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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

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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 NH4-N levels and spent pollinator substrate incubated without larvae had the highest PO4-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.

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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. 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). 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. 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. The BSFL consume between 25 and 300 mg day\(^{-1}\) of substrate, depending on properties such as particle size, fiber content, larval instar, and moisture level. Lalander et al. observed the composition of feedstock affected material reduction during BSFL bioconversion. Black soldier fly bioconversion can reduce and stabilize organic wastes and accelerate substrate bioconversion. The target C/N ratio of 26.5 was used as a model nitrogen source because urea could be obtained consistently and distributed uniformly throughout the feedstocks. 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.

**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. 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. Total sugar was measured using AOAC 980.13. Total starch was determined using an enzymatic-colorimetric method. 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. Total carbon was measured using AOAC 993.12. 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. The acid detergent fiber and acid detergent lignin were measured using AOAC 973.18 through acid and alkaline titration methods. Compositions of samples are listed in Table 2.

### BSF larvae rearing

Larvae rearing methods are described in detail in Palma et al. 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. 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.

#### 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 Scientic, 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. 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. Dry matter was measured based on the gravimetric loss of free water associated with heating to 105 °C for 3 h. Crude fat was measured using AOAC 2003.05 through a Randall modification of the standard Soxhlet extraction method. Total crude protein was measured using AOAC 990.03 through sample combustion, which converts all organic and inorganic substances into combustion gases and detected using gas chromatography.
measured using AOAC 990.03 and calculated from a protein factor of the nitrogen content. Total glucose and TNC were measured using enzymatic hydrolysis where the TNC is the sum of the total glucose, free fructose and free sucrose. 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. Average larval 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 larval 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. 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 (NH₄-N), nitrate-nitrogen (NO₃-N), nitrate-nitrogen (NO₃-N), phosphorus-phosphorus (P₂O₅-P), potassium (K), and calcium (Ca). The nutrient content analyses were conducted at the UC Davis Analytical Laboratories (Davis, CA, USA).

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 |

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

| Almond by-product feedstock sample | Composition of feedstock (g kg⁻¹ dry matter) |
|-----------------------------------|--------------------------------------------|
|                                   | 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 |

aComposition 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.
Black soldier fly larvae rearing

The germination index (GI) was calculated according to the following equation:

\[
GI = \left( \frac{G}{G_0} \right) \times \left( \frac{L}{L_0} \right) \times 100
\]

where \( G \) is the average number of seeds germinated in the treatment, \( L \) is the average root length in the treatment, \( G_0 \) is the average number of seeds germinated in the control, and \( L_0 \) 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.\(^{43}\)

### 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 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.\(^4^\) 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 10 °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.\(^5\) The germination index (GI) was calculated according to the following equation:

\[
GI = \left( \frac{G}{G_0} \right) \times \left( \frac{L}{L_0} \right) \times 100
\]

where \( G \) is the average number of seeds germinated in the treatment, \( L \) is the average root length in the treatment, \( G_0 \) is the average number of seeds germinated in the control, and \( L_0 \) 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.\(^{43}\)

### Data analysis

Responses for average larval harvest weight, specific larval 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.

### Table 3

| Almond by-product feedstock sample | Harvest average weight (g dry larva)\(^{-1}\) \(\times 100\) | Specific larvae growth (g g\(^{-1}\) dry\(a, b\)) | Hull consumption\(^b\) 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) A                                | 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) C                                 | 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 \(P = 0.05\) 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 (\(P > 0.05\)).

### Table 4

| Composition of larvae (g kg\(^{-1}\) dry matter) | Almond by-product feedstock sample |
|------------------------------------------------|-----------------------------------|
| 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    | 449.5 | 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).
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). 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 513.4 g kg⁻¹ dry matter and 513.4 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

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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)ab | Cystine (g kg⁻¹ dry matter)ab |
|-----------------------------------|---------------------------------|-------------------------------|
| 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                 |

*Means and standard deviations in parentheses. Four replicates for all treatments.

*Means followed by the same letter within columns are not statistically different at α = 0.05 based on the Tukey-Kramer HSD test.

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

| pHb | C/Nk | cCER10d (mg CO2 g⁻¹ biomass)b | GI (%)k | Glx2 (%)k | Acetic acidc (mg g⁻¹ biomass)b |
|-----|------|-------------------------------|--------|----------|-----------------------------|
| w/o | w/   | w/o                          | w/     | w/      | w/                          |
| Sample | larvae | larvae | larvae | larvae | larvae | larvae | larvae |
| 1    | 8.19A | 8.60A* | 25.86B | 26.97B* | 127.33A | 62.19A* | 49A | 53A | 7.45C | 6.45CD* |
| 2    | 5.63C | 7.22B* | 21.70B | 22.01C | 93.15B | 65.30A | 18C | 6CD | 12.80AB | 13.39A |
| 3    | 8.22A | 8.48A* | 25.74B | 26.58B* | 95.46B | 76.18A | 43A | 19BC | 6.60C | 9.03BC |
| 4    | 7.05B | 7.66B* | 20.42B | 20.45C | 122.65A | 47.24A* | 0A | 0B | 1C | 0D |
| 5    | 8.16A | 8.80A* | 25.99B | 27.54B* | 95.40B | 53.43A* | 0A | 2B | 12BC | 8.83BC | 3.85D* |
| 6    | 8.01A | 8.46A* | 25.76B | 26.66B | 110.35A | 75.35A | 20B | 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 |

*Means followed by the same letter within columns are not statistically different at α = 0.05 based on the Tukey-Kramer HSD test. Four replicates for all treatments containing larvae and three for replicates without larvae.

*Stars denote significant differences between treatments without and with larvae incubation based on Student’s t-test (P < 0.05).

bCharacteristics 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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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 (Gx2), 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.

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.
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. Chia et al. formulated 12 diets with varying protein and net energy levels consisting of brewers’ spent grains, brewers’ yeast and cane molasses. Significant differences were observed in larval and pre-pupal developmental time among the experimental diets but no significant differences in larval and preupal weight were observed. Meneguz et al. 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. 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. 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. In the present study, larval 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 larval growth. This is consistent with previous findings where developing larvae performed better on a diet with lower sugar content. 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. This suggests sugars may be more beneficial during the latter phases of larval development than during early larval 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) larval 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. 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. 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. 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. 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. 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 larval 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. 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. 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 Gl 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 cCER100 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. A quality score index was determined for the spent substrates following guidelines developed to assess compost maturity. 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.
Soil biosolarization and anaerobic soil disinfestation are soil bio-
minification practices that use solar heating and anaerobic condi-
tions to control soilborne pests, respectively.66, 67 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.68, 69 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 produc-
tion 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 suf-
cient 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
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