

## Article

# Utilization of Carrot Pomace to Grow Mealworm Larvae (*Tenebrio molitor*)

Dominic Rovai <sup>1</sup> , Maxwell Ortgies <sup>1</sup>, Samir Amin <sup>1</sup> , Sara Kuwahara <sup>2</sup>, Gregory Schwartz <sup>2</sup>, Ruta Lesniasukas <sup>3</sup>, Jeff Garza <sup>4</sup> and Amy Lammert <sup>1,\*</sup> 

<sup>1</sup> Department of Food Science and Nutrition, California Polytechnic State University, San Luis Obispo, CA 93407, USA; drovai@calpoly.edu (D.R.); mortgies@calpoly.edu (M.O.); samin02@calpoly.edu (S.A.)

<sup>2</sup> Department of BioResource and Agricultural Engineering, California Polytechnic State University, San Luis Obispo, CA 93407, USA; skuwahar@calpoly.edu (S.K.); gschw01@calpoly.edu (G.S.)

<sup>3</sup> Garza Consulting, Evanston, IL 60201, USA; ruta@the-gc.com

<sup>4</sup> Garza Consulting, Grand Rapids, MI 49501, USA; jeff.garza@the-gc.com

\* Correspondence: alammert@calpoly.edu

**Abstract:** Edible insects are a sustainable food source to help feed the growing population. Mealworms (*Tenebrio molitor*) can survive on a variety of food wastes and alter their composition based on the feed source. Commercial carrot production produces an abundance of carotenoid-rich carrot pomace, which may be beneficial for mealworm larvae growth. This study uses an I-optimal response surface design to assess the effect of dehydrated carrot pomace concentrations (made up with wheat bran as the control) in the substrate and wet carrot pomace as the moisture source (potato and carrot as control moisture sources) in a mealworm-larvae-growing system. Using this design, statistical models were fit to determine the relationship between the substrate and moisture and dependent variables, which include mealworm larvae mortality, days to maturity, weight, protein content, fat content, moisture content, ash content, and total carotenoid content. An optimum diet was proposed, in which the best diet for improving commercial mealworm growth was found to contain 36% dehydrated carrot pomace in the substrate, with wet carrot pomace as the moisture source. This research provides an application for a commercial waste stream and provides insight to help improve the growth of a sustainable protein source.

**Keywords:** mealworms; carrot pomace; sustainability



**Citation:** Rovai, D.; Ortgies, M.; Amin, S.; Kuwahara, S.; Schwartz, G.; Lesniasukas, R.; Garza, J.; Lammert, A. Utilization of Carrot Pomace to Grow Mealworm Larvae (*Tenebrio molitor*). *Sustainability* **2021**, *13*, 9341. <https://doi.org/10.3390/su13169341>

Academic Editor: Attila Gere

Received: 25 July 2021

Accepted: 18 August 2021

Published: 20 August 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

In 1975, it was first suggested that insects could possibly ease global food shortages [1] and that insects represent a sustainable and nutritious food source that ought to receive backing from organizations such as WHO and FAO, which it ultimately did receive [2]. Edible insects are a sustainable and nutritious food source that may help feed a growing population [2]. Mealworm larvae (*Tenebrio molitor*) are a suitable candidate for mass production because of their ability to grow well in high larval density environments [3]. Commercial mealworm rearing operations typically consist of a mealworm bin that has a dry substrate, which acts as the primary food source and bedding, and a moisture source. The substrate is typically a dried grain such as wheat bran, and the moisture source is a vegetable such as carrot or potato. Wheat bran provides all the necessary nutrients for growth, development, and reproduction [4], and vegetables provide water for insects. Mealworms are able to actively absorb moisture from the air [5], but research has found that the addition of a moisture source increases the growth of mealworms [6] and is commonly used in commercial growing. Mealworms are known to have complex dietary flexibility [2] and can also survive by consuming nonconventional livestock feed sources including organic wastes [7] and even polystyrene [8–10]. Edible insect mass production has found

promising results in the use of insects to valorize food waste [11], and the composition of mealworms may be influenced by the composition of the feed source [12].

In the United States, California is responsible for over 85% of carrot production [13]. During carrot processing, it is estimated that 30–50% of the carrot is left over as pomace after commercial juicing [14]. Carrot pomace is high in pro-vitamin A carotenoids, with total carotene values reported up to 2 g/kg dry matter [15]. In addition to providing vitamin A, carotenoids are also beneficial because of their antioxidant activity. In human health, dietary carotenoids have been linked to improved brain cognitive function, heart health, and cancer prevention [16].

Currently, carrot pomace is used as a soil amendment and feed source for cattle. However, because of the nutrients that remain in this waste product from the carrot industry, research has focused on ways to utilize this by-product to supplement the human diet. Studies have looked at incorporating carrot pomace into food products such as baked goods, dressings, and functional drinks to increase the level of dietary fiber and carotenoids [17]. Carrot pomace was found to increase the nutritional value of a cookie product by adding micronutrients, carotenoids, and fiber to the cookies while still showing consumer acceptability at an 8% replacement level for wheat flour and exhibiting acceptable carotenoid retention during baking and storage [18]. The research of food-grade carrot waste for baked goods is one utilization of a waste stream from the carrot industry, but do other opportunities exist? How could the waste stream be used in other areas? Could carrot pomace be utilized as a feed source in mealworm farming?

The generation of carrot pomace during commercial carrot processing represents a loss of nutrients, in addition to a loss of the resources used to grow the carrots. The utilization of carrot pomace as a feed source for mealworm larvae may provide an additional process stream for the pomace and improve the growth of mealworm larvae. This research will evaluate the growth, survival, and nutritional composition of mealworm larvae grown on carrot pomace—as both the substrate and moisture source—and determine the optimum diet based on these parameters.

## 2. Materials and Methods

### 2.1. Mealworms

Mealworm larvae (*Tenebrio molitor*) approximately 1 month old (0.019 g/mealworm) were obtained from Jord Producers (Lindsay, NE, USA). Mealworm larvae were kept in plastic containers (12.2" L × 7.8" W × 5.1" H) with lids with holes in them. Containers were covered in aluminum foil to minimize light exposure and carotenoid degradation. Growing took place in a climate chamber maintained at 24 °C and 55–65% relative humidity.

### 2.2. Diet Preparation

Carrot pomace was received from Bolthouse Farms (Bakersfield, CA, USA). The pomace was dried in a cabinet dryer (Harvest Saver, Model # R-4, Serial # HS188, Commercial Dehydrator System Inc., Eugene, OR, USA) at 100 °F for 24 h, ground in a Vitamix blender, and sieved through a 1 mm sieve. Wheat bran (Cargill Red Flaky Wheat Bran, Minneapolis, MN, USA) was used as the control substrate, as it is common in the industry. Wheat bran and dried carrot pomace were mixed by hand in the appropriate percentages, by weight, indicated by the experimental design (Table 1).

### 2.3. Experimental Design

An I-optimal response surface design was created using Design Expert (Stat-Ease, version: 13.0.2.0, 2021, Minneapolis, MN, USA) and was used to assess the effect of the feed substrate (dried carrot pomace and wheat bran) and moisture source (carrot pomace, carrot, potato) on the nutritional profile—total carotenoid and proximate composition—growth, and survival of mealworm larvae (Table 1). Mealworm larva weight was used as the dependent variable since mealworm composition because of the independent variables was being explored. Five substrate ratios were used, expressed as the percentage of carrot

pomace, with the balance made up of wheat bran. The moisture sources used were potato and carrot, which are commonly used in industry, and carrot pomace. Carrot pomace was evaluated as both the substrate and moisture source to determine how it can be used to improve the mealworm larvae rearing system.

**Table 1.** Mealworm growing experimental design.

Treatment	Percent Dried Carrot Pomace (Balance with Wheat Bran)	Moisture Source
1	25	Carrot Pomace
2	100	Carrot
3	25	Potato
4	50	Potato
5	50	Potato
6	67	Carrot
7	67	Carrot Pomace
8	0	Potato
9	100	Carrot Pomace
10	0	Carrot
11	67	Carrot Pomace
12	25	Carrot
13	0	Carrot Pomace
14	100	Potato
15	67	Carrot

Mealworm larvae were allowed to feed ad libitum, and the feed substrate and moisture source were replaced as necessary based on visual observation of feed and feces accumulation. The end of growth was defined as the time when 5% of the original population of mealworm larvae began to pupate, recorded through daily monitoring of pupa. Post-harvest, mealworm larvae were fasted for 24 h to evacuate the gut and then frozen ( $-80^{\circ}\text{C}$ ). Frozen mealworm larvae were vacuum-sealed in metalized pouches and stored at  $-80^{\circ}\text{C}$  to minimize oxidation prior to analysis.

#### 2.4. Mealworm Larvae Growth

Growth was assessed by calculating the average weight of a sample of mealworm larvae. Once 5% pupation was reached, a 4.8–5.0 g sample of mealworm larvae were weighed and counted to determine the average weight per individual mealworm larvae.

#### 2.5. Mealworm Larvae Mortality

Mortality was assessed by removing and recording the number of dead mealworm larvae (identified by black color) from each mealworm tray. Visual inspection was performed daily.

#### 2.6. Proximate Composition

The proximate composition of mealworm larvae from all the treatments, as well as all feed sources in the mealworm system, were analyzed [19]. A loss on drying method was used to determine the moisture content. Samples were dried at  $38^{\circ}\text{C}$  for 24 h in a cabinet dryer (Harvest Saver, Model # R-4, Serial # HS188) to remove excess moisture before being transferred to a vacuum oven (HFS Vacuum Oven Model # DZF-6050, HFS Inc., Azusa, CA, USA) at  $71^{\circ}\text{C}$  for 16 h. Ash was determined using a muffle furnace (Barnstead Thermolyne 62700, Thermolyne Corporation, Dubuque, IA, USA) at  $550^{\circ}\text{C}$  for 2 h. Fat content was determined using Soxhlet extraction (FOSS Soxtec™ 2043, Eden Prairie, MN, USA) with petroleum ether (AOAC 991.36). Protein content was determined using the Kjeldahl method (FOSS Tecator™ Digester, FOSS Kjeltex™ 8200 Auto Distillation Unit, Eden Prairie, MN, USA) to determine the percentage of nitrogen in the sample, and a standard nitrogen-to-protein conversion factor of 6.25 was used to determine the protein content (AOAC 981.10). Total carbohydrates were calculated by difference.

### 2.7. Total Carotenoid Determination

An extraction solvent was prepared using a 2:1:1 ratio by volume of hexane, acetone, and ethanol [20]. Three grams of mealworm larvae was added to 15 mL of extraction solvent and homogenized at 7500 rpm for 2 min (Sentry Microprocessor, SP Industries Inc., Warminster, PA, USA) in 50 mL centrifuge tubes. Triplicate tubes were prepared for each sample. The centrifuge tubes were centrifuged at 6500 rpm for 5 min at 5 °C (Eppendorf 5910 R, Eppendorf, Hamburg, Germany). The supernatant—containing hexane and non-polar carotenoids ( $\beta$ -carotene)—was removed from the tubes and made up with hexane to 10 mL in a volumetric flask. Absorbance values at  $\lambda_{\max}$  (450 nm) were measured in triplicate (Shimadzu UV-1900i, Shimadzu Scientific Instruments, Columbia, MD, USA). An extinction coefficient of 2505 for  $\beta$ -carotene was used to calculate the concentration of carotenoids in the sample using Beer's law. These steps were also used to determine the carotenoid content of all feed and substrates in the mealworm system, but for high carotenoid samples (carrot and wet and dry carrot pomace), smaller samples were used and they were diluted with hexane to 25 mL in volumetric flasks to ensure absorbance values less than 1.00. To minimize carotenoid degradation during analysis, all steps were carried out under low light conditions, and all solvents were kept on ice during analysis.

### 2.8. Analysis

For each response variable, analysis of variance (ANOVA) was performed, and a model was fitted to determine significant factors in Design Expert (Stat-Ease, version: 13.0.2.0, 2021). As this research involves a biological system with inherent variability, significance was evaluated using a standard  $p$ -value of 0.05 but allowed up to a  $p$ -value of 0.10 if the factors improved the strength of the model overall. Table 2 summarizes transformations used for each response variable. An optimum diet for mealworm larvae was proposed using the mathematical models generated to maximize mealworm larvae weight and minimize mealworm larvae mortality and days to pupation.

**Table 2.** Dependent variable transformations used for statistical analysis.

Dependent Variable	Transformation
Mortality (% initial population)	Inverse square root
Days to 5% pupation	None
Mealworm larvae weight (g)	None
Total carotenoid content ( $\mu\text{g/g}$ )	Square root
Moisture (%)	None
Ash (%)	None
Protein (%)	None
Fat (%)	None

## 3. Results

### 3.1. Feed Composition

The composition of substrate and moisture sources used to grow mealworm larvae can be found in Table 3. Looking at the substrates, they are significantly different from each other for all proximate composition measurements, but the largest differences are the moisture content, protein content, and the total carotenoids. On an as-is basis, the dried carrot pomace used in the substrate contained only half the protein content (7.95%) as the wheat bran (15.92%) but contained a high amount of total carotenoids (1109.46  $\mu\text{g/g}$ ). Dried carrot pomace had lower protein content and higher ash and carbohydrate values. All moisture sources contained high moisture values, with carrot pomace containing the most moisture content (90.36%), followed by carrot (85.24%), and potato (78.51%). All moisture sources contained low values for ash, protein, and fat. Carrot and carrot pomace differed from potato because they contained high values for total carotenoids (Table 3).

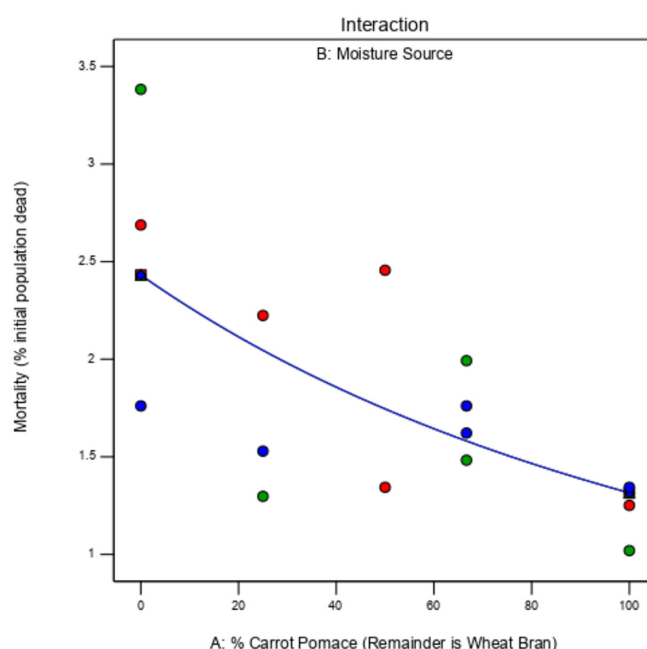
**Table 3.** Composition of substrate and moisture sources used for mealworm larvae growing (on a wet basis). Different letters within a column indicate significant differences.

Feed	Type	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrates (%)	Total Carotenoids (µg/g)
Wheat bran	Substrate	6.47 <sup>D</sup>	4.12 <sup>B</sup>	15.92 <sup>A</sup>	2.34 <sup>A</sup>	71.15 <sup>B</sup>	1.48 <sup>C</sup>
Carrot pomace (dried)	Substrate	1.63 <sup>E</sup>	6.10 <sup>A</sup>	7.95 <sup>B</sup>	1.31 <sup>B</sup>	83.01 <sup>A</sup>	1109.46 <sup>A</sup>
Potato	Moisture	78.51 <sup>C</sup>	1.18 <sup>C</sup>	2.02 <sup>C</sup>	0.03 <sup>C</sup>	18.26 <sup>C</sup>	0.49 <sup>C</sup>
Carrot	Moisture	85.24 <sup>B</sup>	1.13 <sup>C</sup>	1.16 <sup>D</sup>	0.04 <sup>C</sup>	12.43 <sup>D</sup>	127.09 <sup>B</sup>
Carrot pomace (wet)	Moisture	90.36 <sup>A</sup>	0.60 <sup>D</sup>	1.09 <sup>D</sup>	0.05 <sup>C</sup>	7.90 <sup>E</sup>	144.64 <sup>B</sup>

### 3.2. Mealworm Mortality, Growth, and Weight

#### 3.2.1. Mortality

A significant model was fit ( $p = 0.0064$ ) that found the percentage of carrot pomace in the substrate to be a significant factor ( $p = 0.0064$ ) in mealworm larvae mortality (Table 4). As the percentage of carrot pomace in the substrate increased, the mortality decreased (Figure 1).



**Figure 1.** Model predicting mealworm larvae mortality based on the percentage of carrot pomace in the substrate (remainder is wheat bran). Values in red are potato, green is carrot, and blue is carrot pomace. Since there was no significant difference ( $p > 0.05$ ) between the moisture sources, the blue line represented in the figure is the model fit representing the relationship between the mealworm larvae mortality and % carrot pomace in the substrate.

**Table 4.** ANOVA table and fit statistics for mealworm larvae mortality.

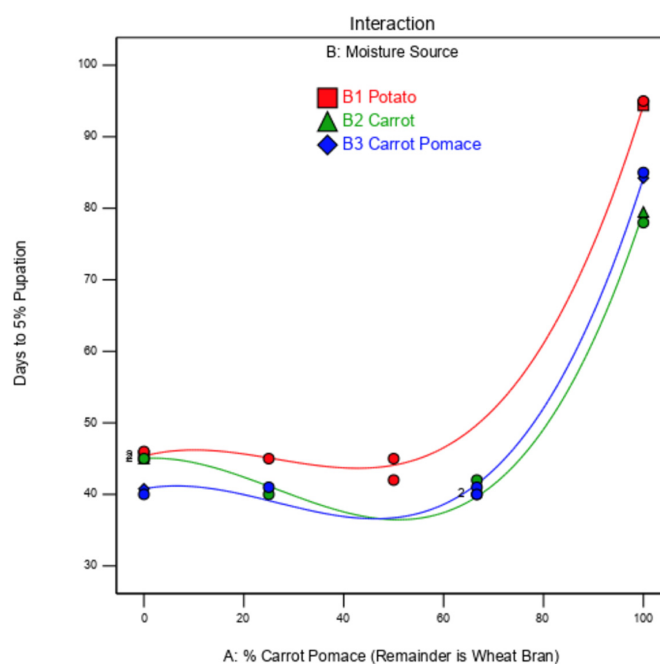
Source	Sum of Squares	df	Mean Square	F-Value	p-Value	Fit Statistics
<b>Model</b>	0.0918	1	0.0918	10.5	0.0064	<b>R<sup>2</sup></b> 0.4469
A-% Carrot Pomace (Remainder is Wheat Bran)	0.0918	1	0.0918	10.5	0.0064	<b>Adjusted R<sup>2</sup></b> 0.4044
<b>Residual</b>	0.1137	13	0.0087			<b>Predicted R<sup>2</sup></b> 0.2704
Lack of Fit	0.0816	10	0.0082	0.7633	0.6753	<b>Adeq Precision</b> 6.619
Pure Error	0.0321	3	0.0107			
<b>Cor Total</b>	0.2055	14				

### 3.2.2. Days to Pupation

A significant model was fit ( $p < 0.0001$ ) that found the interaction between the carrot pomace in the substrate and moisture source ( $p = 0.0087$ ) to significantly influence days to 5% pupation. A cubic relationship between the days to pupation and the percentage of carrot pomace in the substrate was also found to be significant ( $p < 0.0001$ ) (Table 5). Across all moisture sources, days to pupation was lowest, around 50% carrot pomace in the substrate, but increased drastically as the percentage increased to 100% carrot pomace. Across all substrate percentages, mealworm larvae that are grown on potato as the moisture source take longer to reach 5% pupation, compared to carrot and carrot pomace moisture sources. At carrot pomace substrate percentages less than 50%, carrot pomace is the preferred moisture source, while at substrate percentages greater than 50%, carrot is the preferred moisture source (Figure 2).

**Table 5.** ANOVA table and fit statistics for days to pupation.

Source	Sum of Squares	df	Mean Square	F-Value	p-Value	Fit Statistics	
<b>Model</b>	4778.14	7	682.59	218.53	<0.0001	significant	<b>R<sup>2</sup></b> 0.9954
A-% Carrot Pomace (Remainder is Wheat Bran)	2341.3	1	2341.3	749.58	<0.0001		<b>Adjusted R<sup>2</sup></b> 0.9909
B-Moisture Source	157.09	2	78.54	25.15	0.0006		<b>Predicted R<sup>2</sup></b> 0.9746
AB	62.96	2	31.48	10.08	0.0087		<b>Adeq Precision</b> 42.8089
A <sup>2</sup>	1990.36	1	1990.36	637.22	<0.0001		
A <sup>3</sup>	196.13	1	196.13	62.79	<0.0001		
<b>Residual</b>	21.86	7	3.12				
Lack of Fit	14.86	4	3.72	1.59	0.3659	not significant	
Pure Error	7	3	2.33				
<b>Cor Total</b>	4800	14					



**Figure 2.** Model predicting mealworm larvae days to pupation based on the percentage of carrot pomace in the substrate (remainder is wheat bran) and the moisture source (potato in red, carrot in green, carrot pomace in blue). The red, green, and blue lines are the independent model fits for the results of potato, carrot, and carrot pomace moisture sources, respectively.



### 3.2.3. Mealworm Weight

A significant model was fit ( $p = 0.0001$ ) that found both main effects—percentage of carrot pomace in the substrate ( $p < 0.0001$ ) and the moisture source ( $p = 0.0527$ )—as well as their interaction ( $p = 0.0592$ ), to significantly influence mealworm larvae weight. A cubic relationship between the mealworm larvae weight and the percent carrot pomace in the substrate was also found to be significant ( $p = 0.0093$ ) (Table 6). The residuals show a significant lack of fit ( $p = 0.0481$ ), and upon further investigation, the lack of fit seemed to be driven by an outlier. This outlier was investigated, but there was not enough justification to remove it from the data. Thus, the current model accepts the outlier and the lack of fit (Table 6). Across all moisture sources, mealworm larvae weight increased as the percentage of carrot pomace in the substrate increased from zero to about 20%, and then decreased as the percentage increased to 100% carrot pomace. The significant interaction means that the effect of moisture source depends on the percentage of carrot pomace in the substrate. When the percent of carrot pomace in the substrate is zero to 50%, carrot pomace is the best moisture source because it results in higher mealworm larvae weight. Carrot is the best moisture source when the percentage of carrot pomace in the substrate is greater than 50% (Figure 3). Potato as a moisture source resulted in the lowest mealworm larvae weight across all substrate percentages.

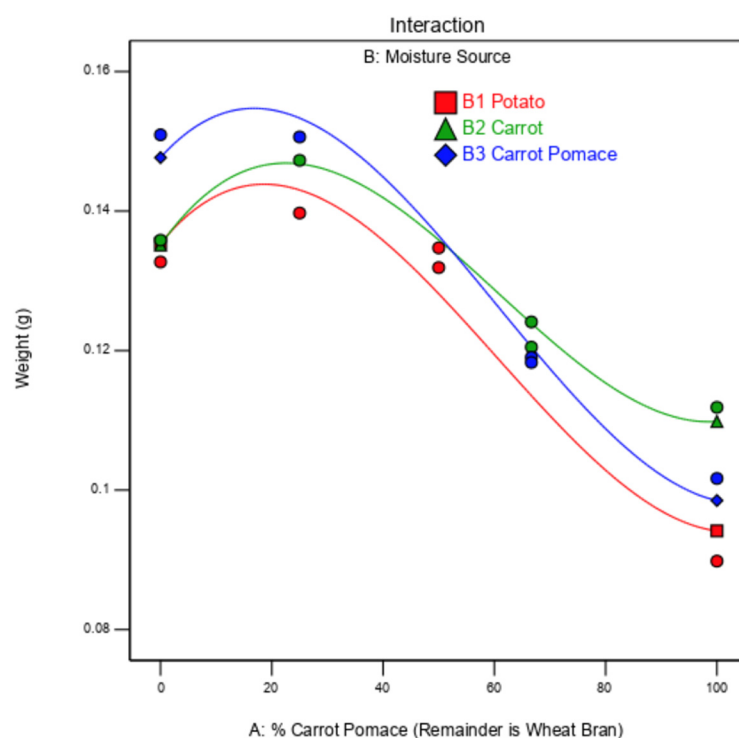
**Table 6.** ANOVA table and fit statistics for mealworm larvae weight.

Source	Sum of Squares	df	Mean Square	F-Value	p-Value	Fit Statistics	
<b>Model</b>	0.0042	7	0.0006	28.72	0.0001	significant	<b>R<sup>2</sup></b> 0.9663
A-% Carrot Pomace (Remainder is Wheat Bran)	0.0033	1	0.0033	159.51	<0.0001		<b>Adjusted R<sup>2</sup></b> 0.9327
B-Moisture Source	0.0002	2	0.0001	4.61	0.0527		<b>Predicted R<sup>2</sup></b> 0.6652
AB	0.0002	2	0.0001	4.35	0.0592		<b>Adeq Precision</b> 17.7392
A <sup>2</sup>	0.0004	1	0.0004	19.51	0.0031		
A <sup>3</sup>	0.0003	1	0.0003	12.61	0.0093		
<b>Residual</b>	0.0001	7	0				
Lack of Fit	0.0001	4	0	9.39	0.0481	significant	
Pure Error	0	3	3.6x10 <sup>-6</sup>				
<b>Cor Total</b>	0.0043	14					

### 3.3. Mealworm Composition

#### 3.3.1. Moisture Content

A significant model was fit ( $p < 0.0001$ ) that found both main effects—percentage of carrot pomace in the substrate ( $p < 0.0001$ ) and the moisture source ( $p = 0.0007$ )—to significantly influence the moisture content. A cubic relationship between the moisture content and the percentage of carrot pomace in the substrate was also found to be significant ( $p = 0.0007$ ) (Table 7). Across all moisture sources, the moisture content of mealworms increased as the percentage of carrot pomace in the substrate increased from zero to 25%, and then slightly decreased until 70%, where it increased again. Mealworm larvae fed carrot pomace or potato had higher moisture content values than those that were fed carrot, across all substrate percentages (Figure 4).



**Figure 3.** Model predicting mealworm larvae weight based on the percentage of carrot pomace in the substrate (remainder is wheat bran) and the moisture source (potato in red, carrot in green, carrot pomace in blue). The red, green, and blue lines are the independent model fits for the results of potato, carrot, and carrot pomace moisture sources, respectively.

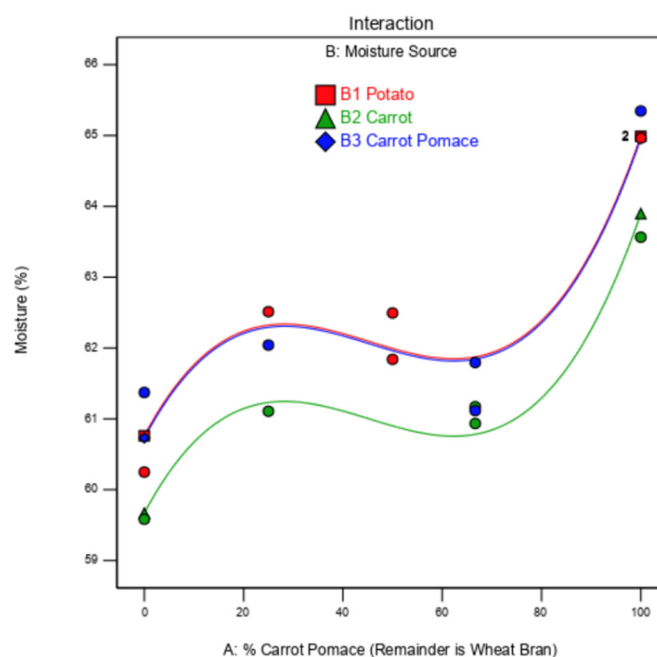
**Table 7.** ANOVA and fit statistics for mealworm moisture content.

Source	Sum of Squares	df	Mean Square	F-Value	p-Value		Fit Statistics	
<b>Model</b>	33.66	5	6.73	29.75	<0.0001	significant	<b>R<sup>2</sup></b>	0.9429
A-% Carrot Pomace (Remainder is Wheat Bran)	20.58	1	20.58	90.92	<0.0001		<b>Adjusted R<sup>2</sup></b>	0.9112
B-Moisture Source	3.8	2	1.9	8.39	0.0088		<b>Predicted R<sup>2</sup></b>	0.8374
A <sup>2</sup>	3.23	1	3.23	14.25	0.0044		<b>Adeq Precision</b>	17.6815
A <sup>3</sup>	5.81	1	5.81	25.65	0.0007			
<b>Residual</b>	2.04	9	0.2263					
Lack of Fit	1.56	6	0.2606	1.65	0.3648	not significant		
Pure Error	0.4735	3	0.1578					
<b>Cor Total</b>	35.7	14						

### 3.3.2. Protein Content

A significant model was fit ( $p < 0.0001$ ) that found the percentage of carrot pomace in the substrate ( $p < 0.0001$ ) to significantly influence the protein content (Table 8). The protein content of mealworm larvae fed wheat bran (0% carrot pomace) was just under 20% and decreased to 16.5% when the substrate was 100% carrot pomace (Figure 5).





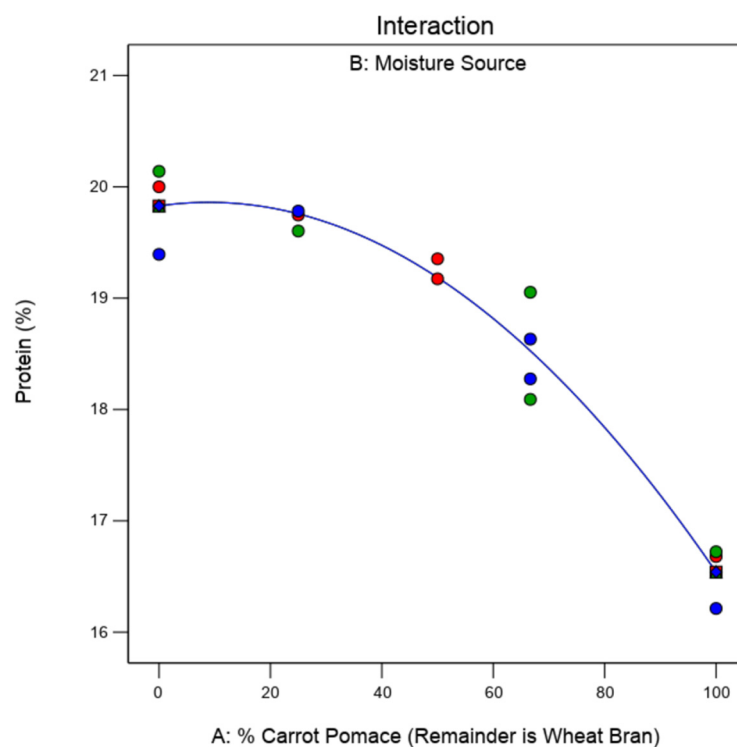
**Figure 4.** Model predicting mealworm larvae moisture content based on the percentage of carrot pomace in the substrate (remainder is wheat bran) and the moisture source (potato in red, carrot in green, carrot pomace in blue). The red, green, and blue lines are the independent model fits for the results of potato, carrot, and carrot pomace moisture sources, respectively.

**Table 8.** ANOVA table and fit statistics for mealworm larvae protein content.

Source	Sum of Squares	df	Mean Square	F-Value	p-Value		Fit Statistics	
<b>Model</b>	21.75	2	10.88	122.04	<0.0001	significant	<b>R<sup>2</sup></b>	0.9531
A-% Carrot Pomace (Remainder is Wheat Bran)	19.42	1	19.42	217.91	<0.0001		<b>Adjusted R<sup>2</sup></b>	0.9453
A <sup>2</sup>	2.79	1	2.79	31.33	0.0001		<b>Predicted R<sup>2</sup></b>	0.923
<b>Residual</b>	1.07	12	0.0891				<b>Adeq Precision</b>	24.6286
Lack of Fit	0.527	9	0.0586	0.3238	0.9174	not significant		
Pure Error	0.5425	3	0.1808					
<b>Cor Total</b>	22.82	14						

### 3.3.3. Total Carotenoid Content

A significant model was fit ( $p < 0.0001$ ) that found both main effects—percentage of carrot pomace in the substrate ( $p < 0.0001$ ) and the moisture source ( $p = 0.0221$ )—and their interaction ( $p = 0.0350$ ) to significantly influence the total carotenoid content (Table 9). As the percentage of carrot pomace in the substrate increased, so did the total carotenoid content. The effect of the moisture source depends on the percentage of carrot pomace in the substrate. While the total carotenoid content increased for all treatments as the percent of carrot pomace in the substrate increased, the increase was greatest for mealworm larvae fed carrot as the moisture source and least prominent for mealworm larvae fed carrot pomace as the moisture source (Figure 6).



**Figure 5.** Model predicting mealworm larvae protein content based on the percentage of carrot pomace in the substrate (remainder is wheat bran). Values in red are potato, green is carrot, and blue is carrot pomace. Since there was no significant difference ( $p > 0.05$ ) between the moisture sources, the blue line represented in the figure is the model fit representing the relationship between the % protein and % carrot pomace in the substrate.

**Table 9.** ANOVA table and fit statistics for mealworm larvae carotenoid content.

Source	Sum of Squares	df	Mean Square	F-Value	p-Value	significant	Fit Statistics	
<b>Model</b>	3.2	5	0.6392	42.02	<0.0001	significant	<b>R<sup>2</sup></b>	0.9589
A-% Carrot Pomace (Remainder is Wheat Bran)	2.82	1	2.82	185.52	<0.0001		<b>Adjusted R<sup>2</sup></b>	0.9361
B-Moisture Source	0.1823	2	0.0912	5.99	0.0221		<b>Predicted R<sup>2</sup></b>	0.8954
AB	0.1515	2	0.0758	4.98	0.035		<b>Adeq Precision</b>	20.6605
<b>Residual</b>	0.1369	9	0.0152					
Lack of Fit	0.0933	6	0.0155	1.07	0.52	not significant		
Pure Error	0.0436	3	0.0145					
<b>Cor Total</b>	3.33	14						

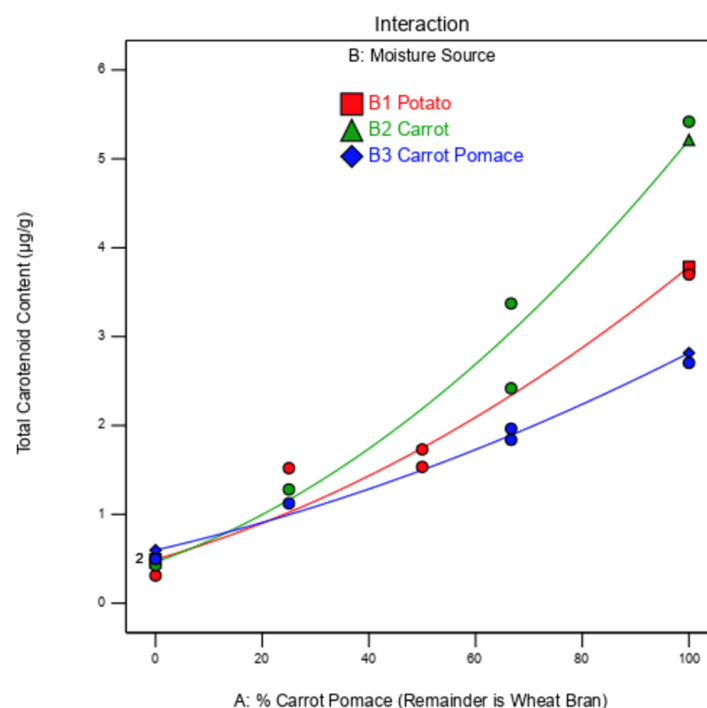
### 3.3.4. Insignificant Factors

No significant models ( $p > 0.05$ ) were found for fat content or ash content.

### 3.4. Optimum Diet

In a mealworm mass rearing operation, a desirable diet will produce as many large mealworm larvae as possible in the shortest amount of time. Thus, optimization aimed to find the diet that maximized mealworm larvae weight and minimized mortality and days to pupation. Based on these criteria, an optimal diet was selected that optimized our objectives, which consisted of 36% carrot pomace in the substrate and carrot pomace as the moisture source. With these diet parameters, Table 10 summarizes the expected growth and composition of the mealworm larvae. Mealworm larvae grown on this diet will reach

5% pupation after 37 days with an average size of 0.148 g, and 98.1% of the starting population surviving. These mealworms will have an average composition of 62.2% moisture, 19.6% protein, and contain 1.2  $\mu\text{g}$  carotenoids per gram of mealworm larvae.



**Figure 6.** Model predicting mealworm larvae total carotenoid content based on the percentage of carrot pomace in the substrate (remainder is wheat bran) and the moisture source (potato in red, carrot in green, carrot pomace in blue). The red, green, and blue lines are the independent model fits for the results of potato, carrot, and carrot pomace, respectively.

**Table 10.** Predicted growth and composition of mealworm larvae fed optimum diet—36% carrot pomace and carrot pomace moisture source.

Factor	Level
Mortality (% initial population dead)	1.91
Days to 5% pupation	37
Weight (g)	0.148
Total carotenoid content ( $\mu\text{g}/\text{g}$ )	1.20
Moisture (%)	62.24
Protein (%)	19.57

## 4. Discussion

### 4.1. Mealworm Larvae Growth, Weight, and Mortality

This study shows that including carrot pomace in the diet of mealworm larvae can positively impact the survival, days to pupation, and weight of mealworm larvae. Research has found that high-protein diets (21.9–22.9% crude protein) are beneficial in decreasing the mortality and growth time of mealworms [21]. Research has found that mealworm larvae development was quicker on high-protein diets and produced mealworms with higher pupal weight [12]. In the current study, high-protein diets were not included. However, carrot pomace—despite its low protein value—was able to improve the growth of mealworms when it supplemented wheat bran as the substrate.

In mealworms, the carotenoids present in carrot pomace may reduce oxidative stress that is known to negatively impact insect growth [22] and stimulate the immune system of the insects [23]. Dehydrated carrot pomace used in the substrate of mealworm growing

contains much less protein than the control (wheat bran) but provides high levels of carotenoids (Table 3). Carotenoids act as antioxidants by quenching singlet oxygen and scavenging peroxy radicals [24] and have been shown to have positive health benefits for humans, such as improvements to health and vision, brain cognitive function, heart health, and cancer prevention [16].

As a moisture source, carrot pomace was either insignificant or beneficial in the mealworm growing system. Compared to mealworm larvae that were fed potato, mealworm larvae fed carrot or carrot pomace as the moisture source reached maturity after fewer days (Figure 2) and had higher weight (Figure 3). Potatoes are known to contain glycoalkaloids, which have been shown to be toxic to insects from the family *tenebrionidae* [25]. However, due to low mortality values across all growth trials, and the insignificance of moisture source on mortality, it is unlikely that there was a toxic effect of glycoalkaloids from the potatoes in this study. As discussed with the carrot pomace used as the substrate, the carotenoids present in carrot products may have had beneficial effects on mealworm larvae growth. One distinct difference of carrot pomace, compared to carrot and potato moisture sources, is the particle size. Carrot and potato were added to mealworm bins as slices, while carrot pomace was commercially processed. In livestock, such as chickens, a smaller particle size of limestone was found to increase the digestibility of calcium and phosphorus [26]. In the current study, the smaller particle size of the carrot pomace moisture source may have increased digestibility. Additionally, significant interactions were observed between substrates and moisture sources—particularly carrot and carrot pomace—when looking at mealworm larvae weight and days to pupation. For these variables, carrot pomace was the preferred moisture source when the substrate contained higher percentages of wheat bran, but as the mealworm larvae growing system contained higher amounts of carrot pomace, carrot was the preferred moisture source. This may be because the carrot pomace was from the same source, and the benefits of carrot pomace as a moisture source were minimized as it was already present in high concentrations in the substrate.

#### 4.2. Mealworm Larvae Composition

The proximate composition of mealworm larvae in this study was reported on an as-is basis, but when dry matter values for protein (45.90–53.22%) and fat (26.55–32.61%) were calculated, they fell within ranges reported in the literature [27,28]. Using the models fitted in this research, there appears to be a trend in the nutritional profile of mealworms based on the composition of the feed source. As the percentage of carrot pomace in the substrate increased, the protein content of mealworm larvae decreased exponentially. The observed trend is likely because the protein content of wheat bran (15.92%) is double that of dehydrated carrot pomace (7.95%) (Table 3). While insects are able to employ regulation strategies to balance nutrient intake [29], a significant trend was still observed between the substrate and protein content. However, despite wheat bran containing two times the protein content of carrot pomace, the difference was only about 3.5% protein between the two end points. Research has found that crude protein content remained relatively consistent with different diets [12], though the substrates in that study contained much higher protein values (11.9–39.1%), compared to the current study (7.95–15.92%).

Some research has found the total fat content of mealworm larvae to remain constant, despite different fat content substrates [30], while other research found the fat content of mealworm larvae—and the fatty acid profile—to be influenced by the fat composition of the feed source [12,31]. In the current study, no significant models were found that predicted the fat content based on the feed sources. This is likely because the fat content in the substrates and moisture sources used was low and similar across all diets.

As the percentage of carrot pomace in the substrate increased, so did the total carotenoid content. This is expected based on the total carotenoid content of the substrates, as dehydrated carrot pomace as a substrate has a considerably higher total carotenoid content compared to wheat bran (Table 3). Research has found that the carotenoid content of insects, including mealworms, can be increased by supplementing the diet [32], though that

study utilized commercial supplementation in commercial insect feed. The current study suggests that carotenoid content in mealworm larvae can also be enhanced by feeding them carotenoid-rich by-products from the food industry. Interestingly, the same trend in carotenoid accumulation was not observed based on the moisture source. As the percentage of carrot pomace in the substrate increased, higher carotenoid moisture sources (carrot pomace) did not result in mealworm larvae with higher total carotenoids than potato (Figure 6). This may be because the carrot pomace used as the substrate (dehydrated) is the same carrot pomace used as the moisture source. As they are the same material, except for water content, the benefits of the moisture source are minimal. Additionally, research has found that insects are known to accumulate carotenoids [33]. Thus, because mealworm larvae that were fed potato took longer to reach maturity (Figure 2), it may have contributed to the higher carotenoid values with substrates containing high percentages of carrot pomace.

#### 4.3. Limitations and Future Work

One of the hurdles of utilizing carrot pomace as the substrate is the need to dehydrate and grind the carrot pomace prior to use. The food industry should assess the economic feasibility of different dehydration methods to efficiently process carrot pomace to be used in a mealworm growing system. While carrot pomace proved beneficial to a point, it was evident that the lack of protein content inhibited mealworm larvae growth and development when the percentage of carrot pomace became too high. Future research should (1) use an alternative carrot pomace source to verify the results, (2) assess the viability of supplementing carrot pomace with a substrate that has a higher protein content than wheat bran, and (3) explore the microbial and pathogen load involved in growing mealworm larvae using carrot pomace. Research has shown that even within a single mealworm-larvae-rearing location, the microbial load can vary between growing cycles in addition to food safety considerations [34].

This research supports the benefits of an agricultural waste product (carrot pomace) on mealworm larvae growth. Research has found that black soldier fly larvae are able to grow off almost any vegetable scrap substrate, but growth performance can be improved by tailoring the composition of scraps to optimize growth [35]. The current study suggests that carrot pomace can be used to optimize mealworm larvae growth. Future research should explore other agricultural waste products as feed sources for mealworm larvae, to help target food waste and look to optimize the growth and sustainability of mealworm larvae growing systems.

#### 5. Conclusions

Carrot pomace can be used to improve the growth of mealworm larvae and should be considered as a viable feed source for mealworm growing operations. The carotenoids in the carrot pomace are beneficial for the growth and survival of mealworm larvae to a certain extent, but the low protein content of the carrot pomace necessitates pairing with another high protein substrate. Future research should explore the growth and survival of mealworm larvae using carrot pomace and other high-protein waste streams.

**Author Contributions:** Conceptualization, D.R., S.A. and A.L.; data curation, R.L. and J.G.; formal analysis, D.R., R.L. and J.G.; funding acquisition, D.R., S.A. and A.L.; investigation, D.R. and M.O.; methodology, D.R., M.O., S.A., R.L., J.G. and A.L.; project administration, D.R. and A.L.; resources, S.A., S.K., G.S. and A.L.; software, R.L. and J.G.; supervision, D.R., S.A., S.K., G.S. and A.L.; validation, D.R. and A.L.; visualization, D.R.; writing—original draft preparation, D.R.; writing—review and editing, S.A. and A.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by the National Institute of Food and Agriculture, the National Needs Graduate Fellowship Program (Award No. 2017-38420-26767), and the California State University Agricultural Research Institute (Award No. 21-03-105). Mealworm larvae were kindly donated by Jord Producers (Lindsay, NE, USA), and carrot pomace was donated by Bolthouse Farms (Bakersfield, CA, USA).

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy and ethical restrictions.

**Conflicts of Interest:** The authors declare no conflict of interest. The sponsors (National Institute of Food and Agriculture, National Needs Graduate Fellowship Program; California Agricultural Research Institute; Jord Producers; Bolthouse Farms) had no role in the design, execution, interpretation, or writing of the study.

## References

1. Meyer-Rochow, V.B. Can insects help to ease the problem of world food shortage? *Search* **1975**, *6*, 261–262.
2. van Huis, A.; van Itterbeeck, J.; Klunder, H.; Mertens, E.; Halloran, A.; Muir, G.; Vantomme, P. *Edible Insects: Future Prospects for Food and Feed Security*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2013.
3. Zaelor, J.; Kitthawee, S. Growth response to population density in larval stage of darkling beetles (Coleoptera; Tenebrionidae) *Tenebrio molitor* and *Zophobas atratus*. *Agric. Nat. Resour.* **2018**, *52*, 603–606. [[CrossRef](#)]
4. Cortes Ortiz, J.; Ruiz, A.T.; Morales-Ramos, J.; Thomas, M.; Rojas, M.; Tomberlin, J.; Yi, L.; Han, R.; Giroud, L.; Jullien, R. Insect mass production technologies. In *Insects as Sustainable Food Ingredients*; Elsevier: Amsterdam, The Netherlands, 2016; pp. 153–201.
5. Hansen, L.L.; Ramlov, H.; Westh, P. Metabolic activity and water vapour absorption in the mealworm *Tenebrio molitor* L. (Coleoptera, Tenebrionidae): Real-time measurements by two-channel microcalorimetry. *J. Exp. Biol.* **2004**, *207*. [[CrossRef](#)]
6. Liu, C.; Masri, J.; Perez, V.; Maya, C.; Zhao, J. Growth performance and nutrient composition of mealworms (*Tenebrio molitor*) fed on fresh plant materials-supplemented diets. *Foods* **2020**, *9*, 151. [[CrossRef](#)] [[PubMed](#)]
7. Ong, S.Y.; Zainab-L, I.; Pyary, S.; Sudesh, K. A novel biological recovery approach for PHA employing selective digestion of bacterial biomass in animals. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 2117–2127. [[CrossRef](#)]
8. Yang, Y.; Yang, J.; Wu, W.; Zhao, J.; Song, Y.; Gao, L.; Yang, R.; Jiang, L. Biodegradation and mineralization of polystyrene by plastic-eating mealworms: Part 1. Chemical and physical characterization and isotopic tests. *Environ. Sci. Technol.* **2015**, *49*, 12080–12086. [[CrossRef](#)] [[PubMed](#)]
9. Yang, S.; Brandon, A.; Andrew Flanagan, J.; Yang, J.; Ning, D.; Cai, S.; Fan, H.; Wang, Z.; Ren, J.; Benbow, E.; et al. Biodegradation of polystyrene wastes in yellow mealworms (larvae of *Tenebrio molitor* Linnaeus): Factors affecting biodegradation rates and the ability of polystyrene-fed larvae to complete their life cycle. *Chemosphere* **2018**, *191*, 979–989. [[CrossRef](#)]
10. Yang, S.; Wu, W.; Brandon, A.; Fan, H.; Receveur, J.; Li, Y.; Wang, Z.; Fan, R.; McClellan, R.; Gao, S.; et al. Ubiquity of polystyrene digestion and biodegradation within yellow mealworms, larvae of *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae). *Chemosphere* **2018**, *212*, 262–271. [[CrossRef](#)]
11. Varelas, V. Food wastes as a potential new source for edible insect mass production for food and feed: A review. *Fermentation* **2019**, *5*, 81. [[CrossRef](#)]
12. van Broekhoven, S.; Oonincx, D.G.A.B.; van Huis, A.; van Loon, J.J.A. Growth performance and feed conversion efficiency of three edible mealworm species (Coleoptera: Tenebrionidae) on diets composed of organic by-products. *J. Insect Physiol.* **2015**, *73*, 1–10. [[CrossRef](#)]
13. Bao, B.; Chang, K. Carrot pulp chemical composition, colour and water-holding capacity as affected by blanching. *J. Food Sci.* **1994**, *59*, 1159–1161. [[CrossRef](#)]
14. Singh, B.; Panesar, P.; Nanda, V. Utilization of carrot pomace for the preparation of a value added product. *World J. Dairy Food Sci.* **2006**, *1*, 22–27.
15. Sharma, K.D.; Karki, S.; Thakur, N.S.; Attri, S. Chemical composition, functional properties and processing of carrot-A review. *J. Food Sci. Technol.* **2012**, *49*, 22–32. [[CrossRef](#)] [[PubMed](#)]
16. Miller, D.D.; Li, T.; Liu, R.H. Antioxidants and Phytochemicals. In *Reference Module in Biomedical Sciences*; Elsevier: Amsterdam, The Netherlands, 2014. [[CrossRef](#)]
17. Bellur Nagarajaiah, S.; Prakash, J. Nutritional composition, acceptability, and shelf stability of carrot pomace-incorporated cookies with special reference to total and  $\beta$ -carotene retention. *Cogent Food Agric.* **2015**, *1*. [[CrossRef](#)]
18. Ahmad, M.; Wani, T.A.; Wani, S.M.; Masoodi, F.A.; Gani, A. Incorporation of carrot pomace powder in wheat flour: Effect on flour, dough and cookie characteristics. *J. Food Sci. Technol.* **2016**, *53*, 3715–3724. [[CrossRef](#)] [[PubMed](#)]
19. AOAC International. *Official Methods of Analysis of AOAC International*; AOAC International: Arlington, VA, USA, 2016.
20. Lee, H.S. Characterization of Carotenoids in juice of red navel orange (Cara Cara). *J. Agric. Food Chem.* **2001**, *49*, 2563–2568. [[CrossRef](#)]
21. Oonincx, D.G.A.B.; van Broekhoven, S.; van Huis, A.; van Loon, J.J.A. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. *PLoS ONE* **2015**, *10*. [[CrossRef](#)]
22. Felton, G.W.; Summers, C.B. Antioxidant Systems in insects. *Arch. Insect Biochem. Physiol.* **1995**, *29*, 995. [[CrossRef](#)]
23. Vigneron, A.; Jehan, C.; Rigaud, T.; Moret, Y. Immune defenses of a beneficial pest: The Mealworm Beetle, *Tenebrio molitor*. *Front. Physiol.* **2019**, *10*. [[CrossRef](#)]
24. Stahl, W.; Sies, H. Antioxidant activity of carotenoids. *Mol. Asp. Med.* **2003**, *24*, 345–351. [[CrossRef](#)]
25. Nenaah, G. Individual and synergistic toxicity of solanaceous glycoalkaloids against two coleopteran stored-product insects. *J. Pest Sci.* **2011**, *84*, 77–86. [[CrossRef](#)]



26. Li, W.; Angel, R.; Plumstead, P.W.; Enting, H. Effects of limestone particle size, phytate, calcium source, and phytase on standardized ileal calcium and phosphorus digestibility in broilers. *Poult. Sci.* **2021**, *100*, 900–909. [[CrossRef](#)]
27. Rumpold, B.A.; Schlüter, O.K. Nutritional composition and safety aspects of edible insects. *Mol. Nutr. Food Res.* **2013**, *57*, 802–823. [[CrossRef](#)] [[PubMed](#)]
28. Ghosh, S.; Lee, S.M.; Jung, C.; Meyer-Rochow, V.B. Nutritional composition of five commercial edible insects in South Korea. *J. Asia-Pac. Entomol.* **2017**, *20*, 686–694. [[CrossRef](#)]
29. Behmer, S.T. *Insect Herbivore Nutrient Regulation*; Annual Review of Entomology: Palo Alto, CA, USA, 2008. [[CrossRef](#)]
30. Dreassi, E.; Cito, A.; Zanfini, A.; Materozzi, L.; Botta, M.; Francardi, V. Dietary fatty acids influence the growth and fatty acid composition of the yellow mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidae). *Lipids* **2017**, *52*, 285–294. [[CrossRef](#)]
31. Fasel, N.J.; Mene-Saffrane, L.; Ruczynski, I.; Komar, E.; Christe, P. Diet Induced Modifications of Fatty-Acid Composition in Mealworm Larvae (*Tenebrio molitor*). *J. Food Res.* **2017**, *6*, 22. [[CrossRef](#)]
32. Finke, M.D. Complete nutrient content of four species of commercially available feeder insects fed enhanced diets during growth. *Zoo Biol.* **2015**, *34*, 554–564. [[CrossRef](#)]
33. Duffey, S.S. Sequestration of Plant Natural Products by Insects. *Annu. Rev. Entomol.* **1980**, *25*. [[CrossRef](#)]
34. Vandeweyer, D.; Crauwels, S.; Lievens, B.; Van Campenhout, L. Metagenetic analysis of the bacterial communities of edible insects from diverse production cycles at industrial rearing companies. *Int. J. Food Microbiol.* **2017**, *261*, 11–18. [[CrossRef](#)]
35. Barbi, S.; Macavei, L.I.; Fuso, A.; Luparelli, A.V.; Caligiani, A.; Ferrari, A.M.; Maistrello, L.; Montorsi, M. Valorization of seasonal agri-food leftovers through insects. *Sci. Total Environ.* **2020**, *709*. [[CrossRef](#)] [[PubMed](#)]