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Proceeding Paper

Sustainable Strategies to Increase the Content of Protein, Unsaturated Fatty Acids and Vitamins in *Tenebrio molitor* Larvae Flours through Vegetable Waste Supplementation †

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Abstract: *Tenebrio molitor* larvae were fed with wheat bran and supplemented (1:1) with tomato or cucumber agricultural waste for 6 weeks. After supplementation, larvae were dried in a pilot-infrared oven (68 °C for 4 h) and ground to obtain the flours. The quality attributes and nutritional value of insect flours differed based on the supplemented diet. Unsaturated fatty acids, proteins, starches and certain vitamins were enhanced in flours from supplemented larvae. Therefore, tomato and cucumber waste can be revalorised as supplements of *T. molitors'* conventional diet to obtain insect flours with higher nutritional values and acceptable-quality attributes.

Keywords: insect farming; mealworm flour; alternative protein; linoleic acid; vitamins; quality attributes; colour; lipid oxidation; vegetable waste

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1. Introduction

A huge amount of vegetable waste is generated by agri-food industries, reaching 3–5% of annual production, which entails economic losses and high environmental impacts. Additionally, in the next few years, population growth will cause higher demand for food protein products, which is difficult to achieve if meat alternatives are not proposed. For this reason, the revalorisation of generated waste is more and more necessary to reduce the carbon footprint of this industry, obtain economic profitability and develop novel and nutritive food products for the future.

Insects are a profitable and environmentally friendly source, as they can transform low-value organic by-products into high-value food or feed [1]. Therefore, insect farming has been proposed as a potential solution for the previously explained issues since it leads to lower emissions of ammonia and greenhouse gases than conventional farms due to its greater efficiency in the conversion of feed into protein, a reduced requirement of land area and water and a lower investment in capital and equipment [2]. *Tenebrio molitor* is one of the most interesting insects to generate food and feed due to its high protein content (~50%), high-quality fat (~30%) and micronutrients such as vitamins or minerals. Furthermore, their ability to feed on any source makes them a perfect candidate for being fed with wastes. Food technologist and agri-food companies are interested in the production of these new sources of alternative proteins with the aim of developing insect meals that are suitable for animal and human nutrition while reducing environmental issues.

Vegetable wastes are an excellent source of bioactive compounds which exert biological activities when consumed, such as antioxidant, anti-inflammatory and hypoglycaemic activities, among others. Most common bioactive compounds in vegetables include phenolic compounds, terpenoids, vitamins and sulphur compounds. Therefore, the consumption of vegetable wastes by *T. molitor* larvae could contribute to improving the nutritional composition, functional value and quality of mealworm flours. The main aim of this study was to evaluate the effect of supplementing the diet of *T. molitor* larvae with cucumber and tomato wastes on the quality attributes and nutritional profile of insect flours.

2. Material and Methods

2.1. Insect Farming

Insect farming was carried out at the Insectalia S.L. company facilities. *T. molitor* larvae were fed with wheat bran (W) as a control diet or supplemented (1:1) with agricultural wastes from tomato (T) or cucumber (C) cultivation. Water was added to the control diet to compensate for the wastes' moisture. Five trays containing 100 g of 40-day larvae were used for each studied diet condition (C + W, T + W, and W). Larvae were grown in a controlled-chamber room in 12 h light–dark cycles (27 °C and 50% humidity) and fed once per week for 6 weeks. After that, they were starved for 48 h, collected and frozen at −20 °C until being processed.

2.2. Insect Processing

Frozen larvae (4 kg) corresponding to each treatment condition were blended in a Thermomix® TM6 to obtain a paste. Paste was extended in an oven tray and dried in an infrared irradiation oven at 68 °C until reaching <5% humidity (~4 h). Thereafter, the dried paste was ground to obtain the corresponding flour. All insect flours (C + W, T + W, and W) were stored at room temperature until analyses were performed.

2.3. Nutritional Value

2.3.1. Fatty Acid Profile

Fatty acid methyl esters (FAMEs) were obtained following the AOAC official method 996.06 and analysed in an Agilent 7890B gas chromatograph coupled to a 7200 quadruple-time-of-flight mass spectrometer with electron impact ionisation. FAMEs were separated in an HP-88 capillary column, using Helium as carrier gas. The oven temperature was increased from 80 °C to 145 °C (8 °C/min), maintained for 26 min, then increased to 200 °C (2 °C/min), maintained for 1 min and finally increased to 220 °C (8 °C/min). Injector and transfer line temperatures were maintained at 250 and 240 °C, respectively. Detector's instrumental conditions were ionisation source temperature, 230 °C; ionisation energy, 70 eV; mass range, 50–500 m/z; and solvent delay, 2 min. Extraction and derivatisation were performed in triplicate. Tentatively identified FAMEs were confirmed by comparison with Supelco's mix of 37 FAMEs.

2.3.2. Vitamin Content

Vitamin content was outsourced and determined by HPLC-MS.

2.3.3. Protein Content

Total protein content was outsourced and determined by Kjeldahl method.

2.3.4. Available Starch Content

Total available starch was measured in the flours following the manufacturer's instructions described in a digestible and resistant starch assay kit (Megazyme; K-DSTRS). Results are expressed as percent (w/w) of fresh weight. Extraction and determination of starch in flour were performed in duplicate.

2.4. Quality Attributes

2.4.1. Moisture

Flour (2 g) was dried in an oven (Memmert GmbH, Schwabach, Germany) at 95 °C for 4 h and the final weight was measured. Flours were evaluated in triplicate.

2.4.2. Colour

The CIELab parameters (lightness, L*; green–red chromaticity, a*; and blue–yellow chromaticity, b*) were utilised to characterise the colour of insect flours by using a colorimeter (PCE-CSM 3, PCE Instruments UK Ltd., Southampton, UK). Nine readings were taken in flour by changing the position of the colorimeter in each measurement.

2.4.3. Lipid Oxidation

Lipid oxidation of flours was evaluated through the spectrophotometric determination of 2-thiobarbituric acid-reactive substances (TBARS) using a lipid peroxidation (MDA) assay kit MAK085 (Sigma-Aldrich, St. Louis, MI, USA). Lipid oxidation was expressed as mg of malondialdehyde (MDA) per kg of flour. Extraction and determination of flours were performed in duplicate.

2.5. Statistical Analysis

Statistical analyses were carried out using the Statgraphics Centurion XVI.I software (Statgraphics Technologies Inc., The Plains, VA, USA). Results were reported as the mean ± standard deviation. Results were subjected to an analysis of variance (ANOVA) followed by Tukey post hoc test to establish statistical differences among mean values. The statistical significance level was set at $p < 0.05$.

3. Results and Discussion

3.1. Nutritional Value

Total protein content was significantly enhanced in flours obtained from larvae fed with T + W and C + W (Table 1). Similar results were reported by Ruschioni et al. [3] when supplementing diets with olive pomace (similar protein content to that in the W diet, 11–15% DW). Controversial results have been reported about the influence of diet on the larvae protein content. Oonincx et al. [4] hypothesised that high-protein diets lead to larvae with a higher protein content. Conversely, Van Broekhoven et al. [5] demonstrated that the protein content in larvae was similar despite diets that differed in their protein content by up to two and three times. In our study, the main dietary source of protein was W, with around 15% in fresh weight (21 and 14 times higher than that in C and T, respectively). The total amount of protein given to the larvae in the diet to obtain 1 kg of flour was approximately 0.87 kg (C + W), 0.92 kg (T + W) and 1.2 kg (W), which shows that larvae supplemented with the C+W diet were the most efficient in bioconverting dietary protein in their own protein. Further studies are necessary to elucidate the effects of diet composition on larvae metabolism, growth and composition. Diet influences the amino acid composition, but there is no established correlation between protein levels in diet and in larvae, although it has been suggested that protein denaturation might play a role [6].

Table 1. Effect of different larvae diets (W: wheat bran diet; C + W: cucumber-waste-supplemented diet; T + W: tomato-waste-supplemented diet) on flour total protein and total available starch contents.

Insect Flour	Total Protein Content (% *w/w* Fresh Weight)	Total Available Starch Content (% *w/w* Fresh Weight)
C + W	49.73 ± 0.19 a	1.03 ± 0.10 a
T + W	49.10 ± 0.18 b	1.09 ± 0.13 a
W	45.58 ± 0.22 c	0.39 ± 0.02 b

Different letters indicate significant ($p < 0.05$) differences among flours.

Starch content was higher in C + W and T + W flours (Table 1). As far as we know, there are no available studies in which the starch content of *T. molitor* flours after supplementation is evaluated. However, some authors have reported that larvae adapt their metabolism to the provided diet, and a high level of starch can reduce their growth [7]; this was observed in our research since the growth of larvae fed with W was lower than that of supplemented larvae. The differences are probably related to changes in larval metabolism, which is adapted to the provided nutrients, although further studies are necessary to understand such changes.

Table 2 shows the main fatty acids found in *T. molitor* flour, with linoleic acid, elaidic acid and palmitic acid being the most abundant. The percent of linoleic acid (referred to as total fatty acid content) was higher in C + W and T + W flours (1.37 and 1.33 times, respectively) than in W flour, whereas the opposite trend was observed for myristic acid. Such modification led to an increase in the percent of polyunsaturated fatty acids in the supplemented flours. Therefore, the fatty acid composition in *T. molitor* flour strongly depends on diet, as previously described [5]. High oleic and linoleic acid contents have been found in insects fed with grains. However, the lipid profile and content in larvae is more affected by the non-fibrous carbohydrate, starch and protein contents [5,8] than by polyunsaturated fatty acids in diets. Fatty acids can be synthesised de novo from carbohydrates, which are regulated by acetyl-CoA carboxylase and fatty acid synthase [9]. Therefore, some metabolic changes could be triggered by the supplementation, although further enzyme expression and activity studies are necessary to confirm this hypothesis.

Table 2. Effect of different larvae diets (W: wheat bran diet; C + W: cucumber-waste-supplemented diet; T + W: tomato-waste-supplemented diet) on flour fatty acid profile.

Insect Flours	Fatty Acids (%) *							
	Myristic Acid C14:0	Palmitic Acid C16:0	Stearic Acid C18:0	Elaidic Acid C18:1n9t	Linoleic Acid C18:2	Saturated Fatty Acids	Monounsaturated Fatty Acids	Polyunsaturated Fatty Acids
C + W	11.81 ± 0.27 b	22.33 ± 0.10 c	5.29 ± 0.06 a	22.14 ± 0.05 c	33.23 ± 0.18 a	39.70 ± 0.11 c	24.73 ± 0.05 c	34.94 ± 0.17 a
T + W	10.95 ± 0.03 c	23.71 ± 0.02 b	5.17 ± 0.02 a	23.20 ± 0.25 b	32.23 ± 0.24 b	40.08 ± 0.07 b	25.68 ± 0.24 b	33.66 ± 0.19 b
W	16.94 ± 0.03 a	24.23 ± 0.46 a	4.72 ± 0.17 b	24.28 ± 0.10 a	24.24 ± 0.08 c	46.09 ± 0.32 a	27.94 ± 0.03 a	25.29 ± 0.05 c

* Fatty acids were quantified in a relative way, expressing the percent concentration of each individual compound with respect to the total content of FAMEs identified in the samples; different letters indicate significant ($p < 0.05$) differences among fatty acid content in flours.

Table 3 shows the vitamin contents in flours obtained after feeding *T. molitor* with different diets. *T. molitor* is a good source of vitamins since it contains high levels of vitamins B2, B3, B5 and C [8]. However, the vitamin content in the diet and the applied drying conditions also influence their content in flours. All the flours had similar vitamin contents, but some differences were found in vitamins C, B3, D3, E and K. Vitamin C was higher in T + W flours, which might be explained by the higher content found in tomato waste (16 mg/100 g FW), whereas cucumber and wheat bran had <1 mg/100 g FW. Similar results after feeding *T. molitor* with *Moringa olifeira* leaves were reported by Kotsou et al. [10]. Vitamin B3 was higher in C + W and T + W flours despite the fact that W had five-fold times the content than agricultural wastes. As vitamin B3 can be synthesised from tryptophan, the higher B3 content could be related to changes in the aminoacidic profile or the biosynthesis pathway in larvae. Higher vitamin D3 and K contents were found in C + W and T + W flours, although a correlation with the content in diets cannot be stated. Regarding vitamin E, the lowest content was found in C + W flour, probably due to the lower concentration in cucumber wastes compared to tomato wastes or W (0.1, 0.2 and 1.0 mg/100 g FW, respectively). The results may be also influenced by the fat contents of flours (C + W: 24.4%; T + W: 27.2%; W: 28.2%) and the possible degradation of vitamin E due to air, temperature, light exposure and the role of this antioxidant vitamin in lipid peroxidation.

Table 3. Effect of different larvae diets (W: wheat bran diet; C + W: cucumber-waste-supplemented diet; T + W: tomato-waste-supplemented diet) on flour vitamin content.

Insect Flours	Water-Soluble Vitamins (mg/100 g Fresh Weight)						Fat-Soluble Vitamins (mg/100 g Fresh Weight)				
	C	**B1**	**B2**	**B3**	**B5**	**B6**	**A1**	**D2**	**D3**	**E**	**K**
C + W	0.32 ± 0.06 a	1.10 ± 0.22 a	1.5 ± 0.3 a	12.0 ± 2.4 a	7.1 ± 1.4 a	0.34 ± 0.07 a	0.037 ± 0.007 a	<0.05 a	0.15 ± 0.03 b	0.38 ± 0.08 a	0.008 ± 0.002 b
T + W	0.48 ± 0.08 b	1.10 ± 0.22 a	1.6 ± 0.3 a	11.0 ± 1.8 ab	6.5 ± 1.0 a	0.34 ± 0.05 a	0.034 ± 0.005 a	<0.05 a	0.15 ± 0.03 b	0.70 ± 0.14 b	0.005 ± 0.001 b
W	0.29 ± 0.1 a	0.93 ± 0.19 a	1.4 ± 0.3 a	8.0 ± 2.2 b	6.3 ± 1.3 a	0.4 ± 0.03 a	0.038 ± 0.006 a	<0.05 a	0.10 ± 0.02 a	0.59 ± 0.11 ab	0.004 ± 0.001 a

Different letters indicate significant ($p < 0.05$) differences among flours.

3.2. Quality Attributes

Table 4 shows the effect of different diets on the moisture and colour of flours obtained from *T. molitor*. Moisture was similar among flours since applied processing conditions for drying were maintained to obtain flours with <5% humidity. Regarding the colour parameters, flours obtained from larvae fed with T + W and C + W showed slightly higher L* and b* values than those fed with W. However, the a* and a*/b* ratios were lower in C + W and T + W flours. Despite the slight but significant differences detected in the colours of the flours, all of them were of acceptable quality to be used as food or feed ingredients.

Table 4. Effect of different larvae diets (W: wheat bran diet; C + W: cucumber-waste-supplemented diet; T + W: tomato-waste-supplemented diet) on flour moisture and colour parameters.

Insect Flour	Moisture (%)	L*	a*	b*	a*/b*
C + W	3.69 ± 0.17 a	37.84 ± 0.11 a	19.83 ± 1.11 c	20.13 ± 0.44 a	0.99 ± 0.08 c
T + W	3.88 ± 0.25 a	38.00 ± 0.22 ab	24.06 ± 1.33 b	18.79 ± 0.35 b	1.28 ± 0.08 b
W	3.81 ± 0.11 a	37.18 ± 0.35 b	26.23 ± 0.77 a	14.84 ± 0.5 c	1.77 ± 0.02 a

Different letters indicate significant ($p < 0.05$) differences among flours. L*: luminosity; a*: green–red chromaticity; b*: blue–yellow chromaticity; a*/b*: ratio among green–red and blue–yellow chromaticity.

C + W flour showed the highest lipid peroxidation, followed by T + W flour (Figure 1). The obtained results could be related to the fatty acid profile of flour, since the higher content in long-chain highly unsaturated fatty acids (Table 2) makes the flour more susceptible to oxidation. Additionally, vitamin E acts as an antioxidant inhibiting unsaturated fatty acid oxidation, so its lower content in C + W flour might be related to its susceptibility to lipid peroxidation. In addition, lipid oxidation can be triggered by different factors during flour processing and storage, such as heat, light or oxygen.

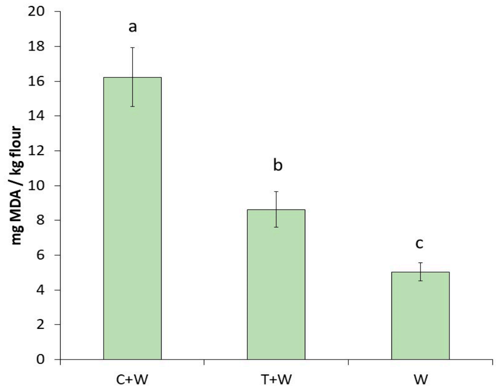

Figure 1. Effect of different larvae diets (W: wheat bran diet; C + W: cucumber-waste-supplemented diet; T + W: tomato-waste-supplemented diet) on flour lipid peroxidation (TBARs). Different letters indicate significant ($p < 0.05$) differences among flours.

4. Conclusions

Tomato and cucumber wastes have been demonstrated to be excellent supplements for obtaining *T. molitor* flours with enhanced content in vitamins, protein and unsaturated fatty acids while maintaining similar colour attributes to those obtained after conventional diets. Therefore, the revalorisation of vegetable waste through *T. molitor* bioconversion is a feasible strategy for obtaining high-added-value food products through a sustainable approach.

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