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Bioconversion of orange pomace using *Hermetia illucens* larvae: development and nutritional composition of the larvae

Bioconversão de bagaço de laranja através do uso de larvas de Hermetia illucens: desenvolvimento e composição nutricional das larvas

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ABSTRACT

Oranges, a popular fruit, generate large amounts of waste, with half discarded as pomace after juice extraction, contributing to 110–120 million tons of citrus waste annually, and posing environmental challenges, especially regarding soil and water pollution. Therefore, this study evaluates bioconversion using larvae of *Hermetia illucens* (BSFL) fed with orange pomace, analyzing the effectiveness of the process and the resulting larvae's nutritional quality. Preliminary tests showed that pure orange pomace does not provide the necessary nutritional support for BSFL development. Thus, BSFL was fed with pomace supplemented with farinaceous at different concentrations: LA25 (25% pomace, 75% farinaceous residue), LA50 (50% pomace, 50% farinaceous residue), LA75 (75% pomace, 25% farinaceous residue), and LA0 (100% farinaceous residue). Larval performance, bioconversion development, and BSFL nutritional quality were evaluated. Results showed that BSFL can effectively convert orange pomace, utilize its nutrients, and reduce its pollutant potential. Overall, using only pure orange pomace did not support larval growth. However, increasing levels of farinaceous residue altered development, bioconversion parameters, and BSFL nutritional quality ($p \leq 0.05$). It was observed that 25% of the farinaceous residue (LA75) significantly improved BSFL's overall performance ($p \leq 0.05$), also enhancing the valorization of this residue concerning all evaluated parameters.

Keywords: agroindustrial residue; larval biomass; alternative protein; BSFL; animal feed.

RESUMO

A laranja, uma fruta popular, gera grandes quantidades de resíduos, com metade descartada como bagaço após a extração do suco, contribuindo para os 110-120 milhões de toneladas de resíduos cítricos anualmente, e criando desafios ambientais, especialmente em relação à poluição do solo e da água. Sendo assim, este estudo tem por objetivo avaliar a bioconversão utilizando larvas de *Hermetia illucens* (BSFL) alimentadas com bagaço de laranja, analisando a eficácia do processo e a qualidade nutricional das larvas resultantes. Testes preliminares demonstraram que o bagaço de laranja puro não oferece o aporte nutricional necessário para o desenvolvimento da BSFL. Com isso, as dietas foram complementadas com resíduo farináceo e definidas com base em diferentes concentrações do bagaço de laranja: LA25 (25% de bagaço, 75% de resíduo farináceo), LA50 (50% de bagaço, 50% de resíduo farináceo), LA75 (75% de bagaço, 25% de resíduo farináceo) e LA0 (100% de resíduo farináceo). Foram avaliados o desempenho larval, o desenvolvimento em bioconversão e a qualidade nutricional da BSFL. Os resultados mostraram que a BSFL pode efetivamente converter bagaço de laranja, aproveitar seus nutrientes e, portanto, reduzir seu potencial poluente. De forma geral, utilizando somente o bagaço de laranja puro, não houve crescimento larval, embora níveis crescentes de inclusão do resíduo farináceo tenha alterado parâmetros de desenvolvimento e de bioconversão, bem como a qualidade nutricional da BSFL ($p \leq 0{,}05$). Assim, observou-se que a inclusão de 25% do resíduo farináceo (LA75) melhorou o desempenho global da BSFL ($p \leq 0{,}05$), propiciando também a valoração deste resíduo em relação a todos os parâmetros avaliados.

Palavras-chave: resíduo agroindustrial; biomassa larval; proteína alternativa; BSFL; alimentação animal.

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Introduction

Orange is a widely consumed fruit that generates significant agricultural waste. After orange juice extraction, approximately 50% of the fruit is discarded as pomace, known as Citrus Processing Waste (CPW) (Cypriano et al., 2017).

110 to 120 million tons of citrus waste are generated yearly from citrus processing industries, creating enormous challenges regarding soil pollution, groundwater contamination, and the overall management of wet/semi-solid waste (Mahato et al., 2020).

Currently, most of the waste is improperly processed. It can be considered an environmental problem due to its high ease of fermentation and biodegradability. Citrus waste is used as input in animal feed or dumped in landfills (dos Santos et al., 2018).

The citrus fruit processing industry faces significant adversity due to the high costs of treating the generated waste. Consequently, organizations in this sector are progressively dedicated to seeking more efficient approaches. This involves adopting sustainable technologies, optimizing processes, and exploring economically viable alternatives for treating and utilizing these wastes (Zema et al., 2018).

Several innovative methods utilizing fly larvae have been recently developed, such as the Black Soldier Fly (BSF — *Hermetia illucens*), a promising alternative in waste management (Ebeneezar et al., 2021).

BSF larvae (BSFL) can reduce 45 to 80% of the available organic waste for bioconversion in a relatively short period of 2 to 5 weeks (Terfa, 2021; Jenkins et al., 2023). The utilization of BSFL provides a fresh perspective on sustainable waste management practices, as their use may outperform the current conventional technologies, such as vermicomposting and bacterial decomposition, regarding cost-effectiveness, ecological footprints, and economic potential (Lalander et al., 2019; Singh and Kumari, 2019).

Bioconversion using BSFL is a promising environmental and technological method, as it not only reduces volumes of biodegradable organic waste, thus avoiding final disposal in landfills as aforementioned, but also mitigates greenhouse gas emissions and environmental contamination by toxic leachate (Chiam et al., 2021).

Furthermore, value-added products are generated, such as larvae (a source of animal protein), oil (cosmetics, feed, food, and fuel), and frass (organic fertilizer) (Lalander et al., 2019).

Therefore, this study assessed the feasibility of the bioconversion process using BSFL fed with orange pomace, simultaneously examining the effectiveness of this process and the resulting larvae's nutritional quality.

Material and Methods

Orange pomace and BSF larvae

The citrus pomace, composed of peel, pomace, and seeds from juice processing, was acquired from a local agro-industry. To ensure consistency in the experiments, the residue was dehydrated in a forced-air oven at 65°C for 72 hours and ground in a Willey knife mill with a 1 mm mesh. The young BSFL were obtained from a colony established in the Entomology Laboratory at *Universidade de Santa Cruz do Sul* — UNISC. This colony was maintained in a climate-controlled room at 28°C and 60% relative humidity. The neonates were fed chick starter feed until they reached six days of age. At that point, they demonstrate optimal digestive capacity and are ready to be transferred to their respective treatments.

Preliminary experiments

Preliminary trials were conducted to assess whether orange pomace served as a suitable food source for the development of BSFL. After 15 days of experimentation, it was observed that pure orange pomace (LA100) hindered larval development. Consequently, the diets employed in this study were supplemented with nutritional enrichment from a starchy residue obtained from a biofactory of biological agents (composed of 97% wholemeal flour and 3% yeast). The diets were defined based on different concentrations of orange pomace: LA25 (25% orange pomace supplemented with 75% starchy residue), LA50 (50% pomace supplemented with 50% starchy residue), LA75 (75% pomace supplemented with 25% starchy residue), and LA0 (100% starchy residue). All diets underwent nutritional analysis (crude fat, crude protein, crude fiber, and ash content) according to official methods of the Association of Official Analytical Chemists (AOAC, 2016), as explained by Komilus and Mufit (2021).

Growth and bioconversion performance

For each treatment, three experimental containers (three replicates/diet) were filled with 30 g of the different formulations (dry weight/residues), supplemented with 70% water (21 mL), and precisely 100 six-day-old larvae were introduced.

To assess the growth capacity of BSFL, 30 larvae were randomly collected every two days during the experiment, cleaned with paper towels, weighed, and returned to the diet.

Treatments were halted, and larvae were harvested when the first pre-pupae (30% of the total) were observed. The pre-pupae were then kept until the total emergence of adults.

The overall performance of bioconversion by BSFL was evaluated using the following parameters, as described by Bosch et al. (2020):

Biomass grain (g, fresh weight) $= (final\ larval\ weight - initial\ larval\ weight) x\ 100$;

Biomass yield (g, grams) $= \frac{g\ biomass\ produced}{g\ substrate\ consumed}$

Bioconversion efficiency (%) $= \frac{(lf - li)}{d} x100$

where *d* is the amount of diet provided, *lf* is the final larval weight, and *li* is the initial larval weight.

$$\textbf{Substrate reduction (\%)} = \frac{total\ residue\ added - residue\ after\ treatment}{total\ residue\ added} x100$$

$$\textbf{Bioconversion rate} = \frac{waste\ consumed}{total\ larval\ biomass}$$

Nutritional composition of BSFL

Three plastic trays measuring 12.8 cm in height × 29.0 cm in width × 40.8 cm in length (three replicates/diet) were used for each treatment. Into these trays, 3 kg of the different formulations (dry weight - residues) were added, supplemented with 70% water (2.1 liters). Then, 2 g of initial larvae (six days old) per kilogram of wet residue (±3,500 larvae) were inserted.

The experiment was terminated when 30% of the larvae reached the pre-pupal stage. Subsequently, all pre-pupae and larvae were manually harvested with a sieve, washed, dried with paper towels, and inactivated by freezing at -20°C for 1 hour. After inactivation, the larvae underwent a blanching process (40 seconds in water at 100°C) to inactivate/reduce any possible microbial load before storage.

The larvae were dehydrated in a forced-air oven at 70°C for 48 hours. After drying, they were ground in a Willey knife mill with a 1 mm mesh, and subjected to nutritional analysis (crude fat, crude protein, crude fiber, and ash content) according to the official methods of the Association of Official Analytical Chemists (AOAC, 2016), as explained by Komilus and Mufit (2021).

Data analysis

The data described in this study and the means and standard deviations are from triplicate samplings. Each parameter was analyzed using one-way ANOVA and post hoc Tukey's test to detect statistically significant differences among the samples ($p<0.05$). The statistical analysis was conducted using BioStat 5.3 — Biology Statistical Software (AnalystSoft Inc.).

Results and Discussion

The results of this study demonstrate that BSF larvae can effectively convert orange pomace, utilize its nutrients, and consequently reduce its pollution potential.

The nutritional contents are detailed in Table 1. The performance of the larvae regarding growth capacity, conversion of diet into larval body mass, and reduction of overall substrate is presented in Table 2.

Based on the development indices (Table 2), it was observed that, regarding survival rate, larval development, and adult emergence, LA25, LA50, and LA75 showed promising results, with significant differences ($p\leq0.05$) compared to LA0.

According to Table 1, the treatment with the LA0 diet had the highest concentration of protein and the lowest concentration of lipids. According to Parodi et al. (2020), a high concentration of crude protein in the larval diet increases the excretion of uric acid during the peak of larval metabolism, leading to metabolic stress, as adaptation becomes necessary to process and eliminate the excess nitrogen from the breakdown of crude protein. This metabolic stress can impair the normal development of larvae, resulting in mortality and low adult emergence.

Although not showing significant differences ($p\geq0.05$) compared to the LA25 and LA50 treatments, the LA75 treatment demonstrated an improvement in the life cycle of BSFL larvae, especially in terms of survival and adult emergence. These results are mainly related to the specific nutritional composition of this diet, as evidenced in Table 1, where a proper balance between carbohydrate and lipid levels is observed.

Maintaining this balance between carbohydrates and lipids in the larvae diet is crucial to ensure their healthy development and the nutritional quality of the larvae, as highlighted by Cammack and Tomberlin (2017).

Table 1 – Nutritional quality of the diets used in the study (g/100 g).

Bromatology	LA0	LA25	LA50	LA75
Total carbohydrates (g)	68.3	70.58	72.85	75.13
Crude fiber (g)	11.0	11.27	11.33	11.50
Crude fat (g)	1.10	1.41	1.70	2.01
Crude protein (g)	16.5	13.88	11.25	8.63

Table 2 – Development and bioconversion indices of BSFL raised on experimental diets (mean±standard deviation; n=3).

Parameters	LA0	LA25	LA50	LA75
Survival rate (%)	44 ± 1.15^{a}	68 ± 6.50^{b}	67 ± 2.51^{b}	83 ± 10.01^{b}
Development (days)	41 ± 6.65^{a}	19 ± 1.15^{b}	19 ± 1.15^{b}	21 ± 1.00^{b}
Adult emergence (%)	38 ± 2.64^{a}	65 ± 8.50^{b}	63 ± 2.0^{b}	71 ± 7.63^{b}
Biomass gain (g)	1060.1 ± 52.86^{a}	892.8 ± 51.15^{b}	848.2 ± 74.06^{b}	906 ± 74.05^{b}
Biomass yield (g)	0.40 ± 0.03^{a}	0.36 ± 0.02^{ab}	0.32 ± 0.02^{b}	0.35 ± 0.03^{ab}
Bioconversion efficiency (%)	35.33 ± 1.76^{a}	29.76 ± 1.70^{b}	28.27 ± 2.46^{b}	30.2 ± 2.46^{b}
Substrate reduction (%)	87.75 ± 2.69^{a}	83.45 ± 1.17^{a}	86.74 ± 0.8^{a}	85.29 ± 0.69^{a}
Bioconversion rate (%)	2.48 ± 0.18^{a}	2.78 ± 0.21^{ab}	3.07 ± 0.27^{b}	2.84 ± 0.24^{ab}

Mean and standard deviation (n=3). Different superscript letters in the rows denote statistically significant differences. One-way ANOVA; Tukey's test ($p<0.05$).

Concerning the other parameters (biomass gain, biomass yield, bioconversion efficiency, substrate reduction, and bioconversion rate), LA0 showed superior results compared to the others ($p \leq 0.05$); however, it should be considered that this treatment took twice the time (41 days), resulting in lower daily gains compared to the other treatments.

The average weight gain observed over time increased exponentially during the initial 12 days for LA25, and 17 days for LA50 and LA75 (Figure 1). A decline followed this increase, a pattern typically associated with healthy development. At the end of the larval cycle, during the pre-pupation stage, larvae stop feeding. This cessation of feeding is crucial for accumulating sufficient reserves to support the transition to adulthood (Dortmans et al., 2017). On the other hand, LA0 showed a development without growth peaks and a decreasing phase and with a prolonged cycle (Figure 1), indicating deficient nutritional input, as mentioned in the present discussion.

The nutritional composition of BSF larvae in the tested substrates is described in Table 3. The different treatments influenced ($p \leq 0.05$) the nutritional composition of the larvae.

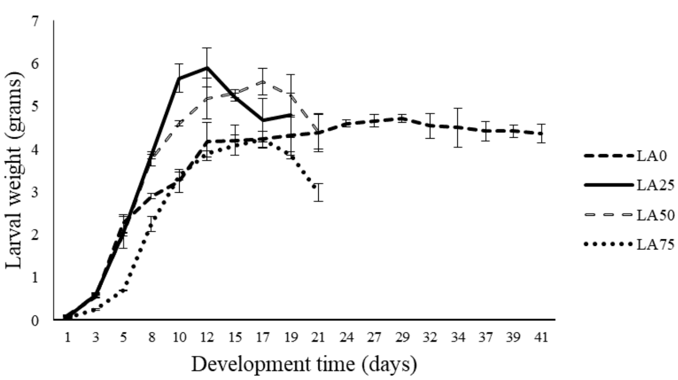

Figure 1 – Average weight gain of 30 BSF larvae cultivated in different treatments using orange pomace. Error bars represent the standard error calculated from 3 replicates/treatments.

Table 3 – Nutritional content of BSFL in different treatments (g/100 g).

Bromatology	LA0	LA25	LA50	LA75
Total carbohydrates	12.9±1.76^a	18.8±2.71^b	23.3±2,31^b	20.1±2.10^b
Mineral matter	5.0±0.05^a	5.5±0.1^b	5.6±0,1^b	6.9±0.15^c
Crude fiber	5.4±0.05^a	5.4±0.41^a	5.8±0,20^a	7.6±0.96^b
Crude fat	37.6±1.62^a	36.9±0.28^a	30.5±1,41^b	26.9±1.55^c
Crude protein	33.8±0.45^a	34.8±2.10^a	34.2±0,96^a	38.4±1.74^b
Calcium	0.66±0.01^a	0.9±0.03^b	0.85±0.11^b	1.15±0.05^b
Total phosphorus	0.67±0.01^a	0.57±0.02^b	0.56±0.01^b	0.65±0^a

Mean and standard deviation (n=3). Different superscript letters in the rows denote statistically significant differences. ANOVA method; Tukey's test ($p<0.05$).

The mineral content increased significantly ($p \leq 0.05$) in the nutritional composition of BSFL, with the growing use of orange pomace: the highest concentration was observed in LA75 (Table 3), consequently resulting in higher values for calcium and phosphorus.

This study demonstrates that BSFL converting orange pomace into larval biomass was highly effective in crude fat accumulation. According to Lu et al. (2022), fat levels vary from 29.0 to 51.3%, corroborating our findings (26.9 to 37.6%).

Furthermore, it is evident that as the inclusion of orange pomace in BSFL diets increases, there is a significant decrease ($p \leq 0.05$) in the concentration of crude fat in their nutritional composition. This is mainly observed in treatments with shorter larval life cycles, such as LA25 and LA50, where the lowest values were reached, as described in Table 2. These findings are consistent with the results of Wang and Shelomi (2017), who also identified a sharp reduction in crude fat levels in early pre-pupae. The marked decrease in crude fat levels can be explained by the accelerated life cycle of BSFL, leading to the dissociation of the fat body during pupation and a sudden increase in energy demand for metamorphosis. This process results in the reduction of crude fat concentrations in the nutritional composition of these larvae, as previously reported by Wang and Shelomi (2017).

The carbohydrate levels in BSFL are low, including part of the chitin and total fibers. These values are determined by subtracting the total (100%) from the sum of proteins, lipids, and ashes (Wu et al., 2020). This study's results for BSFL align with previous research, which reported around 11.22% (Wu et al., 2020) and 11.5% (da Cruz et al., 2022). The variation in carbohydrate levels is related to diet, breeding method, and larval stage. It is essential to point out that carbohydrates include chitin, a nutrient with several functional benefits, such as potential prebiotic, antimicrobial, antiviral, and antifungal agent. Despite often being stigmatized as an anti-nutritional factor, chitin is categorized as indigestible fiber and presents multiple benefits (Hahn et al., 2018).

It is widely recognized that fibers are difficult for most insects to degrade. However, according to studies conducted by Jeon et al. (2011) and Lee et al. (2014), the intestinal microflora of BSFL exhibits cellulase enzyme activity. This explains the efficiency of utilizing biomass rich in crude fiber. This capacity results in higher crude protein production, as evidenced in the LA75 treatment (Table 3).

Regarding the protein content, LA75 differed significantly ($p \leq 0.05$) from the others, corroborating the results presented by Cammack and Tomberlin (2017), where diets with balanced nutrients provided to BSFL present larvae with better protein quality.

The protein gain through the bioconversion process is of utmost importance, highlighting the real valorization of orange pomace. In the present study, protein content in the residues ranged from 8.63 to 16.5%, ending with larval biomass from 33.8 to 38.4% protein. We emphasize the importance of the conversion factor (6.5) adopted in the protein determination method to facilitate comparison with the literature. This value represents a widely used standard in various studies for analyzing and discussing alternative protein sources. According to the literature, it is relevant to consider that these values may be prone to overestimation due to the presence of chitin, which contains nitrogen in its composition.

BSFL demonstrates potential as a source of food protein compared to conventional products. According to Nowak et al. (2016) and Wu et al. (2020), it contains over 5 g/100 g of edible fraction based on weight (EP), and up to 10 g/100 g EP under high protein intake conditions. It is important to note that protein levels can vary depending on the feed substrate and breeding conditions, which directly influence the nutritional composition.

BSFL contains many high-quality proteins, containing almost all essential amino acids, except methionine. In addition to consumption as fresh biomass, they can be used in other areas related to food, such as flours, feeds, and supplements. It is essential to point out that one of the indicators for this is the digestibility rate, which presents rates of 86–89% according to the literature (Adámková et al., 2020).

Conclusions

Orange pomace tends to have high moisture content, making it challenging to handle and process. This excess moisture can increase processing costs and hinder storage. Its acidic nature also complicates its use in new processes.

Our study demonstrates that BSFL is effective in treating orange pomace waste. We found that the type of feed substrate strongly influences the species' development and the nutritional characteristics of the larvae. However, we can use different residues in the same diet (residue mix) to improve these parameters. This is demonstrated in our results, where adding 25% farinaceous residue to the orange pomace yielded promising outcomes regarding bioconversion, larval development, and nutritional quality.

Using BSFL in orange pomace treatment offers various environmental benefits and paves the way for sustainable development. Our findings contribute significantly to a promising future in waste management. In addition to reducing the volume of this waste (by more than 85%), we also have by-products resulting from the bioconversion process. The main one is BSF larvae, which have emerged as a promising food source for various livestock, including broiler chickens, laying hens, fish, and pigs. These larvae have excellent nutritional quality, being a source of high-quality protein, with a content of over 38% in our study.

Authors' contributions

COSTA E SILVA, D.: conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization and writing – original draft. SILVA, R.M.: formal analysis, software and writing – review & edition. KÖHLER, A.: funding acquisition, project administration, resources, supervision and writing – review & edition. VARGAS, D. P. de: funding acquisition, project administration, resources, supervision and writing – review & edition.

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