applied sciences

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Review

Current and Potential Applications of Vibrational Spectroscopy as a Tool in Black Soldier Fly Production and the Circular Economy

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Abstract: Edible insects are characterized by their low environmental footprint compared with traditional sources of animal and plant proteins. This is due to the high feed conversion efficiency of edible insects. The black soldier fly (*Hermetia illucens*) larvae (BSFL) are one of the preferred candidates to be used as alternative sources of protein, due to their ability to add value to a wide range of organic and food waste streams. The ability of BSFL to convert organic matter into protein has resulted in a viable and sustainable ingredient to be utilized in animal feed or human food. BSFL have also been considered as key components of the circular economy due to their intrinsic characteristics and properties. The evaluation of the chemical composition, nutritive value, and functional properties of BSFL have been achieved by the utilization of traditional methods of analysis, although most of these procedures do not agree with the requirements of a circular economy due to their intrinsic characteristics (e.g., destructive, energy use, highly reactive reagents, etc.). Therefore, green analytical technologies have been evaluated, of which infrared (IR) spectroscopy has several advantages. This article reviews current and potential applications of IR spectroscopy combined with chemometrics to analyze the proximate composition, functional characteristics, and traceability of BSFL and frass samples.

Keywords: black soldier fly larvae; chemometrics; circular economy; spectroscopy; composition

Citation: Alagappan, S.; Kolobaric, A.; Hoffman, L.C.; Cozzolino, D. Current and Potential Applications of Vibrational Spectroscopy as a Tool in Black Soldier Fly Production and the Circular Economy. *Appl. Sci.* **2024**, *14*, 7318. https://doi.org/10.3390/app14167318

Academic Editors: Guillermo Petzold and Mauricio Opazo-Navarrete

Received: 1 July 2024
Revised: 9 August 2024
Accepted: 13 August 2024
Published: 20 August 2024

1. Introduction

The demand for animal proteins is estimated to increase due to the rapid growth in population and underlying socio-economic conditions [1–5]. Concomitantly, feeding livestock utilizing traditional feed ingredients has become challenging because of climate change and other factors or disruptions that are affecting agri-production systems [1–5].

Developments in alternative and sustainable sources of protein can aid in the mitigation of the risks associated with climate change on traditional agriculture [6,7]. Recent years have seen an increase in research on the use of edible insects as a sustainable substitute for expensive sources of protein (e.g., soy meal, fish meal) in feed formulations and food ingredients for humans [5,8–10]. Edible insects are characterized by their low environmental footprint compared with traditional sources of animal and plant proteins, due to their high feed conversion efficiency, fast growth rate, and high fertility [1–4,11,12].

Some insect species, such as black soldier fly larvae (BSFL), are excellent in recycling organic matter from different food waste types, as reviewed in the literature [1–5,12]. The production of alternative proteins by blending high nutrient density ingredients with a low environmental footprint is of interest, as they have the potential to reduce the environmental impact of various animal (e.g., meat, dairy) and crop production systems (e.g., cereals, horticulture) along whole processing and value chains [1–5,12].

The evaluation of black soldier fly larvae (BSFL) (*Hermetia illucens*) as sustainable sources of protein has been proposed by different focused research teams across the world [1–5,12]. Overall, BSFL have been one of the preferred candidates as alternative sources of protein due to their ability to add value to a wide range of organic and food waste streams [1–5,12].

The chemical and nutritional composition of BSFL reared on different organic waste sources is comparable to what is present in several conventional animal feed ingredients and agricultural by-products; however, its composition differs depending on the organic waste material, the growing conditions, and the morphological stage of the larvae. These steps meet most of the requirements of the circular economy by transforming waste into high-quality protein as well as other nutrients (e.g., lipids and fatty acids, chitin) [13–19]. The utilization of BSFL has also provided both a technology to efficiently process organic food waste sources as well as a practical alternative to manage different types of food and organic waste where different by-products can be obtained, such as protein, fatty acids, biodiesel, chitin, and biofertilizer (e.g., frass) [20–27]. The inclusion of BSFL into organic food waste recycling systems has proven to be a cost-effective and sustainable process [20–26]. This process determines the possibility of resource recovery and generates valuable products, which consequently establishes a diverse range of economic opportunities for waste management sectors, the food industry, and entrepreneurs [25,28,29].

It is well recognized that the chemical composition and nutritive value of BSFL depends on the type of organic food waste involved. Different alternatives have been explored to increase the nutritional value of the larvae by combining different feeding substrates or even by completing some pre-processing of the waste streams as evaluated by different researchers [25,28,29]. Traditionally, the evaluation of the composition, nutritive value, and functional properties of BSFL have been achieved by the utilization of traditional methods of analysis, and most of them do not agree with the requirements of a circular economy due to their intrinsic characteristics (e.g., destructive, energy use, highly reactive reagents, chemical waste, etc.). Therefore, green analytical technologies have been proposed, of which infrared (IR) spectroscopy has several advantages [30–33]. IR techniques such as mid (MIR), near (NIR), and hyperspectral imaging spectroscopy are easy to use, require minimal or no-sample preparation, and require no chemical reagents [30–33]. These techniques can be used in the laboratory or in an industrial setting [30–33].

A review of the literature was carried out using the Web of Science where a search using the following keywords was performed: black soldier fly larvae, black soldier fly, *Hermetia illucens*, insect protein, insect composition, nutritive value, vibrational spectroscopy, NIR spectroscopy, MIR, ATR, infrared, spectroscopy, chitin, chemometrics. A total of 52 papers containing these words were selected after screening for their suitability and were used in this review. This article reviews current and potential applications of IR spectroscopy combined with chemometrics to analyze the proximate composition, functional characteristics, and traceability of BSFL.

2. Spectroscopy and Data Analytics

A wide range of analytical techniques and methods have been proposed or utilized to characterize the chemical composition, nutritive value, and other characteristics of insects, including BSFL [34,35]. Considering the principles behind the circular economy, the group of techniques based on IR spectroscopy seems to better fit as an analytical method due to properties that include being non-destructive, rapid, easy-to-use and reagent-free [30–34,36–39].

Vibrational spectroscopy encompasses different methods that utilize energy in the electromagnetic spectrum [30–34,36,37]. The infrared (IR) region is divided into the mid infrared (MIR) (400 to 4000 cm^{-1}) and near infrared (NIR) region (12,820 to 4000 cm^{-1}) [31,32]. This technique measures the combination and overtone bands of vibrations of important bonds (aliphatic C–H, aromatic or alkene groups, C–H, amine groups, N–H and O–H) that absorb in the NIR range [30–34,36–39]. The different molecules and chemical components present in a sample contribute to a characteristic position, shape, and size of the analyte's absorption bands [30–34,36–39]. MIR spectroscopy is characterized by the measurement of the fundamental absorption of molecules present in the sample [30–34,36–39]. In MIR spectroscopy, different techniques are available, including the Fourier transform (FT) IR techniques, which include sampling systems such as internal reflection spectroscopy, attenuated total reflection (ATR), diffuse reflection spectroscopy, or specular reflection, as well as the combination of internal and external reflection called diffuse reflection (DRIFT) [30–34,36–39].

The combination of the MIR or NIR spectra with chemometric methods has been utilized to determine different parameters in a wide range of samples, including insects [24,34]. Advances in miniaturization and portability has contributed to the increase in the usage of these instruments, with them being able to move from the laboratory into the field, where on-site and in-field applications have been developed and implemented in the agriculture and food industries, including insects [35,40–42]. These instruments also provide the same precision and performance as the laboratory bench instruments [40–43]. More recently, hyperspectral imaging systems using wavelengths in the VIS (400 to 750 nm) or NIR spectral regions have been utilized to analyze different types of samples, including insects [24,34].

The chemometric techniques utilized can be distinguished as targeted and untargeted depending on their ability to identify constituents, composition, or other properties, such as origin, process characteristics, and traceability monitoring [43,44]. Techniques such as principal components analysis (PCA) [45] and partial least squares (PLS) regression are usually applied to reduce dimensionality in the data set and to develop calibration models for a specific chemical constituent or property [45–48]. Other algorithms are also explored, such as support vector machines (SVM), interval PLS (iPLS), and genetic algorithms (GA) [47]. The interpretation of the models or calibrations is usually achieved by the interpretation of statistics, such as the coefficient of determination in either calibration or cross-validation (R^2), the standard error of the cross validation (SECV) or prediction (SEP), root mean square of standard error of prediction (RMSEP), and the residual predictive deviation value (RPD) [49].

The developments in instrumentation and computational power have resulted in IR spectroscopy being evaluated to predict proximate composition and functional properties, as well as functioning as a tool for traceability in the production of BSFL. The following sections discuss some of the applications reported in the scientific literature.

3. Applications of Infrared Spectroscopy in BSFL as Feed or Food

3.1. Proximate Composition

The prediction of proximate composition is where IR spectroscopy methods have been applied most often [18,19,50,51]. The ability of portable NIR instruments in the two different wavelength regions of 900–1700 nm and 1350–2562 nm, combined with PLS and SVM regression, was evaluated to predict protein and lipid content (%) in BSF larvae flour [19]. The PLS and SVM algorithms yield similar regression models for the determination of the percentage of protein, using both instruments with a residual predictive value (RPD = SD/SECV) > 2.5 and a root mean standard error of prediction (RMSEP) of 1.9%. The SVM regression model yields a better prediction for the total lipid content with an RMSEP of 3.51% and an RPD value of 4.32 [19].

A bench laboratory NIR instrument was used to predict the chemical composition and nutritive value of BSFL (fifth and sixth instars) as well as frass sampled from soy waste and customized bread and vegetable-based diets [50,51]. PLS regression yields an R^2_{cal} of 0.90 and an RPD value of 3.6 for the acid detergent fiber (ADF) and an R^2_{cal} of 0.76 as well as an RPD value of 2.1 for total carbon (TC). Moderate accuracies were observed for the prediction of crude protein (CP) (R^2_{cal} of 0.63; RPD value of 1.4), crude fat (CF) (R^2_{cal} of 0.70; RPD value of 1.6), neutral detergent fiber (NDF) (R^2_{cal} of 0.60; RPD value of 1.6), starch (R^2_{cal} of 0.52; RPD value of 1.4), and sugars (R^2_{cal} of 0.52; RPD value of 1.4) [50,51].

The evaluation of hyperspectral imaging in the NIR range in combination with PLS and SVM to predict the proximate composition of BSFL (intact and individual samples) was also reported [18]. The authors have applied a wavelength selection step using iPLS and GA algorithms. The PLS and SVM algorithms reported an RMSEP value ranging between 1.57 and 1.66% and RPD values ranging between 2.0 and 2.5 for the prediction of CP (% protein range 25.5-43.5%) [18]. The authors also showed that iPLS yielded better regression statistics than wavelengths selected using GA, as indicated by the lower error in prediction. Chemical maps based on hyperspectral images showed the non-uniform distribution of the protein in a single larva or a batch of larvae samples [18].

Short wavelengths in the near infrared (SWIR) combined with hyperspectral imaging were evaluated to predict the proximate composition of dried BSFL samples, including moisture, CP, crude fat (CF), crude fiber, and ash content [52] The BSFL samples were dried at various temperatures and times, and images were collected using a SWIR hyperspectral imaging camera. In this study, PLS regression was used to develop calibration models for moisture, CP, CF, crude fiber, and ash content with R^2 values > 0.89 and RMSEP values within 2% based on the images (data points) [52]. Table 1 summarizes the calibration statistics reported on the prediction of proximate analysis using different spectral techniques and chemometric methods.

Table 1. Calibration and cross-validation statistics reported for the measurement of proximate composition in black soldier fly larvae using different vibrational spectroscopy technologies, hyperspectral, and near infrared.

Technique	Parameter	Sample	Algorithm	R^2	RMSEP (%)	Ref.
SWIR Hyperspectral	Moisture	Data points or images	PLS	0.93–0.97	1.83–2.59	[52]
	CP			0.6–0.93 #	0.55–0.99	
	CF			0.88–0.91 #	1.34–1.67	
	Fiber			0.85–0.87 #	0.46–0.53	
	Ash			0.92–0.96 #	0.25–0.32	
Hyperspectral Imaging (1000 to 2500 nm)	CP	19 generations of larvae	PLS	0.70–0.78	1.42–1.62	[18]
			SVM	0.70–0.80	1.37–1.66	
Portable NIR [A]	CP	BSFL flour	PLS and SVM	0.74–0.86	1.18–1.36	[19]
	Lipids		PLS and SVM	0.76–0.96	3.05–7.41	

R^2: coefficient of determination in cross-validation; RMSEP: root mean square of the standard error in cross validation; CP: crude protein; CF: crude fat; PLS: partial least squares, SVM: support vector machines; # Different pre-processing methods were used by the authors. [A] Two instruments were compared by the authors of this study.

3.2. Functional and Technological Properties—Protein

BSFL have been shown to have functional and technological properties that make them ideal to include in different food systems [5,11], including as a partial substitute for meat in a beef burger patty [53]. In fact, the use of FTIR spectroscopy was reported to reveal different structural changes between flours and protein concentrates of BSFL samples [53–55]. The study of Mshayisa and collaborators (2021) showed that BSFL flour fractions and protein concentrates have great potential as novel functional ingredients for use in food applications [54].

Attenuated total reflectance (ATR) in combination with FTIR spectroscopy was evaluated as a tool to monitor changes in the composition of BSFL collected at two growing stages (fifth and sixth instar) and two waste stream diets (bread and vegetables, soy waste) [56]. The ratio between the absorbances corresponding to the amide groups at 1635 cm^{-1} and lipids between 2921 and 2849 cm^{-1} was used to analyze and classify the BSFL samples according to the type of diet (high proportion of carbohydrates (e.g., bread–vegetable mix) compared with soy waste) [56].

The chemical composition and structural properties of the BSFL flours (full fat and defatted) were assessed using ATR-FTIR spectroscopy [57]. The FTIR spectra showed that the defatting of the samples caused structural modifications, and these can be observed in changes in the absorbance values around the amide I at 1650 cm^{-1} and amide II at 1540 cm^{-1} wave numbers [57]. The analysis of the FTIR spectra showed that defatting influences specific functional groups as well as the nutritional value of the BSFL [57].

3.3. Assessing Components with Specific Functional Properties—Chitin

The characterization and determination of chitin in BSFL is one of the fields in which infrared and Raman spectroscopy have been extensively evaluated [58–63]. The different published studies showed that changes in intensity at specific wave numbers can be used to identify and characterize chitin in BSFL samples from different treatments and processes [58–63]. For example, the use of IR spectroscopy has been reported as a tool to optimize the processing steps of chitin extraction in BSFL where steps such as demineralization, deproteinization, and bleaching as well as methods to optimize the extraction of high-purity chitin from BSFL samples were evaluated [63].

A comparative study of chitin and chitosan and their broad-spectrum antimicrobial activities were evaluated using FTIR spectroscopy [62,63]. The FTIR spectra was interpreted where chitin showed characteristic chitin peaks at 1650 and 1550 cm^{-1} wave numbers, corresponding to amide I stretch and amide II bending, respectively [62,63]. Changes in these wave numbers were associated with the inhibition of the growth of some of the pathogenic bacteria evaluated [63]. The FTIR spectra of extracted chitin was also characterized, showing the specific functional groups at wave numbers associated with O-H stretch, C=O stretch, N-H bend, CH_2 bending, CH_3 deformation, C-N stretch, and C-O-C stretch [64].

3.4. Monitoring the Formation of Maillard Reactions

The utilization of FTIR spectroscopy to monitor the structural changes of BSFL proteins under different temperatures (50, 70, and 90 °C for 2–10 h at 2h intervals) was reported [54]. Differences in the amide I and amide II region were evaluated, allowing for the separation of native BSFL and BSFL-Glu conjugates by using PCA, utilizing the FTIR spectra as input [54]. The first two principal components showed a separation of samples according to the temperature [54]. Soft independent modelling of class analogy (SIMCA) was used to classify the BSFL larvae samples with an efficiency of 91% [54]. The authors concluded that FTIR spectroscopy combined with both PCA and SIMCA can be used to differentiate between native and glycated protein samples extracted from BSFL samples [54].

4. The Analysis of Molds and Yeast

Assessing the presence of bacteria and fungus is of importance to guarantee the safety of the products to feed animals [65]. The ability of NIR spectroscopy to predict yeast and mold counts (YMC) in the feed, larvae, and the residual frass was reported [65]. Predictive models were developed using PLS regression where an R^2_{CV} of 0.98 (SECV: 0.20), R^2_{CV} of 0.81 (SECV: 0.90), and R^2_{CV} of 0.91 (SECV: 0.27) for feed, frass, and larvae were reported, respectively [65]. Nonetheless, the standard error of prediction (SEP) was considered moderate (range from 0.45 to 1.03) by the authors of this study [65].

5. Process Control and Traceability

The control of the quality and safety of food ingredients and commodities are still based on the utilization of discontinuous analyses, where traditional analytical methods used in the laboratory (or sometimes as at/on-line measurements) are commonly used [66,67]. However, this approach is no longer satisfactory to fulfill the needs of a modern food or waste industry [66,67]. In particular, because of higher safety and quality standards imposed as well as high-throughput demands in the production facilities, the number of samples to be analyzed is increasing yearly [66,67]. It is in this context that IR spectroscopy and process analytical technologies (PAT) have been implemented to address these needs along the production, supply, and value chains, leading to a better understanding and control of raw materials, intermediate products in the production process, as well as the final products to be packaged and delivered [66,67]. Overall, the main objective of PAT is to reach real time analysis to better monitor a process, to avoid waste and efficiencies during the process, and to detect faults [66,67]. One of the fields in which IR spectroscopy is proven to contribute the most is during the processing of larvae and along the supply and value chain of BSFL [4,5,29,51,68]. Process analytics have become essential to increase the efficiency and sustainability of the processing. The use of IR spectroscopy can support decision management systems during the evaluation of the most suitable processing methods and control systems during the quality control of either feed or BSFL samples [18,19,52].

The benefits of traceability technologies have been identified and discussed by different authors [68–70]. Traceability is a common practice by the industry, as it is an integral part of logistics management [68–70]. Traceability systems require knowledge about the processes from different points of view, including economic, legal, technological, and social issues [68–70]. The integration of traceability with processing and logistic activities has become important for the manufacturing industries [68–70]. BSFL production does not avoid these requirements where the traceability of the production system (e.g., assuring the consistency and integrity of the organic waste utilized during the process) is considered to be of importance to implement sustainable rearing systems [68]. The ability of NIR spectroscopy combined with a classification method such as PLS-DA was used to trace the food waste used to grow BSFL [51,68]. Different BSFL (5th and 6th instar) samples collected from commercial production facilities were analyzed where the PLS-DA models were able to distinguish different larval instars. The classification models were able to predict the feed source used for rearing the fifth instar larvae (R^2 of 0.89) and sixth instar prepupae (R^2 of 0.91) [51]. Figure 1 illustrates the key steps or control points where infrared spectroscopy could play a role in black soldier fly production and the circular economy.

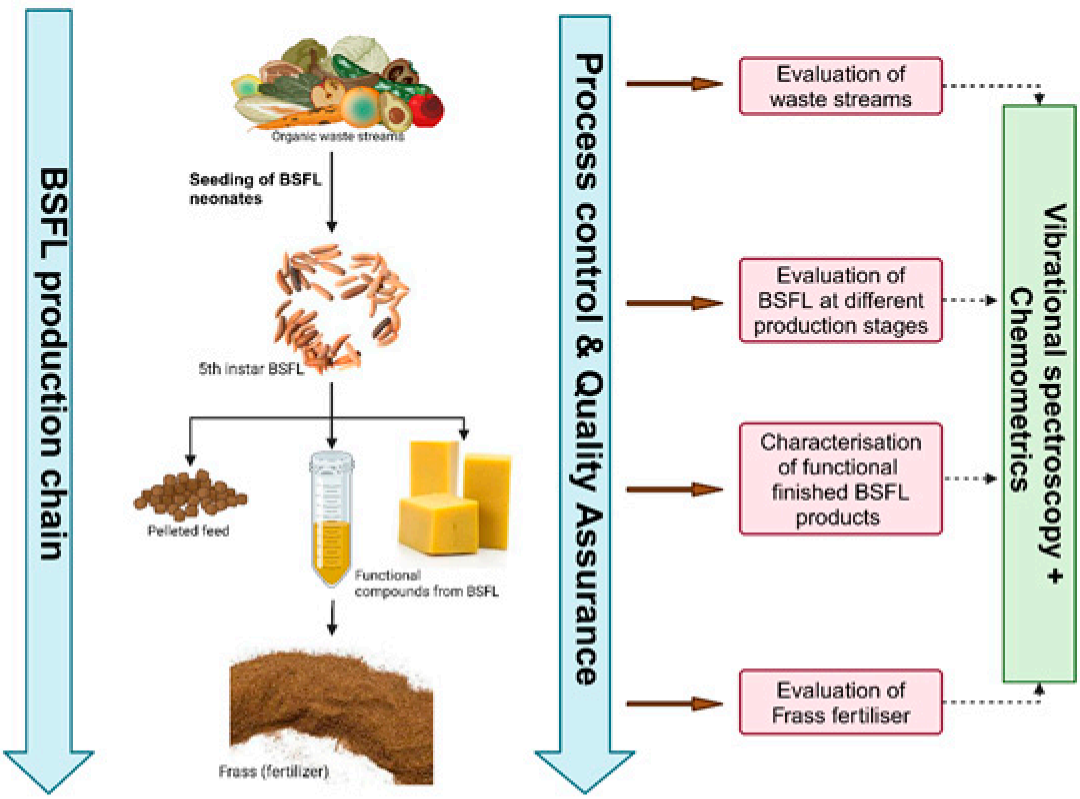

Figure 1. Schematic representation of the key steps where infrared spectroscopy techniques will play a role in black soldier fly production and the circular economy: process control and traceability.

6. Final Considerations

Several studies have shown the ability of vibrational spectroscopy (e.g., MIR, NIR, hyperspectral imaging) to predict the proximate composition of BSFL samples from research and production facilities, as well as to monitor functional and other properties in the feed. The capability of IR spectroscopy to classify BSFL samples according to their food waste origin (e.g., waste stream or diet) has proven that these techniques can be implemented to trace the production of this type of insect sample. Overall, these techniques have demonstrated that they could monitor the development and growth stages of BSFL.

The combination of IR spectroscopy with chemometrics also allowed for the detection of molds and yeast in the samples. These tools have the possibility to support risk evaluation systems as well as to provide objective measurements to identify hazards associated during the processing of BSFL. Consequently, these methods can assist in improving the safety and quality of BSFL as an ingredient intended to be used by the animal feed industry.

Despite the different reports on the use of IR spectroscopy in food safety and freshness prediction, the implementation of this technology is not fully embraced by the insect food and feed industries. Presently, traditional methods such as microbiological counts, temperature recording during pre-processing (e.g., blanching and drying), or the measurement of water activity are chosen over spectroscopy by the insect industry. So far, spectroscopy and IR are still considered "black box' techniques due to many reasons. Examples include the lack of training in the new methods, the preference of the familiar classical food safety methods over the new technologies (e.g., microbiological counts, proximate composition), and the lack of understanding of spectroscopy and chemometrics.

It is without a doubt that vibrational spectroscopy (e.g., MIR, NIR, and Raman) combined with data analytics will play an important role in BSFL production and the circular economy.

Funding: Internal University of Queensland Institutional funds.

Conflicts of Interest: The authors declare no conflict of interest.

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