b,c]		Ca[a] (g kg^{-1} dry matter)[b,c]	
Sample	w/o larvae	w/ larvae	w/o larvae	w/ larvae	w/o larvae	w/ larvae	w/o larvae	w/ larvae	w/o larvae	w/ larvae	w/o larvae	w/ larvae
1	18.8AB	18.0B*	485.3A	484.8A	1370.0B	615.0C*	34.7AB	35.4C	326.7 BC	355.0B	3.5AB	3.4AB
2	21.6AB	21.3A	469.3BC	469.0B	7596.7A	5695.0A*	37.0A	38.5BC	366.7BC	227.5B*	2.8B	2.8C
3	18.3B	17.6B*	471.0B	466.5BC	743.3B	755.0C	41.3A	44.6A	400.0B	405.0B	3.2AB	2.8C
4	22.4A	22.3A	456.3D	455.5C	6513.3A	5052.5A	40.5A	44.6A*	376.7BC	515.0B*	3.3AB	3.3B
5	17.7BC	16.9B	459.0CD	463.0BC	926.7B	400.0C	43.7A	43.4AB	976.7A	825.0A	3.6AB	3.2B
6	18.4AB	17.6B	474.0AB	469.5B	1396.7B	1420.0BC	35.5AB	36.0C	393.3B	485.0B*	3.8A	3.6A
7	14.2C	12.3C	474.7AB	469.5B	1310.0B	2595.0B*	24.0B	17.9D	263.3C	245.0B	2.8B	2.3D

[a]Characteristics of spent feedstocks: total nitrogen (N), total carbon (C), extractable ammonium (NH_4-N), extractable potassium (K), extractable phosphate (PO_4-P), and total calcium (Ca).
[b]Means followed by the same letter within columns are not statistically different at $\alpha = 0.05$ based on the Tukey–Kramer HSD test. Four replicates for all treatments containing larvae and three for replicates without larvae.
[c]Stars denote significant differences between treatments without and with larvae incubation based on Student's *t*-test ($P < 0.05$).

samples ($P < 0.05$). In the treatments without larvae, the cCER was significantly lower for sample 7 than samples 1, 4 and 6 ($P < 0.05$). The non-diluted GI had high phytotoxicity levels in all the samples without larvae. When extracts were diluted (GIx2), samples 1 and 3 had significantly lower phytotoxicity ($P < 0.05$) than the rest of the samples with the exception of sample 7. Acetic acid levels for sample 4 were significantly higher than all the samples ($P < 0.05$) with the exception of sample 2.

Incubation of feedstock with larvae had significant impacts on many of the soil amendment properties of the spent substrate; however, the impacts depended on the type of feedstock tested. With the exception of sample 7 (mixed shells), spent substrate samples had significantly higher pH when incubations contained larvae compared to incubations without larvae ($P < 0.05$). Incubations with samples 1, 3, and 5 resulted in spent substrate with significantly higher C/N for treatments with larvae than without larvae ($P < 0.05$). In general, sample incubations with larvae resulted in lower cCER than the same sample incubated without larvae, but differences were significant for only samples 1, 4, and 5 ($P < 0.05$). The presence of larvae in incubations with sample 1 significantly decreased spent substrate phytotoxicity ($P < 0.05$). This effect was not observed in the diluted extracts.

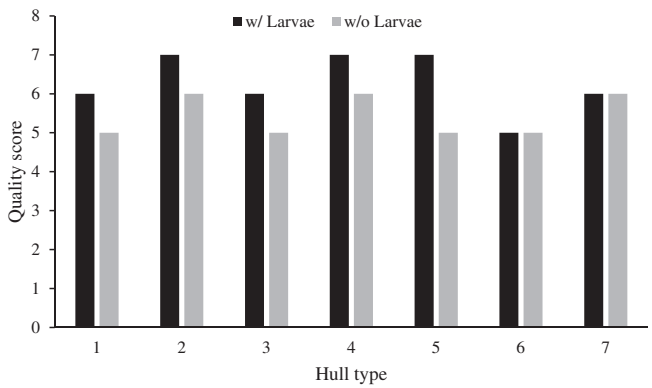

Figure 1 Compost quality score estimated for each spent substrate sample incubated with (w) or without (w/o) larvae from the sum of scores for the parameters defined by the California Compost Quality Council (Table S1).

Finally, for incubations with samples 1 and 5, acetic acid content in the spent substrate was significantly lower in treatments with larvae compared to treatments without larvae ($P < 0.05$).

The feedstock sample had a significant effect on several parameters related to the soil amendment potential of the spent substrate (Table 7). In the samples incubated without larvae, total N was significantly higher in sample 4 than in samples 3, 5, and 5 ($P < 0.05$). In samples with larvae, samples 2 and 4 showed significantly higher total N than the other samples ($P < 0.05$). Total C in samples 1, 6 and 7 incubated without larvae was significantly higher than samples 4 and 5 ($P < 0.05$). Sample 1 incubated with larvae showed significantly higher total C than all other samples ($P < 0.05$). NO_3-N levels were below the detection limit in all the samples (data not shown). NH_4-N levels in spent substrate ranged from 400 g kg^{-1} for sample 5 treated with larvae to 7597 g kg^{-1} for sample 2 treated without larvae. Potassium levels were between 18 g kg^{-1} for sample 7 treated with larvae to 45 g kg^{-1} for samples 3 and 4 treated with larvae. Spent substrate from sample 5 treated with and without larvae had PO_4-P levels on the order of 900 g kg^{-1} which were significantly higher than PO_4-P levels in all other treatments ($P < 0.05$).

Incubation of hulls with larvae had a significant effect on the total N, NH_4-N and Ca content in spent substrate ($P < 0.05$). Samples 1 and 3 incubated with larvae had significantly lower total N than the same samples without larvae ($P < 0.05$). Samples 1 and 2 had significantly lower levels of NH_4-N in incubations with larvae than without larvae, whereas sample 7 had significantly higher levels of NH_4-N in the samples incubated with larvae than without larvae ($P < 0.05$). Incubation of hulls with larvae resulted in significantly higher levels of K than incubation without larvae for sample 4 ($P < 0.05$). Incubations with larvae resulted in significantly lower PO_4-P levels than incubations without larvae for sample 2, whereas incubations with samples 4 and 6 had higher levels of PO_4-P in the samples incubated with larvae ($P < 0.05$).

Impact of feedstock sample and larvae rearing on spent substrate soil amendment quality

To assess the quality of the spent substrate as a soil amendment, a quality score index was determined for the spent substrates following guidelines developed to assess compost maturity

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(Table S1, Fig. 1).[41] According to this index, only substrates with C/N ratio < 25 can be considered mature enough for further consideration in the quality evaluation. This value was only achieved for samples 2 and 4 incubated with and without larvae, meaning that most of the spent substrates would not satisfy the minimum requirements for compost quality. For this analysis, samples with C/N ratio < 25 were given a score of 2 and samples with C/N ratio > 25 a score of 1. The highest total scores were observed for samples 2, 4 and 5 incubated with larvae. The presence of larvae during incubation improved the score for all the samples except for samples 6 and 7 where the score was the same with or without larvae.

DISCUSSION

Larvae growth and composition

Many studies have shown that rearing substrate composition affects larval growth and substrate bioconversion.[14-17, 45-50] Chia *et al*. formulated 12 diets with varying protein and net energy levels consisting of brewers' spent grains, brewers' yeast and cane molasses.[51] Significant differences were observed in larval and pre-pupal developmental time among the experimental diets but no significant differences in larval and prepupal weight were observed. Meneguz *et al*. (2018) found that BSFL development (weight and length) was significantly impacted by feeding substrate and that a fruit-vegetable mixture diet performed better than a pure fruit diet.[52] It was reported that larvae weight was 33% higher in the fruit-vegetable diet after 4 days of growth and remained higher until approximately day 20 when larvae weight on the fruit diet surpassed that of the mixed diet. It was also observed that larvae reached the prepupae stage at a faster rate and with lower mortality when grown on the fruit-vegetable diet compared to the pure fruit diet.[52] Jucker *et al*. reported similar findings where final larval weight was 5.4% greater for larvae grown on a vegetable diet compared to a fruit diet higher in sugars.[53] In the present study, larvae growth varied with initial feedstock composition. An increase in the initial sugar and starch content and decrease in the neutral detergent fiber content in the feedstocks tested resulted in a decrease in average larval harvest weight and specific larvae growth. This is consistent with previous findings where developing larvae performed better on a diet with lower sugar content.[53] Another study introduced glucose to the larvae of silkworm and found a metabolic shift in the fat body from lipogenesis (formation of fat) to glycogenesis (formation of glycogen) during the last instar.[54] This suggests sugars may be more beneficial during the latter phases of larvae development than during early larvae development to produce glycogen reserves for energy during post larval feeding. Additional work is needed to determine ideal carbohydrate levels that promote larval development.

With the exception of growth on mixed shells (sample 7) larvae growth increased with increasing fiber content in the substrate. Gold *et al*. reported that microbes in the larval gut and excretions can hydrolyze fibers, making the nutrients available for larval development.[55] Another study isolated *Bacillus subtilis* from the gut of BSFL and observed BSFL growth on chicken manure – typically a mixture of lignocellulosic bedding material such as straw, hay or rice hulls – that was treated with and without the bacterium.[13] BSFL weight increased by 15.9%, BSFL conversion rate increased by 12.7% and chicken manure reduction rate increased by 12.7% for treatments inoculated with *B. subtilis* compared to non-inoculated treatments.[13] Spranghers *et al*. studied rearing substrates that included digestate and chicken feed and reported that increasing soluble fiber content from 5 to 57 g kg^{-1} increased BSFL yield from 90.8 to 219.8 g kg^{-1}.[10] Observations from previous studies support findings from the present study that BSFL are able to access nutrients from a wide range of substrates including almond by-products.

Very few studies have reported on the effect of substrate composition on amino acid content in harvested larvae. Spranghers *et al*. reported that methionine content in harvested larvae varied when larvae were reared on feedstocks containing chicken feed, digestate, vegetable waste, and restaurant waste.[10] In this prior study, increasing non-fiber carbohydrates in the initial feedstock from 449 g kg^{-1} dry to 618 g kg^{-1} dry decreased methionine content in harvested larvae from 7.6 g kg^{-1} dry to 7.1 g kg^{-1} dry. The observations from prior research are consistent with the present study that demonstrated that larvae methionine content decreased when starch and sugar (non-fiber carbohydrates) increased in the initial feedstock.

The fat content in larvae was also impacted by the fat content of feedstocks. Liland *et al*. observed that the total fatty acids in harvested BSFL increased with increasing total fatty acids of a feeding substrate containing brown algae enriched wheat bran.[49] The total fatty acid content in the larvae was 82.7% higher when larvae were reared on the substrate consisting of 10% brown algae containing 47.6 g kg^{-1} dry total fatty acids compared to 100% brown algae containing 19.7 g kg^{-1} dry total fatty acids. The study also found that fatty acid 20:5n-3 (EPA) content in larvae increased linearly with EPA content in the feeding substrate.[49] The results indicate that the larvae fat content and fatty acid profile could be varied by altering the composition of the feeding substrate; however, further work is needed to determine the fatty acid profile of larvae grown on almond by-products.

Potential value of spent material as soil amendment

In general, low biological stability of the spent substrate was observed for all the samples as indicated by the phytotoxicity tests. In agreement with the composition analysis, spent substrate from nonpareil hulls had the lowest stability highlighted by lower pH, lower GI index, and higher cCER and ammonia levels compared to spent substrate associated with the other feedstocks.

The presence of larvae in incubations did improve the biological stability of the spent substrate. The most consistent effect of larvae was an increase in the pH of the spent substrate. Moreover, all the larvae-incubated samples had lower $cCER_{10d}$ values than corresponding samples without larvae, although the differences were only significant for samples 1, 4, and 5. This suggests that larvae enhanced the decomposition of substrate as observed in prior studies.[13]

A quality score index was determined for the spent substrates following guidelines developed to assess compost maturity.[41] The highest total scores were observed for spent samples 2, 4, and 5 incubated with larvae. The presence of larvae during incubation improved the score for all the varieties except for samples 6 and 7 where the score was the same with or without larvae. These results confirm the significant role larvae can play in shortening the duration for treatment of organic wastes to obtain compost-like products. However, the relatively fast rearing time for larvae results in a short treatment period for the substrates, which does not facilitate achievement of sufficient quality standards. Studies are needed to further investigate the impacts of larvae rearing variables on both spent substrate quality and larvae yield.

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Soil biosolarization and anaerobic soil disinfestation are soil biofumigation practices that use solar heating and anaerobic conditions to control soilborne pests, respectively.[56, 57] These practices also benefit from non-stable organic matter to enhance pest-control efficacy through the production of ammonia and VFAs that are induced by the degradation of the non-stable organic matter.[56, 58, 59] Our results suggest that the low biological stability of the spent substrate from insect rearing could be leveraged in soil application practices that rely on biological activity from amended organic matter.

CONCLUSIONS

Our results show that composition of almond hulls and shells as rearing substrates can have significant effects on larvae production and the available phytonutrients in the spent substrate. By-products containing relatively lower levels of sugar, and starch and higher levels of fibers were more favorable for larvae growth and methionine content than by-products containing relatively higher levels of sugar and starch with lower levels of fibers. The short rearing period used in this study did not facilitate sufficient decomposition of the by-product substrate to achieve compost-like quality standards. However, the study had promising results for the potential role of larvae rearing in shortening the duration for treatment of agricultural by-products to obtain quality soil amendments.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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