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REVIEW ARTICLE

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Vibrational spectroscopy using portable devices combined with traditional machine learning: a powerful tool for insect rearing and food quality assessment

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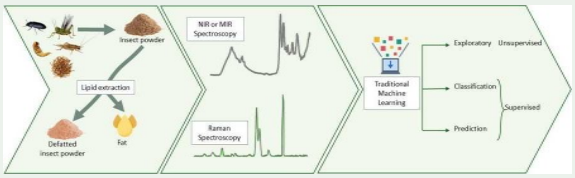
ABSTRACT

The global population will reach 10 billion by 2050, requiring a 70% increase in food and feed production. Alternative nutrient sources, such as edible insects, are being studied to meet this demand due to their high nutritional value and minimal environmental impact. Insects are rich in protein, lipids, and micronutrients, and their nutritional composition changes depending on species, feeding material, and processing methods. Insect proteins are bioavailable and contain essential amino acids, while lipids offer a balanced ratio of unsaturated and saturated fatty acids. Chitin, a major component of insect exoskeletons, is also relevant due to its biological activity. Insect production requires optimized rearing, processing, and quality control methods. Vibrational spectroscopy can be used to ensure the quality and nutritional integrity of insect-based products. Infrared and Raman spectroscopy are two key types of vibrational spectroscopy that provide valuable information about the molecular composition of insect-based products. The incorporation of vibrational spectroscopy into insect farming and production systems could streamline quality control processes, ensure food safety, and support sustainable practices. Portable and miniaturized spectroscopic devices offer a cost-effective solution for on-site monitoring, providing farmers and manufacturers with a convenient tool to maintain the authenticity, quality, and nutritional integrity of insect-based foods.



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Introduction

Entomophagy has a long history in various cultures, particularly across Asia, Africa, and Latin America (*1*, *2*). Modern research confirms the environmental advantages of insect farming, including reduced greenhouse gas emissions and high feed conversion efficiency compared to traditional livestock (*3*, *4*). Insects like *Hermetia illucens* and *Tenebrio molitor* require significantly less land and water and can be cultivated on organic by-products, making them vital components in a circular economy (*5*).

Aside from sustainability, insects contribute to human health through antioxidant, anti-inflammatory, and antihypertensive properties (*6–8*). Regulatory agencies in the EU and North America have approved several insect species for food and feed applications, with growing commercial interest in crickets, mealworms, and black soldier fly larvae (*9*, *10*).

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In this context, there is a growing need for efficient and accessible techniques to evaluate the quality and safety of insect-based products. Proximate analysis of insects typically measures protein using nitrogen-based methods like Kjeldahl or Dumas, and lipids using solvent extraction methods such as Soxhlet or Folch. While accurate, these techniques are time-consuming, labor-intensive, and require specialized equipment and trained personnel. They also involve hazardous solvents, making them less safe and impractical for on-site testing. There is a growing need for faster, safer, and more accessible methods to evaluate the quality of insects and insect-based products. Vibrational spectroscopy, particularly near-infrared (NIR) and mid-infrared (MIR), has emerged as a rapid, non-destructive alternative for food analysis (*11–13*). These methods can assess protein, lipid, and carbohydrate content, differentiate species, and detect adulterants in insect powders (*14–16*).

Recent studies demonstrate the ability of NIR and MIR spectroscopy, when combined with machine learning and chemometric models, to accurately classify insect species and quantify nutritional components, such as proteins and lipids (*17–19*). Portable and handheld spectrometers enable in situ analysis, which is particularly useful for real-time monitoring during the rearing and processing stages (*20*, *21*).

This literature review aims to investigate the use of vibrational spectroscopy as a tool for analyzing insect-based food and feed products. The review focuses on two main objectives:

(1) Evaluate NIR and MIR spectroscopy for quantifying protein and lipid content in insect powders, highlighting spectral features and model performance metrics.
(2) Analyze the viability and performance of portable spectroscopic devices for real-time, non-destructive quality control in insect production systems, including during rearing, processing, and product development stages.

By synthesizing findings from current literature, this review provides valuable information for the use of vibrational spectroscopy in insect-based product analysis.

Why are insects on the rise?

The Food and Agriculture Organization (FAO) of the United Nations estimates the global population will reach 10 billion people by 2050, requiring a 70% increase in food and feed production (*22*). Consequently, it is necessary to explore alternative nutrient sources due to the rising demand for livestock feed, land for cultivation, and water. Edible insects present significant potential due to their high nutritional value and minimal environmental impact, positioning them as a viable solution to address these food security challenges. Entomophagy has been a traditional dietary practice in numerous cultures across Asia, Latin America, and Africa for centuries (*1*, *2*). Insects offer several environmental advantages as a nutrient source. Van Huis and Oonincx (*23*) reported that insects emit comparable or lower levels of greenhouse gases and ammonia than cattle or pigs, and require less land and water compared to conventional protein sources (*4*, *23*). Oonincx et al. (*3*) found that *Locusta migratoria* produces 26% and *Tenebrio molitor* produces 36% of the CO_2 per kilogram of mass gain compared to cattle. Insects exhibit high efficiency in biotransforming a wide range of organic matter into edible body mass, resulting in a high feed conversion ratio, requiring less than 2 grams of feed to produce 1 gram of weight, compared to 8 grams for cattle. This efficiency is partly attributed to insects being ectothermic, which reduces their energy requirements for thermoregulation (*24*).

Moreover, insects can be reared on organic side-streams (bio-waste biomass from food and agriculture) and can convert them into high-value food and feed resources. Additionally, insects have rapid growth and reproduction rates, making them a prolific and sustainable protein source. For example, the life cycle of *Hermetia illucens* spans approximately 45 days, developing through four stages: egg, larva, pupa, and adult, with egg-laying commencing 2-4 days after reaching adulthood (*5*).

Beyond their nutritional and production benefits, insects have been demonstrated to offer health advantages, including antioxidant capacity, anti-inflammatory properties, and blood pressure reduction (*6–8*).

The European Food Safety Authority (EFSA) has approved six insect-based novel foods for human consumption under the European Union's Novel Foods Regulation 2015/2283 (Figure 1). These approved insect species for human consumption include *Tenebrio molitor* (yellow mealworm), *Locusta migratoria* (grasshopper), *Acheta domesticus* (house cricket) and *Alphitobius diaperinus* (lesser mealworm).

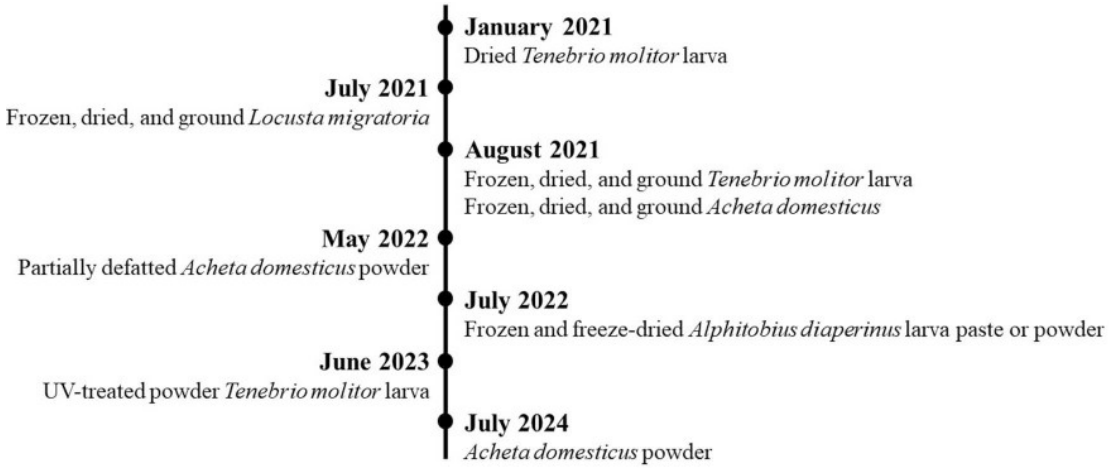

Figure 1. Insects authorized by European Food Safety Authority (EFSA) as Novel Foods.

In North America, insect production for human consumption primarily focuses on crickets and beetles. The market is largely dominated by the *A. domesticus* and G*ryllodes sigillatus* (banded cricket), with the *T. molitor* being the main beetle species sold (*9*).

Regulatory guidelines from the U. S. Food and Drug Administration (FDA) stipulate that insects sold as food must be raised specifically for human consumption in facilities adhering to good manufacturing practices. Manufacturers are also required to ensure the safety and quality of their products (*10*). Additionally, companies must meet all food safety and handling regulations, including critical control points, good manufacturing practices, hazard analysis, and risk-based preventive controls (*10*).

The rising costs and limited availability of conventional feed resources like soymeal and fishmeal for livestock and aquaculture highlight the need for alternative solutions (*25*). Insect rearing offers a promising solution. Frass, a by-product of insect farming, consists of insect excrement, feeding substrate, insect parts, dead eggs, and some dead insects. It can be used as a fertilizer, adding value to waste and promoting a circular economy. Frass enriches soil with nutrients, boosts microbiological activity, and increases plant resilience to stress factors like drought, flooding, and pathogens (*26*).

For animal feed, a broader range of insect species is accepted in various forms, including whole insects, live insects, insect proteins, insect fats, and hydrolyzed insect proteins. In Europe, the following eight insect species have been approved for use in animal feed: *Hermetia illucens* (black soldier fly), *G. sigillatus* (banded cricket), *Gryllus assimilis* (Jamaican field cricket), *A. domesticus* (house cricket), *A. diaperinus* (lesser mealworm), *T. molitor* (yellow mealworm), *Musca domestica* (housefly), *and Bombyx mori* (silkworm). These species are recognized for their potential to provide high-quality and nutritious protein in animal feed.

In Brazil, several insect species, including *H. illucens* and *T. molitor*, are approved for use in aquaculture, pig, and poultry farming without restrictions, according to Normative Instruction No. 110 (24 November 2020). Over the past decade, research into *H. illucens* larvae as animal feed has increased significantly. In the United States, the Association of American Feed Control Officials (AAFCO) mandates that *H. illucens* larvae ingredients must come from insects fed with 'feed grade material.' These larvae can only be sold if they are artificially dried and defatted through mechanical extraction. *H. illucens* larvae are now an AAFCO-certified ingredient for feeds intended for salmonoids, poultry, swine, and adult dogs (*27*). Additionally, in December 2023, AAFCO authorized the commercialization of defatted mealworm proteins (Protein70) in the United States.

Chemical composition and nutritional value

Insects are a highly diverse group of organisms, resulting in varying nutritional values depending on factors such as species, sex, life stage, diet, and environmental and breeding conditions (*2*). Additionally, their chemical composition can be influenced by the processing methods used. Despite this variability, insects

generally provide a good balance of energy and protein, with favorable amino acid and fatty acid profiles, as well as high concentrations of essential micronutrients, including minerals and vitamins (*28*, *29*).

Protein content and value

Insect protein is highly valued for its biological value and bioavailability, constituting the main component of insects in dry matter (*28*). Certain insect species contain protein levels equal to or higher than traditional animal protein sources such as beef or pork, as well as novel alternative sources like yeast, microalgae, or fungi (Table 1). The quality of insect protein is determined by its composition; for human consumption, it provides essential amino acids that the human body cannot synthesize, making up 50% to 80% of the total protein fraction (*28*, *30*). A selection of amino acid profiles is presented in Table 2. Protein content is estimated by measuring total nitrogen and applying a nitrogen-to-protein conversion factor (Kp) of 6.25. However, this factor tends to overestimate actual protein content because it does not account for non-protein nitrogen in insects. Boulos et al. (*31*) calculated a Kp factor of 5.33 based on protein content determination in seven different batches of three insect species. Janssen et al. (*32*) determined a Kp factor of 5.60 ± 0.39 after protein extraction and purification for larvae from *T. molitor*, *A. diaperinus*, and *H. illucens*.

The growing interest in insect proteins has driven research into their techno-functional and physicochemical properties, including solubility, emulsifying capacity, foaming ability, water and oil holding capacity, rheology, gelling, surface hydrophobicity, and antioxidant activity (*33–36*).

Wang et al. (*36*) demonstrated the use of *A. diaperinus* protein concentrate to stabilize double emulsions, showing stability comparable to whey protein and outperforming pea protein, a plant-based alternative protein source. Their study also evaluated the performance of *A. diaperinus* protein under environmental stress conditions (temperature, pH, and osmotic pressure), showing superior performance at high temperatures and similar stability to whey protein in both acidic and alkaline environments. Additionally, digestibility and potential health benefits have been explored. Some peptides derived from insects have demonstrated bioactivity, such as antihypertensive, antimicrobial, and antioxidant effects (*37*).

Lipid composition and fatty acid profile

Lipids are the second most abundant component in insects, comprising up to 50% on a dry basis. They serve as metabolic energy storage and structural components of cell membranes (*28*, *38*, *39*). Insect lipids can be phospholipids, free fatty acids, sterols, or partial glycerides, with triacylglycerols being the most prevalent (*40*). Insects acquire lipids through dietary intake or de novo synthesis. These lipids are stored in the fat body, a multifunctional organ crucial for metabolism and the primary site for nutrient and energy reserve storage. The β-oxidation of triacylglycerols produces nearly twice the energy of carbohydrates and does not require water, making it highly efficient for providing energy during extended nonfeeding periods, growth, and essential events such as reproduction, flight, and immune response (*41*, *42*).

Fatty acid profiles and fat content in insects are highly variable (Table 3). The ratio of saturated fatty acids (SFA) to unsaturated fatty acids (UFA) determines the melting point of lipid fractions. Most insect lipids are rich in UFA, including monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), making them liquid at room temperature, similar to vegetable oils. In contrast, some insect lipids, such as those from *H. illucens*, are abundant in SFA, solid at room temperature, and referred to as fat, akin to animal-based lipids

Table 1. Protein content of traditional and alternative protein sources (*1*, *2*).

	Protein content
Beef	17–23%
Pork	18–21%
Chicken	17–23%
Salmon	20%
Yeast	26-70%
Microalgae	40–70%
Insects (dry matter)	50–82%

Table 2. Amino acid profile of different insects extracted under several conditions. Values are represented as g/100 g of protein based on the previous results (*3–5*).

	Acheta domesticus larvae	*Tenebrio molitor* larvae	*Alphitobius diaperinus* larvae	*Hermetia illucens* larvae	*Locusta migratoria* larvae
Essential aa					
Histidine	2.1	3.6	4.0	3.9	2.0
Isoleucine	4.2	5.0	4.6	4.6	5.2
Leucine	7.3	8.3	7.3	7.5	9.0
Lysine	5.6	6.1	7.1	6.9	3.9
Methionine	1.5	1.5	1.6	2.0	2.5
Phenylanine	3.3	3.6	5.2	4.5	5.4
Threonine	3.5	4.5	4.3	4.3	3.3
Tryptophan	0.6	1.5	1.5	19	0.0
Valine	6.0	6.4	5.8	6.1	6.6
No essential aa					
Arginine	6.3	5.6	5.3	5.1	10.8
Asparagine+Aspartic acid	9.0	9.2	9.4	10.6	8.1
Glutamine+Glutamic acid	8.0	12.3	13.0	13.7	10.9
Serine	4.9	5.0	4.4	4.5	4.5
Glycine	6.0	5.0	4.2	4.9	5.3
Alanine	7.0	7.4	6.6	6.2	8.0
Cystine	2.1	1.1	0.9	0.9	0.0
Proline	6.7	7.9	6.4	5.8	6.4
Tyrosine	4.1	5.8	8.5	6.5	8.3

Abbreviations used: aa: amino acid.

Table 3. Fatty acid composition (% fatty acids/total fatty acids) of insects for food and feed.

		Tenebrio molitor	*Alphitobius diaperinus*	*Acheta domesticus*	*Gryllodes sigillatus*	*Hermetia illucens*	*Bombyx mori*
SFA	C12:0	0.00	0.00	0.00	0.00	64.83	0.00
	C14:0	3.11	0.65	1.80	0.80	9.27	0.10
	C16:0	18.52	25.18	25.99	24.61	8.89	24.2
	C18:0	2.43	8.55	6.09	10.61	1.19	4.5
	Other SFA	0.22	0.76	0.49	1.31	0.00	0.00
	Total	24.28	35.14	34.37	36.53	84.17	28.8
MUFA	C16:1n7	2.09	0.22	2.09	1.40	3.14	1.70
	C18:1n9	49.50	38.49	29.14	25.15	7.08	26.0
	Other MUFA	0.92	1.08	1.69	1.21	0.00	0.00
	Total	52.51	39.77	32.92	27.76	10.21	27.7
PUFA	C18:2n6	21.82	23.28	29.11	33.74	4.96	7.30
	C18:3n3	0.84	1.14	1.56	1.60	0.65	36.3
	Other PUFA	0.00	0.00	0.64	0.00	0.00	0.00
	Total	22.66	24.42	31.31	35.34	5.62	43.6
SFA/UFA		0.34	0.55	0.54	0.57	5.32	0.40
ratio n6/n3		25.98	20.42	13.26	21.09	7.63	0.20

(*43*). Despite their diversity, the main SFAs in insects include palmitic acid (C16:0) and stearic acid (C18:0). The primary components of UFA are palmitoleic acid (C16:1n7) and oleic acid (C18:1n9) for MUFA, and linoleic acid (C18:2n6) and α-linolenic acid (C18:3n3) for PUFA (*28*). Linoleic acid, an omega-6 fatty acid, and α-linolenic acid, an omega-3 fatty acid, are considered essential fatty acids because they cannot be synthesized by humans and must be obtained through diet (*44*). In the case of *H. illucens* fat, additional SFAs such as lauric acid (C12:0), myristic acid (C14:0), and palmitic acid (C16:0) are present.

The ratio of SFA to UFA is related not only to the solidity of the lipids at room temperature but also to health impacts. SFA intake is associated with higher cholesterol levels and coronary diseases, while PUFA intake is linked to their prevention. Moreover, the omega-6 to omega-3 fatty acids (n6/n3) ratio is another commonly used index related to human health. Studies have suggested that a high n6/n3 ratio in the diet is potentially linked to the development of health problems, including cardiovascular, inflammatory, and autoimmune diseases (*45*). Recent studies have indicated that omega-3 fatty acids in *T. molitor* larvae lipids can be enhanced by omega-3 fatty acid supplementation in the diet. Oonincx et al. (*46*) achieved a ratio below 5, recommended for human health, by enriching standard diets with 1-2% of flaxseed oil.

Functional compounds, chitin

Following proteins and lipids, fiber is a major component of insects. The most common form of fiber is chitin, which is the primary constituent of the exoskeleton. Chitin and its derivative, chitosan (produced through the deacetylation of chitin), have garnered significant economic interest due to their beneficial properties and biological activity (*47*). Finke (*48*) estimated the chitin content of various insect species to range between 11.6 and 137.2 mg/kg on a dry matter basis, indicating that insects could serve as a viable alternative source of chitin compared to the crustaceans currently utilized.

Processing methods

Insect manufacturing is essential for producing suitable products for human food, animal feed, and other uses. The process includes rearing, harvesting, pre-processing, final processing, packaging, and storage to ensure product safety and quality (Figure 2). It can be divided into primary production, which involves insect acquisition, and processing activities, which include killing and post-killing steps (*49*).

During primary production, parameters such as temperature, humidity, ventilation, light, and enclosed space must be controlled according to the needs of each insect species to prevent escapes. The substrate also varies; *H. illucens* are fed with wet substrate, while mealworms and crickets are fed with dry material. Harvesting occurs after separating the insects from their feeding substrate and frass, using practices like starvation or isolation by lowering oxygen concentration. Harvesting involves collecting larvae or adults, either manually or automatically, using sieves or nets. This process occurs when holometabolous insects reach the larval stage (e.g. mealworms and flies) and when hemimetabolous insects (e.g. crickets and grasshoppers) reach the young nymph or adult stage. Before killing, chilling is commonly performed, especially in freeze-drying cases. The killing method depends on the species; typically, mealworms are killed using hot water, boiling vapor, or freezing, while *H. illucens* are usually killed by mincing and hot water. High temperatures in this phase kill microbial flora and instantly kill the insects, reducing the risk of microbiological dangers in the final product. Alternatively, freezing at temperatures below 5°C maintains the nutritional values for further use (*49*, *50*).

Several post-killing processing methods can be applied to insects, including thermal, chemical, or mechanical techniques. These methods serve various purposes, such as decontamination, water removal, or fractionation. Extraction methods can be classified into wet or dry pathways based on when the drying step is performed. In the wet mode, moisture is removed in the last step after separating the final product (*51*). Conversely, the dry method involves drying the whole insect before processing or fractionation (*52*). Techniques such as freeze-drying, heat-based dehydration, microwaves, or sun drying are used to remove water and prevent microbiological proliferation.

The final insect product can be whole insects in various forms, such as dried, frozen, or chilled, or a homogeneous powder or paste achieved through grinding or milling. It is well documented that several fractions, including protein, lipids, chitin, and derivatives, can be obtained (*49*, *50*). The first step in fractionation is defatting, which can be achieved through various methods such as mechanical pressing, organic solvent extraction (Soxhlet and Folch), aqueous extraction, supercritical CO_2, or triple partitioning (*44*). The choice of lipid extraction method affects the yield, quality, and composition of the extract. Defatting the insect

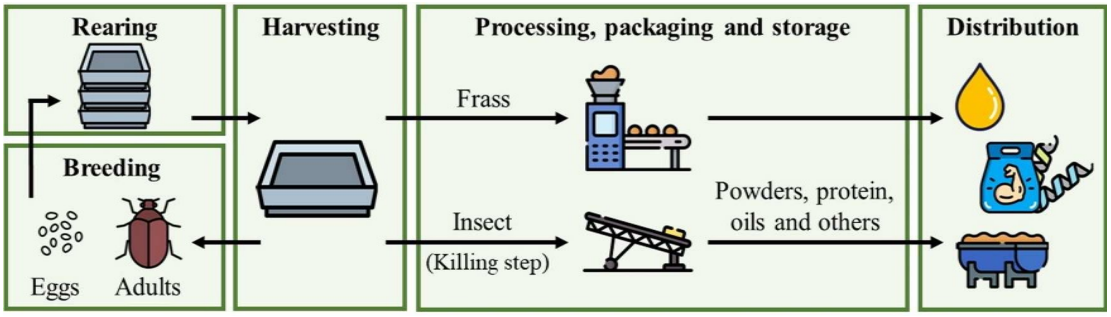

Figure 2. Insect production activities. Adapted from IPIFF (*1*).

powder is necessary for protein extraction and improves the manufacture of the powder by reducing its stickiness, facilitating storage, and extending product shelf life (*53*).

In the protein extraction step, common methods include enzymatic extraction, pH shift, and ionic strength. The yield can be affected by previous pretreatment, and protein conformation can be modified by high temperatures. The final extract is chitin, which can be obtained after defatting, deproteinization, and demineralization with hydrogen chloride and high temperature. Chitin and its derivative, chitosan, are of interest due to their significant biological activity, prompting investigations into alternatives to chemical demineralization (*54*). While the aforementioned methods are the most used, emerging techniques are being studied to improve process efficiency, environmental friendliness, and cost-effectiveness. Examples include high hydrostatic pressures, pulsed electric fields, ultrasound, and cold atmospheric pressure plasma (*50*).

All parameters and conditions throughout the entire process impact the physicochemical properties, proximate composition, color, and taste, microbial load, and shelf life of the product (*50*–*52*, *55*).

Insect lipids as food, feed, and use in industry

Insect studies primarily focus on protein extraction; however, lipids are also produced during protein isolation. These lipids have potential applications across various industries, making them valuable products rather than mere by-products. One of the main objectives is to integrate insects into the diet as a source of nutrients and energy. Despite the benefits of consuming insects, consumer acceptance remains one of the largest barriers to the adoption of this novel food, a phenomenon known as neophobia (*56*). Many consumers perceive insects as dirty, disgusting, and dangerous (*57*). Research suggests that presenting insects as an ingredient in convenience foods increases the likelihood of acceptance, as the initial barrier of rejection is more easily overcome (*58*, *59*). Additionally, effective communication and information dissemination can help society accept entomophagy as a positive practice (*60*).

Lipids play a crucial role in the physical, chemical, and sensory characteristics of foods, further highlighting their importance in food applications. They are suitable replacements for vegetable oils or animal fats as ingredients. Smetana et al. (*61*) formulated margarine by substituting the hard fraction and liquid oil fraction with *H. illucens* fats and *T. molitor* oil, respectively. The resultant margarine, formulated with 75% insect lipids, exhibited a reduced environmental impact compared to butter, while retaining comparable spreading properties to plant-based margarine and improving the yellowish coloration of the margarine. Delicato et al. (*62*) incorporated fat from *H. illucens* larvae as a replacement for butter in bakery products. They observed that substituting 25% with insect fat did not affect consumer acceptance, and they were even able to substitute 50% in waffle production. Additionally, texture and color were hardly affected, indicating that *H. illucens* fat has similar properties to butter. Cheseto et al. (*63*) analyzed other insect species in bakery products, replacing olive and sesame oils with lipids from two commonly consumed African grasshoppers (*Schistocerca gregaria* and *Ruspolia differens*). The study demonstrated that while color was not greatly affected, taste was influenced by insect oils.

Crude insect fats and oils have aftertastes, making it necessary to refine them to improve their organoleptic properties and reduce off-flavors (*64*). To this end, Tzompa-Sosa et al. (*65*) investigated the sensory perception of replacing vegetable oil with crude and deodorized *T. molitor* oil in crackers and hummus formulations, both fresh and baked. They concluded that a high percentage of vegetable oil could be substituted with deodorized *T. molitor* oil, but not crude oil, which impacts sensory perception. Tzompa-Sosa et al. (*66*) also observed the impact on sensory profiling of using crude and deodorized yellow mealworm oil as frying oil. The study showed that deodorized *T. molitor* oil is a viable alternative to vegetable oil for frying.

Insect lipids have not yet been accepted for human consumption in the EU but are allowed for feed applications. They can fully address all feed markets, including aquaculture, pet food, poultry, pigs, and other animal nutrition (*25*). *T. molitor* oil was evaluated as a replacement for palm oil and poultry fat in broiler chicken diets. Benzertiha et al. (*67*) observed an improvement in the fatty acid profiles of muscle tissues due to the increased n-3 and n-6 fatty acids. Furthermore, no adverse effects were observed on chicken performance, nutrient digestibility, and blood parameters.

Due to their variable composition, insect lipids have potential applications in various industries, including cosmetics, energy, and industrial sectors (*68*). Verheyen et al. (*69*) evaluated lipids from *H. illucens*, *L.*

migratoria, and *A. domesticus* for potential use in cosmetics. They found that lipids from *L. migratoria* and *A. domesticus* were suitable for skincare products, while lipids from *H. illucens*, which are rich in lauric acid, were more appropriate for soaps and shower gels.

Insect lipids also have the potential for biodiesel production. The palmitic and stearic acids present in large amounts in insects have high calorific value and good viscosity for biodiesel production. Long-chain SFA tend to produce biodiesel with poor cold flow properties, while polyunsaturated fatty acids PUFA have been found to have poor oxidative stability. Notably, *H. illucens* is rich in medium-chain SFA, such as lauric acid, myristic acid, and palmitic acid, making its lipids potentially ideal substrates for producing high-quality biodiesel (*70*, *71*).

Novel analytical methods

Vibrational spectroscopy

The demand for new, cost-effective, accessible, and rapid techniques to study food and feed products is greater than ever. Traditional analytical methods, while accurate, are often time-consuming, expensive, and require complex procedures, limiting their feasibility for small-scale producers or *in situ* analysis. In this context, vibrational spectroscopy has emerged as a promising alternative. With advancements in miniaturized and portable equipment, vibrational spectroscopy allows for real-time monitoring and quality control with minimal sample preparation. This technology is increasingly recognized for enhancing analytical efficiency and accessibility, making it a developing area of modern analytical chemistry (*11*–*13*, *68*, *72*).

Vibrational spectroscopy includes several techniques that analyze molecular vibrations, including Raman and infrared (IR) spectroscopy. These methods provide valuable insights into molecular structures and compositions, making them widely applicable across various disciplines. Fingerprinting capability of IR spectroscopy is one of the most valued factors, as is its potential for both qualitative and quantitative applications when combined with advanced pattern recognition techniques (*11*–*13*, *72*).

Near-infrared (NIR), mid-infrared (MIR), Raman spectroscopy, and hyperspectral imaging (HSI), have proven to be highly effective and widely used as rapid and sensitive methods for evaluating agro-food products (*73*, *74*).

Infrared spectroscopy

The IR spectroscopy studies the interaction between matter and electromagnetic radiation within the IR range of the electromagnetic spectrum, spanning from 10 to 14,300 cm^{-1}, positioned between visible light and microwaves. This spectral range is subdivided into three regions: near infrared (NIR), mid-infrared (MIR), and far infrared (FIR), in the intervals of 14,300-4000 cm^{-1}, 4000-400 $cm^{-1,}$ and 400-10 cm^{-1}, respectively. Each region offers unique insights into molecular vibrations and provides valuable information for analytical purposes (*75*).

IR radiation is absorbed, either wholly or partially, by the chemical bonds within the sample, altering their energy states. Each frequency of infrared radiation carries a specific energy, allowing frequencies to be associated with a molecular bond. The bonds are not static; they experience various types of vibrations: stretching (symmetric and asymmetric) and bending (scissoring, rocking, twisting, wagging) (*76*, *77*).

The MIR region can be divided based on the types of bonds present. These include the functional groups region, which includes the single bonds region (4000–2500 cm^{-1}), the triple bonds region (2500–2000cm^{-1}), and the double bonds region (2000–1500 cm^{-1}); and the fingerprint region, located between 1500 and 650 cm^{-1}. This region contains a complex series of absorptions, producing a unique spectral pattern for the sample. The fingerprint region is highly specific to each molecule, making it useful for structural identification and comparison of different substances (*78*, *79*). MIR has less penetrative power due to the longer wavelengths used, which means that the information gathered primarily pertains to the sample surface. In contrast, NIR penetrates deeper into the sample, resulting in a more representative analysis. The NIR region is divided into two sub-regions: short-wave NIR (SW-NIR, 14,300 to 9,000 cm^{-1}), and long-wave NIR (LW-NIR, 9,000 to 4,000 cm^{-1}). SW-NIR includes 3rd and 4th overtones; radiation in this range offers stronger penetration and lower heating effects (*80*–*84*). LW-NIR contains bands from the 1st and 2nd overtones, as well as combination bands, but it exhibits poorer penetration into samples.

The NIR spectra, compared to MIR, exhibit less selectivity due to the nature of the bands in that region. Overall, it is feasible to determine whether the composition of two samples is the same by comparing their infrared spectra. Minor differences in the vibrational frequency of the identical bond between molecules may occur because the identities and locations of other molecules can slightly modify the wavenumber of a chemical bond (*85*).

A spectrum also contains quantitative information. According to Lambert-Beer's law, the intensity of the IR bands is correlated with the concentration of the molecule (*86*). The level of absorbance is directly proportional to the number of bonds irradiated, or equivalently, to the concentration of a functional group. However, IR spectroscopy responds specifically to chemical bonds, meaning that monoatomic atoms and ions do not absorb IR radiation. The intensity of the absorption is proportional to the dipole moment of the molecule; hence, vibrations in homonuclear diatomic molecules, which lack a dipole moment, are not active in the IR spectrum (*75*). This limitation means that homonuclear diatomic molecules, such as N_2 and O_2, cannot be detected using IR spectroscopy. Additionally, water exhibits very broad absorption bands, complicating the analysis of aqueous samples and complex mixtures due to overlapping signals. Despite challenges, IR spectroscopy stands out because of its ability to analyze samples in any state of aggregation (solid, liquid, or gas) with minimal or no sample preparation in just a few seconds. This versatility makes IR spectroscopy a valuable tool for rapid analysis in both scientific research and industrial settings.

In Fourier Transform (FT) IR spectroscopy (*87*, *88*), the incoming light beam is directed through an interferometer, where its spectral information is encoded by varying the optical path lengths. As the beams recombine, their interference, both constructive and destructive, produces an interferogram that contains the encoded spectral data. The interferogram is then sent to the sample compartment, where the sample's molecular structure absorbs specific energy frequencies. Following absorption, the detector records an interferogram that represents energy distribution over time for all frequencies, with a reference beam serving as a background measurement. A Fourier transformation is subsequently applied to mathematically isolate the sample spectrum from the background, producing the final IR spectrum (*89*).

Raman spectroscopy

Raman spectroscopy is an analytical technique that, like IR spectroscopy, provides information about molecular vibrations and chemical structures of a sample. It is based on the scattering of monochromatic light, typically from a laser. When a laser beam interacts with a molecule, two types of scattering can occur: elastic (Rayleigh) scattering and inelastic (Raman) scattering (*90*).

During inelastic scattering, the frequency of the re-emitted photons shifts relative to the original monochromatic frequency. This frequency difference, known as the Raman shift, is related to the vibrational and rotational states of the molecules, providing chemical structural information (*90*). If the scattered light exhibits a lower frequency than the incident light, the molecule absorbs energy and moves to a higher vibrational level, a phenomenon known as Stokes Raman scattering. If the scattered light shows an increased frequency (and shorter wavelength), the molecule loses energy and transitions to a lower vibrational level, resulting in anti-Stokes Raman scattering.

A Raman spectrum typically displays the intensity of scattered light as a function of the Raman shift, where each band corresponds to a specific vibrational mode of the molecules in the sample (*76*). The intensity of Raman bands is proportional to the concentration of scattering molecules, providing quantitative information about the sample composition.

Hyperspectral imaging

Hyperspectral imaging (HSI) is an analytical method that integrates imaging technology with spectroscopy, enabling the acquisition of both spatial and spectral data from a given object (*91*). HSI can collect data across various regions of the electromagnetic spectrum, including ultraviolet (50,000–25,000 cm^{-1}), visible (26,320–12,500 cm^{-1}), near-infrared (NIR, 14,300-4000 cm^{-1}), and short-wave infrared (4000–10,310 cm^{-1}). HSI offers flexibility in terms of the spectral region collected, spatial resolution, and field of view (FOV). This adaptability allows it to be used for a wide range of applications, making it suitable for different types of samples and analytical needs. The process of hyperspectral image analysis involves several steps (*92*). The process begins with the segmentation of a calibrated hyperspectral image, followed by the extraction of spectra.

The final step involves the selection of optimal wavelengths using weighting coefficients. The resulting data is then processed to create a spectral image that can be analyzed for various properties.

Instrumentation

In IR and Raman spectroscopy, the sample is irradiated with light. Most of the light passes through the sample, causing specific molecular bonds to vibrate and absorb some energy. The reflected or transmitted light is then collected by a detector, which generates an electronic signal proportional to the light's intensity. This signal is processed to produce a corresponding electronic output, which is then converted into the spectrum of the sample. Vibrational spectroscopy devices consist of a light source, a wavelength selector, and a detector (*93*).

The integration of FT with NIR, MIR, and Raman spectroscopy enhances the analytical capabilities by improving resolution and sensitivity and allowing faster data acquisition. FT-NIR is particularly effective for analyzing overtone and combination vibrations of molecular bonds, making it valuable for determining the composition and concentration of organic compounds in the pharmaceutical and food industries. FT-MIR provides detailed spectral information on molecular vibrations, facilitating functional group identification and chemical characterization. FT-Raman spectroscopy improves signal-to-noise ratio, making it particularly useful for analyzing samples that are difficult to examine using conventional Raman methods, such as those that exhibit fluorescence or have low scattering efficiency. These FT-based techniques enable real-time monitoring and advanced analysis across various fields.

Trends in analytical chemistry are moving towards simple and less time-consuming analytical methods, leading to the development of portable and handheld spectrometers. These devices offer flexibility for *in situ* measurements, enabling real-time data acquisition (*94*, *95*). IR spectroscopy is particularly suited for miniaturization due to its simplicity, speed, selectivity, and minimal sample preparation requirements. In recent years, portable MIR and NIR spectrometers have become commercially available, serving a wide range of analytical applications. Portable devices are typically classified into suitcase-format devices (>4 kg) and handheld devices (<1 kg). While NIR spectrometers have been successfully miniaturized to palm-sized formats, the miniaturization of MIR spectrometers faces challenges due to moving parts and quantum-type pyroelectric detectors that require cooling to reduce thermal noise (*93*, *96*, *97*). These portable devices can be operated by non-expert users, function in harsh conditions outside of controlled laboratory environments, and provide outputs that may be a value or an identification rather than a spectrum (*94*).

The development of portable and handheld spectrometers has been enabled by advances in semiconductor and photonic technology, coupled with software development for ease of use by non-expert users, communication technologies (Bluetooth, Wi-Fi), battery power, chemometrics, algorithms, and cloud integration (*85*). Despite the significant reduction in size and weight, infrared spectroscopy capabilities have led to successful qualitative and quantitative calibration results for a broad range of applications (*97*). Due to miniaturization and lower costs, portable and handheld NIR spectrometers have become the most commercially developed (Table 4). However, portable FT-MIR devices, incorporating Michelson interferometers, are also commercially available (Table 5) (*98*).

Strategies to reduce the size of spectrometers primarily focus on light-splitting and detecting components. Key wavelength selection techniques include the Hadamard mask, Fabry-Pérot filter, micro opto electro-mechanical systems (MOEMS), linear variable filters (LVF), and digital micromirror devices (DMD). Additionally, advances in novel technologies such as thin-film filters, lasers, LEDs, alternative light sources, and high-performance detector arrays have further enabled the reduction in size and enhancement in performance of IR spectrometers (*96*). As shown in Table 4, many of these miniaturized devices primarily use InGaAs detectors. Although cost and power consumption are major drivers in miniaturization, single-element detectors, which are more cost-effective and energy-efficient, often result in noisier spectra (*99*). Another approach is the use of LEDs, as seen in the SciO instrument, which offers extremely low power consumption, compactness, and durability; however, this approach only partially covers the NIR region.

Advances in micro-electro-mechanical systems (MEMS) and their integration with micro-optics (MOEMS) have significantly reduced the size and production cost of spectrometers. MEMS technology integrates mechanical and electrical components at a microscopic scale, enabling the development of compact, robust, and highly sensitive IR sensors. This technology enables the rotation of miniature gratings to obtain a spectrum within milliseconds while improving the signal-to-noise ratio through the co-addition

Table 4. Examples of miniaturized NIR devices available on the market.

Spectrometer	Manufacturer	IR Source	Wavelength Selector	Detector	Wavelength Region (nm)	Spectral Resolution (nm)
AvaSpec- Mini-NIR	Avantes	Tungsten halogen	-	InGaAs	900–1700	12
C14486GA	Hamamatsu	Tungsten halogen	MEMS	InGaAs	950–1700	5
C15511-01	Hamamatsu	Tungsten halogen	MEMS	InGaAs	1100–2500	5.7
MicroNIR 1700 ES	VIAVI	Tungsten halogen	LVF	InGaAs	950–1650	12.5
MicroPHAZIR	Thermo Fisher Scientific	Tungsten halogen	MEMS	InGaAs	1600–2400	11
nanoFTIR NIR	SouthNest Technology	Tungsten halogen	MEMS	InGaAs	800–2600	6
Proxiscout Scanner	Büchi Labortechnik AG	Tungsten halogen	MEMS	InGaAs	1350–2550	16
NIRONE	Spectral Engines	Tungsten halogen	MEMS	InGaAs	1100–1350 1350–1650 1550–1950 1750–2150 2000–2500	12–16 13–17 15–21 16–22 18–28
NIRscan	Texas Instruments	Tungsten halogen	MEMS	InGaAs	950–1700	10
NIR-S-G1	InnoSpectra	Tungsten halogen	MEMS	InGaAs	950–1700	10
SCiO	Consumer Physics		LED	Si photodio de	740–1070	-

of multiple scans (*100–102*). LVFs are optical bandpass filters with transmission characteristics that vary along their length due to changes in the thickness of the coating. This enables precise selection of wavelengths in a compact form without moving parts (*93*). Fabry-Pérot interferometers consist of two mirrors separated by a cavity. Incident light undergoes multiple reflections between the mirrors, creating constructive or destructive interference depending on the wavelength (*103*). Hadamard-transform spectrometers use a single-pixel photodetector operating at any wavelength. The light beam is encoded after passing through the sample, the grating, and the mask, and then arrives at the detector, with the spectrum being restored through a Hadamard transform (*103*). LVFs, Hadamard, and DMD are wavelength selectors that eliminate the need for moving parts, thereby significantly reducing the size and production costs of devices (*93*).

The technologies described have been integrated into several devices available on the market (Tables 4 and 5). Examples include the NIRONE sensor, equipped with a Fabry-Pérot interferometer; the MicroNIR by VIAVI, which combines an InGaAs detector with an LVF; the NIRScan, which incorporates a DMD; the MicroPhazir, which utilizes a MEMS Hadamard mask; and the NeoSpectra Scanner (new Proxiscout Scanner), which employs MEMS Michelson interferometers. The increasing popularity of miniaturized Raman spectrometers is attributed to their compact size and ease of portability (Table 6), making them ideal for a wide range of applications, including field analysis and agricultural settings (*104*). These devices typically integrate key components such as lasers, optics, and detectors into a single, compact unit, which not only reduces the overall size of the system but also simplifies its complexity.

Table 5. Examples of miniaturized mid-infrared devices available on the market.

Spectrometer	Manufacturer	IR Source	Wavenumber Selector	Detector	Wavenumber Region (cm^{-1})	Spectral Resolution (cm^{-1})
Agilent Cary 630	Agilent	Wire-wound element	Michelson interferometer	dTGS	KBr 7000-350 ZnSe 5100-600	≤2
Agilent 4500	Agilent	Wire-wound element	Michelson interferometer	dTGS	4000–650	2–6
Agilent 4300	Agilent	Wire-wound element	Michelson interferometer	dTGS	5200–650	4–16
Agilent 5500	Agilent	Wire-wound element	Michelson interferometer	dTGS	4000–650	2–16
TruDefender FTX	ThermoFisher	Tungsten halogen	Michelson interferometer	dTGS	4000–650	4
Alpha II	Bruker	SiC globar	Michelson interferometer	dTGS	4000–400	2
MOBILE-IR II	Bruker	SiC globar	Michelson interferometer	MCT	6000–670	2
ARCoptix FTIR-rocke	ARCoptix	SiC globar	Michelson interferometer	MCT	5000–1660 /6600–1200 5000–830 5000–650	0.5-4

Table 6. Examples of miniaturized Raman devices available on the market.

Type	Spectrometer	Manufacturer	Laser (nm)	Laser Power (mW)	Spectral Resolution (cm^{-1})	Spectral Range (cm^{-1})
	RX210	Renishaw, plc.	500			100–2000
Portable	EZRaman-1 Dual	Enwave Optronics, Inc.	532	50	6	100–3200
			785	350	7	250–2350
	i-Raman Prime	Methrom	532	35	8	150–3350
			785	340	8	150–3400
	Xantus-2	Rigaku Corporation	532–1064	60–490	10-15/15-18	200-3000 /200-2000
			785–1064	60–490	710/15-18	200–2000
	i-Raman EX	Methrom	1064	500	10	100–2500
Handheld	FirstGuard	Rigaku Corporation	532	10–60	10–15	200–3000
			785,1064			
	RaPort	Enspectr	532	60	3	140–4180
	RSL-1	Digilab Raman	785	500		
	FirstDefender XL	Thermo Fisher Scientific	785	300	7-10.5	250–2900
	Bravo	Bruker	785,853	100	10–12	300–3200
	MIRA *P*	Methrom	785	100	8–10	400–2300

Chemometrics

The application of vibrational spectroscopy generates large datasets comprising hundreds or thousands of samples and variables, making data analysis challenging. Effective tools are required to manage and analyze this data, enabling the extraction of as much information as possible. Chemometrics, a multivariate statistical analysis of chemical data, involves the selection of optimal experimental designs and data processing methods for chemical investigations through mathematical and statistical techniques. It focuses on identifying and evaluating relevant information from unprocessed data (*105*, *106*).

Preprocessing is essential before any data analysis to reduce variation from experimental conditions. Miniaturized devices are exposed to fluctuating environmental factors, such as temperature and humidity, which can lead to spectral drift. Therefore, preprocessing is a key step to mitigate these effects. This includes mean centering, standardization, normalization, smoothing, transformation, or data reduction (*107*). The most common multivariate methods can be classified into three categories: exploratory or pattern recognition, classification or discrimination, and prediction (*108*). There are two primary types of qualitative methods based on multivariate data analysis: supervised and unsupervised methods. Unsupervised approaches aim to identify clusters or associations between samples without prior knowledge of class or group membership. Conversely, supervised methods require class data, enabling qualitative information to be incorporated into the classification process, to achieve the best possible separation of groups and maximize the classification method's predictive capability. These methods undergo a training phase to construct a classification or discriminating model based on pattern recognition methods (*105*, *108*). Quantitative analysis, on the other hand, links spectra to quantifiable properties of samples. Its objective is to establish relationships between a response variable and several independent variables, in this case, the wavenumbers (*108*).

Principal component analysis (PCA) is one of the most common unsupervised exploratory pattern recognition techniques in multivariate data analysis. It reduces the number of variables, known as dimension reduction, by establishing new variables called principal components (PCs). Each PC is a linear combination of the initial variables (wavenumbers), with the first principal component (PC1) explaining the largest variance, the second PC (PC2) explaining the second largest variance, and so forth. The main outputs of PCA are the principal component scores, which represent the projections of the samples from the original data space onto the PCs, and the loadings. Loadings are used to identify which regions in the dataset have the largest effect on each component and contribute to the separation (*81*, *109*). Another common exploratory methodology is hierarchical cluster analysis (HCA), which utilizes agglomerative clustering techniques. In HCA, the samples are grouped based on their similarities, and the results are typically represented as a dendrogram. Samples that are closer together in the dendrogram exhibit greater similarities, while those that are farther apart are less similar (*79*).

Some supervised classification analyses include Soft Independent Modeling of Class Analogy (SIMCA), Linear Discriminant Analysis (LDA), and Partial Least Squares Discriminant Analysis (PLS-DA). SIMCA utilizes PCA models for each class to identify similarities between classes. It considers each class separately through its own model of principal components. The discriminating power is used to assess which variables are most

important for separating the classes, with higher values indicating greater power for discriminating among classes (*79*, *105*). LDA, a PCA-based method, selects directions, which are combinations of the original variables, that achieve maximum separation among different classes (*79*). PLS-DA identifies key spectral differences between specified groups and creates a discriminant model based on these differences, maximizing covariance between the independent and dependent variables (*105*, *108*).

In partial least squares regression (PLSR), a quantitative supervised multivariate analysis, a large number of variables are compressed into a small number of latent variables (PLS factors) to explain the maximum variance. The goal is to establish a relationship between the spectral data (independent variables) and the quantitative data (dependent variables). The variance included in the model and the total number of samples significantly influence the robustness of the model (*79*, *108*).

Model validation is essential to prevent overfitting, where the model becomes excessively complex due to the inclusion of too many factors, and to assess the predictive performance on future samples. Overfitting occurs when irrelevant variables encode noise or lack discriminating power. Conversely, if the number of principal components/latent variables/factors is too small, important information may be lost, leading to low predictive power (*110*). Some options, such as leave-one-out, leave-n-out, or cross-validation, have become more common for conducting internal validation of the model. However, only when the dataset is sufficiently large that it is highly recommended to employ an independent dataset. The dataset is divided into calibration and test sets. The calibration set should be representative of the entire sample population and is used for adjusting the parameters of the model. The test set measures the predictive ability of the model (*111*). Chemometric models are often developed and validated using a single 'master' instrument. Nonetheless, directly applying these models to predict quantitative traits from data collected by a secondary instrument can lead to prediction errors. To address this, calibration transfer algorithms are used to mathematically align spectral responses. This process is applicable when transferring models between different benchtop or miniaturized devices. Alternatively, robust calibration strategies that incorporate data from multiple instruments can also be used to effectively minimize inter-device variability.

Application of vibrational spectroscopy with insects

Infrared spectroscopy

The application of vibrational spectroscopy for insects incorporates a broad range of areas, from entomology and agriculture to food science and environmental monitoring. The application of IR spectroscopy initially was found to be useful in taxonomic studies, age, and sex determination of insects, and later expanded into metabolomics (*112–114*). With the increasing interest in insects for various applications, including food and feed production, there is a growing need for efficient control measures throughout the production and post-production processes. Addressing this demand requires exploring novel techniques that enable rapid, *in situ*, and cost-effective analysis, particularly for food manufacturers and farmers. In this context, vibrational spectroscopy emerges as a promising alternative to conventional analytical methods.

Vibrational spectroscopy, particularly NIR and MIR techniques, has become essential for analyzing both proteins and lipids in insect-based products, allowing species identification, quality control, and nutritional assessment. Several studies have focused on different insect species and showed how these methods can be used for analysis, with the incorporation of advanced data models for more accurate predictions (Tables 7 and 8). For insect species identification, Mellado-Carretero et al. (*14*) used ATR-FTMIR spectroscopy combined with SIMCA, a supervised classification algorithm, to authenticate several edible insect powders. The insect species studied were *T. molitor, A. diaperinus, G. sigillatus, A. domesticus, and L. migratoria*. The ATR-FTMIR spectra were used to capture characteristic spectral bands, particularly associated with chitin and lipids, which differ significantly across species. SIMCA helped build models that discriminated between species based on their unique spectral features. Robertson et al. (*15*) analyzed the molecular structure and chemical composition of insect powders, specifically *H. illucens* larvae, *T. molitor*, *G. sigillatus*, and *G. assimilis*, using ATR-FTMIR spectroscopy combined with multivariate statistical methods. The study showed distinct differences in the chemical profiles of these insect species, which were linked to variations in digestibility and protein hydrolysis. PCA was used to identify these differences, highlighting variations in carbohydrate, protein, and lipid content that are critical for understanding the nutritional value of insect powders. The

Table 7. Recent research activity of MIR spectroscopy combined with chemometrics in insects.

Application	Instrument	Analysis and Results
Insect powders discrimination by origin and species (*6*)	Portable ATR-FT-MIR (Agilent Cary 630)	SIMCA
Predict the percentage of insect powder in dough and snack (*7*)	Portable ATR-FTMIR (Agilent Cary 630)	PLSR Range: 0-13.9%, R^2_P = 0.97-0.99, $RMSE_P$ = 1.08-1.90
Monitor compositional changes in *H. illucens* larvae feeding with different waste (*8*)	Portable ATR-FTMIR (Bruker Alpha MIR)	PCA and PLS-DA
Qualitative characterization of green ant from Australia by the chemical composition (*9*)	Benchtop ATR-FTMIR (PerkinElmer Spectrum 100™)	PCA
Determination of insect lipids and their fatty acid profile in dough (*10*)	Portable ATR-FTMIR (Agilent Cary 630)	PLSR Insect lipid R^2_{CV} = 0.95, $RMSE_{CV}$ = 0.71, RPD = 5.21; Unsaturated and saturated fatty acid $R^2_{CV} \geq 0.98$, $RMSE_{CV} \leq 1.88$, RPD≥5.70 Fatty acid profile $R^2_{CV} \geq 0.98$, $RMSE_{CV}$ = 2.01, RPD≥5.55
Predict fat content in edible insect powders (*11*)	Portable ATR-FTMIR (Agilent Cary 630)	PLSR R^2_{CV} = 0.89, $RMSE_{CV}$ = 3.22, RPD = 2.31
	Portable ATR-FT-MIR (Agilent 4500)	PLSR R^2_{CV} = 0.89, $RMSE_{CV}$ = 3.22, RPD = 2.31
Predict essential amino acids on insects (*12*)	Benchtop FTMIR (WQF-510)	PLSR $R^2_P > 0.65$, $RMSE_P < 0.71$,RPD>1.37; Decision tree $R^2_P > 0.79$, $RMSE_P < 0.41$,RPD>1.14; Radial basis artificial neural network $R^2_P > 0.74$, $RMSE_P < 0.60$, RPD>1.40
Classification of adulterated edible insect flours (*13*)	Benchtop FTMIR (PerkinElmer Spectrum Two™)	SIMCA Sensitivity = 66.66-86.67%, Specificity = 73.33-95.24%, Efficiency = 69.92-90.85%
Identify differences on molecular structure of insect powders (*14*)	Benchtop ATR-FTMIR (Nicolet iS5)	PCA

combination of FT-MIR and PCA proved to be an effective, rapid technique for classifying insect-derived products, aiding in quality control and fraud prevention in the animal feed industry. Both studies demonstrated the potential of vibrational spectroscopy, combined with advanced statistical and chemometric tools, to provide a reliable, non-destructive approach for species identification and quality assurance in insect-based products. These methods are essential for ensuring the integrity and nutritional value of insect powders used in food products and animal feed.

In addition to species identification, MIR spectroscopy has been found valuable in quantifying the proportion of insect powders in food products, such as baked goods or protein bars. This application ensures that manufacturers meet nutritional claims and labeling requirements regarding the inclusion of insect ingredients. It also allows for the detection of non-target species or adulterants, maintaining product integrity and consumer trust. García-Gutiérrez et al. (*16*) used ATR-FTMIR spectroscopy with PLSR models to analyze insect powders in food products such as doughs and 3D-printed snacks. The insect species analyzed were *A. diaperinus* and *L. migratoria*. The composition of the doughs included chickpea flour, which was partially replaced by insect powders in varying amounts (0–13.9%). The study demonstrated that PLSR models could effectively predict insect powder concentrations in doughs, allowing for accurate identification of the insect content in both raw and cooked food products. The results also showed that this method could successfully discriminate between different insect powders in food matrices, highlighting the potential for routine quality control in the food industry. Ni et al. (*17*) used NIR spectroscopy to study various insect protein powders derived from *H. illucens*, *A. domesticus*, and *T. molitor*. The study utilized PLSR models to differentiate between these species and detect adulteration levels in insect protein powders. The study achieved high prediction accuracy, with R^2 values ranging from 0.98 to 0.99, showing the model's

Table 8. Recent research activity of NIR spectroscopy combined with chemometrics in insects.

Application	Instrument	Results
Quantitative analysis of insect protein content in handcrafted fitness bars (intact and milled) (*15*)	Benchtop FTNIR (Büchi NIRFlex N-500)	PLSR Range: 19.3–23.0%, $R_P^2 = 0.94$, $RMSE_P = 0.21$
	Handheld FTNIR (MicroNIR 1700 ES)	PLSR Range: 19.3–23.0%, $R_P^2 = 0.62$, $RMSE_P = 0.53$
	Handheld FTNIR (SCiO Sensor)	PLSR Range: 19.3–23.0%, $R_P^2 = 0.55$, $RMSE_P = 0.57$
	Handheld FTNIR (Tellspec Enterprise Sensor)	PLSR Range: 19.3–23.0%, $R_P^2 = 0.55$, $RMSE_P = 0.57$
Quantify different insect powders in wheat flour (*16*)	Benchtop FTNIR (Bruker MPA Multipurpos FTNIR)	PLSR Range: 5-50%, $R^2 = 0.99$, $RMSE_{CV} = 0.65$, RPD = 21
Predict the fat content and the fatty acid profile in living *T. molitor* larvae (*17*)	(PSS 2120, Polytec GmbH)	PLSR Fat content Range: 5.7-16.1%, $R_P^2 = 0.99$, $RMSE_P = 0.27$, RPD = 8.33; Fatty acid profile $RMSE_P = 0.26$-2.11, RPD = 1.96-5.55
Fatty acid profiling and amino acid quantification in instars of *T. molitor* larvae (*18*)	(PSS 2120, Polytec GmbH)	PLSR Fatty acid profile $R_P^2 = 0.57$-0.96, $RMSE_P = 0.071$-0.81, RPD = 1.51-4.98; Amino acid content $R_P^2 = 0.51$-0.95, $RMSE_P = 0.076$-0.54, RPD = 1.43-4.42
Detection of insect adulteration in simulated matrix of insect powders (*19*)	Portable NIR (ASD LabSpec)	PLSR Range: 0-100% $R_{CV}^2 = 0.98$-0.99, $RMSE_{CV} = 1.85$-4.11, RPD = 7.66-17.06
Prediction of protein and lipid content in *H. illucens* larvae flour (*20*)	Portable NIR-S-G1 (innoSpectra)	Fat content PLSR, $R_P^2 = 0.76$, $RMSE_P = 6.44$, RPD = 2.35 SVMR, $R_P^2 = 0.91$, $RMSE_P = 3.76$,RPD = 4.04; Protein content PLSR, $R_P^2 = 0.81$, $RMSE_P = 1.31$, RPD = 2.42, SVMR, $R_P^2 = 0.0.85$, $RMSE_P = 1.19$, RPD = 2.66
	Handheld NIR (NeoSpectra Scanner)	Fat content PLSR, $R_P^2 = 0.85$, $RMSE_P = 5.73$,RPD = 2.65 SVMR, $R_P^2 = 0.94$, $RMSE_P = 3.51$,RPD = 4.32; Protein content PLSR, $R_P^2 = 0.83$, $RMSE_P = 1.27$, RPD = 2.50, SVMR, $R_P^2 = 0.85$, $RMSE_P = 1.19$, RPD = 2.66
Classify insect powder samples and predict macronutrients in different insect powders (*21*)	Portable FTNIR (NeoSpectra Micro Development Kit)	PLSR Macronutrients prediction $R_P^2 = 0.71$-0.94, $RMSE_P = 1.00$-2.00, RPD = 1.80-3.80
	Handheld NIR (SCiO Sensor)	PLSR Macronutrients prediction $R_P^2 = 0.94$-0.99, $RMSE_P = 0.18$-1.04, RPD = 3.65-11.01
Predict total lipids and fatty acids in *H. illucens* larvae (*22*)	NIR-hyperspectral imaging (SisuCHEMA)	Fat content PLSR, $RMSE_P = 5.25$, RPD = 2.9 SVMR, $RMSE_P = 4.76$,RPD = 3.2; Fatty acid content PLSR, $RMSE_P = 0.15$-3-00,RPD = 1.8-3.3 SVMR, $RMSE_P = 0.09$-1.38, RPD = 2.5-5.5
Determination of protein content in single *H. illucens* larvae (*22*)	NIR-hyperspectral imaging (SisuCHEMA)	PLSR, $R_P^2 = 0.731$-0-751, $RMSE_P = 1.61$-1.66, RPD = 2.18-2.26, SVMR, $R_P^2 = 0.75$-0.773, $RMSE_P = 1.57$-1.634, RPD = 2.22-2.31
Determination of insect lipids and their fatty acid profile in dough (*10*)	Handheld FTNIR (NeoSpectra Scanner)	PLSR Insect lipid $R_{CV}^2 = 0.99$, $RMSE_{CV} = 0.39$, RPD = 5.53, Unsaturated and saturated fatty acid $R_{CV}^2 \geq 0.88$, $RMSE_{CV} \leq 5.64$, RPD $\geq$ 2.64,

(*Continued*)

Table 8. Continued.

Application	Instrument	Results
		Fatty acid profile $R^2_{CV} \geq 0.90$, $RMSE_{CV} \leq 4.42$, $RPD \geq 1.97$
Predict fat content in edible insect powders (*11*)	Handheld FTNIR (NeoSpectra Micro Development Kit)	PLSR $R^2_{CV} = 0.98$, $RMSE_{CV} = 1.26$, $RPD = 7.42$
	Handheld FTNIR (NeoSpectra Scanner)	PLSR $R^2_{CV} = 0.98$, $RMSE_{CV} = 1.26$,$RPD = 7.42$

effectiveness in identifying insect species and estimating adulteration levels in protein powders. This model enabled the detection of compositional differences, including rearing methods and processing, which could be used to verify the authenticity of insect protein products in the food and feed industries. Foschi et al. (*18*) applied ATR-FTMIR spectroscopy to authenticate insect flours and detect adulteration. Mixtures of *A. domesticus* and *A. diaperinus* flours were prepared to mimic adulterated samples, along with pure flours of the same insects. The research highlighted significant differences in the spectral characteristics between pure insect flours and those adulterated with whole grain flours. Specifically, it identified key absorption bands associated with lipids, chitin, and proteins. The study used sequential preprocessing through orthogonalization discriminant analysis (SPORT-DA) and SIMCA to effectively classify and differentiate the samples. For *A. domesticus* flour, the SPORT-DA model achieved the best results, accurately classifying 100% of the test samples. The SIMCA model provided the most accurate predictions for *A. diaperinus*, correctly classifying 90% of the test samples. Hou et al. (*19*) explored FTMIR spectroscopy combined with machine learning techniques to predict the amino acid content in insect-derived products. In this study, 200 samples from 9 commercial insect species, *T. molitor*, *Zophobas morio*, grasshoppers (*Acrida cinerea* and *L. migratoria*), *Bombyx mori*, *Teleogryllus emma*, *Chrysomya megacephala, Musca domestica*, and *H. illucens*, were tested. Radial basis artificial neural network (RBA-NN) accurately predicted amino acid profiles, achieving R^2 of 0.97 for phenylalanine content. This model outperformed others, PLSR and decision tree, especially when using the first derivative of the FTMIR spectra. Beć et al. (*115*) focused on FTNIR spectrometers for *A. diaperinus* protein content analysis in handcrafted insect bars. To predict protein content, the study used both PLSR and Gaussian process regression (GPR) calibration methods. These models were used to analyze the spectral data obtained from the spectrometers. The root mean square error of prediction (RMSEP) values ranged from 0.55% to 0.66% for intact bars and 0.23% to 0.34% for milled samples, demonstrating the models' ability to reliably quantify protein content. The study also showed that the GPR calibration improved the predictive performance, highlighting the model's flexibility in handling different spectrometer types for on-site protein content analysis.

On the lipid side, Mantilla et al. (*116*) explored the lipid content of edible Australian green ants (*Oecophylla smaragdina*) using FTMIR spectroscopy. The study used PCA to process the spectral data. PCA helped identify variations in the chemical composition of different anatomical parts of the insects, with the abdomen showing the highest lipid content. Key absorbance peaks were identified for lipids and fatty acids, with significant differences found in the aliphatic CH_2 groups (lipids and fatty acids) and amide I and II groups (proteins and chitin). PCA clustering allowed the separation of insect body parts based on their chemical profiles, and it also showed that storage conditions affected the lipid composition, emphasizing the importance of understanding storage impacts.

Méndez-Sánchez et al. (*117*) used both ATR-FTMIR and FTNIR spectroscopy to quantify fat content in insect powders, specifically from *T. molitor* and *A. diaperinus*. The study applied PLSR to the spectral data obtained from handheld FTNIR spectrometer. The models developed demonstrated strong correlations (R_p up to 0.99) between the spectral data and the fat content in the insect powders, with standard errors of prediction (SEP) around 1.05%. This method allowed for the real-time, on-site analysis of fat content in insect powders, providing a fast and cost-effective approach to nutritional assessment. The study also highlighted that handheld FTNIR device showed superior performance for field-based applications, which is crucial for on-the-go monitoring of insect-based food products. Méndez-Sánchez et al. (*20*) evaluated the effectiveness of portable ATR-FTMIR and handheld FTNIR spectrometers for quantifying insect lipids and

their fatty acid profiles in chickpea-based dough. The study found that the FTNIR spectrometer was particularly effective at predicting total insect lipid content showing a low SE_{CV} = 0.39% and strong correlation (R_{CV} = 0.99), while the FTMIR spectrometer showed superior performance in predicting individual fatty acids particularly saturated and unsaturated fatty acids with values of standard error in prediction ($SE_P \leq 2.81\%$) and an $R_P \geq 0.97$. The insect species tested included *T. molitor*, *A. diaperinus* and *A. domesticus*. These results confirmed the strong predictive capabilities of both spectroscopic methods for lipid quantification in insect-based products.

Finally, vibrational spectroscopy, particularly NIR, is not only useful for protein and lipid analysis but also plays an essential role in ensuring that insect-based food products meet nutritional labeling and regulatory requirements. By quantifying the proportion of insect powders in food products, it supports manufacturers in verifying their nutritional claims, particularly regarding protein, fat, and carbohydrate content. This information is crucial for product formulation and marketing, ensuring that insect-based foods align with consumer expectations and industry standards. Additionally, the technique's ability to detect non-target species or adulterants helps maintain product integrity and supports consumer trust in insect-based food products.

Some researchers have focused on applying vibrational spectroscopy to study the chemical composition of live insect larvae (*118*). This application is particularly useful during the rearing stage, as it allows for the real-time monitoring of larvae growth and health. By tracking these parameters, it becomes possible to optimize environmental conditions and feeding strategies to achieve the best quality and nutritional content in the larvae.

One of the major advantages of vibrational spectroscopy is its non-destructive nature. Unlike traditional laboratory techniques, this method allows researchers to analyze insect samples without altering their physical state, ensuring that the larvae can still be used or sold after analysis. This non-invasive characteristic makes vibrational spectroscopy a preferred tool for routine quality control during the rearing of larvae. By incorporating this technique throughout the rearing process, farmers can ensure that larvae consistently meet the necessary standards for quality and composition before harvest. Kröncke et al. (*119*) investigated the impact of different dietary substrates on the fat and fatty acid composition of *T. molitor* larvae, emphasizing how nutrition influences the larvae's nutritional profile. The study used NIR spectroscopy to analyze fat content and fatty acid composition, showing that different diets lead to significant variations in the larvae's fat composition, including saturated, monounsaturated, and polyunsaturated fatty acids. The NIR technology allowed for the non-destructive, rapid measurement of the larvae's nutritional composition without killing them. Kröncke et al. (*120*) also explored nutrient variations in *T. molitor* larvae across different developmental instars, finding that younger larvae have higher moisture and protein content, while fat content increases as larvae mature. The study also highlighted the utility of NIR spectroscopy in predicting amino acid and fatty acid content, offering valuable insights for insect farmers to optimize harvesting times based on nutritional yield. The study suggests that earlier instar stages are preferable for harvesting, balancing growth rates with economic factors in insect farming. Hoffman et al. (*121*) used ATR-FTMIR spectroscopy to monitor and classify *H. illucens* larvae based on their growth stages (5th and 6th instars) and dietary waste streams. The study achieved 100% classification accuracy, distinguishing larvae based on their diet types, bread-vegetable mixture and soy waste. The MIR spectra revealed key differences in the larvae's chemical composition, with certain absorbance bands correlating to water, protein, and lipid content, allowing the researchers to identify spectral signatures associated with each diet type. Using PLS-DA regression, the study achieved strong classification and prediction performance, with R^2 values greater than 0.80 for predicting diet type. The research also demonstrated that a higher carbohydrate proportion in the diet (like the bread-vegetable mix) yielded different spectral characteristics compared to the soy waste diet.

Cruz-Tirado et al. (*122*) used NIR-HSI as a non-contact and non-destructive analytical method to estimate the protein content in individual, intact *H. illucens* larvae. The larvae were placed on a Teflon® plate, and NIR-HSI images were captured using a SisuCHEMA SWIR hyperspectral camera, with a spectral range of 928 to 2524 nm. The study involved capturing nineteen hyperspectral images of 380 larvae, followed by image processing that included background removal using K-means clustering and morphological corrections. These steps improved the quality of the data and enabled more accurate analysis. Chemometric techniques, including PLSR and support vector machine regression (SVMR), were tested to develop predictive models for estimating protein content. PCA revealed the heterogeneous distribution of proteins and lipids, with protein accumulation being higher at the edges of the larvae. The study highlighted the potential of NIR-HSI as a

rapid, non-destructive method for estimating protein content and observing its distribution, providing valuable insights for quality control in insect protein production. In a follow-up study by Cruz-Tirado et al. (*21*), NIR-HSI was used to predict lipid content and fatty acid composition in *H. illucens* larvae. Both PLSR and SVMR models were applied, with SVMR outperforming PLSR in predicting fatty acids, achieving excellent predictive accuracy for total lipid content and specific fatty acids like lauric, myristic, palmitic, and oleic acids. The lipid content in *H. illucens* larvae was found to reach up to 63.90%, with variations depending on their feeding and conditioning systems. The study demonstrated that variable selection methods such as Bootstrapping soft shrinkage (BOSS) and iPLS improved model performance. The findings emphasize the advantages of NIR-HSI for assessing the nutritional quality of insect-based products.

Vibrational spectroscopy can be applied to monitor changes in the chemical composition of insect-based products during processing (e.g. drying, baking). This helps ensure that the final product meets quality standards. While not as common, there is potential for vibrational spectroscopy to be used in assessing microbial contamination in insect products. Rapid detection of pathogens can enhance food safety.

One of the significant advantages of vibrational spectroscopy is that it allows for non-destructive testing of samples. This means that products can be analyzed without altering their physical state, preserving them for sale. Vibrational spectroscopy provides a fast and cost-effective method for analyzing insect products compared to traditional laboratory techniques, making it suitable for routine quality control in food production. Overall, the application of vibrational spectroscopy in the insect food sector enhances food safety, ensures compliance with regulations, and supports the growing market for edible insects as a sustainable source of protein.

The main MIR bands typically identified in insects (Table 9) include chitin bands associated with CN stretching vibrations around 900–1100 cm^{-1}, CO stretching around 1150–1200 cm^{-1}, and the amide I band, which appears as a doublet at 1655 and 1625 cm^{-1}. Protein bands, linked to C = O stretching vibrations of peptide bonds, are found around 1650 cm^{-1}, while NH bending and CN stretching vibrations are observed around 1540–1560 cm^{-1}. Lipid bands are associated with CH stretching around 2800–3000 cm^{-1}, and carbohydrate bands, including CC stretching, appear in the range of 1000–1200 cm^{-1}. These carbohydrate bands are attributed to various components, including glucose derivatives found in the insect cuticle.

In NIR spectroscopy (Table 9), bands related to chitin are also identified, with CH stretching typically found around 4250–4100 cm^{-1}, while OH and NH stretching are often observed in the 5300–5900 cm^{-1} range. Protein-related NH bending occurs around 6700–6300 cm^{-1} and 5100–4350 cm^{-1}, while lipid bands are characterized by CH stretching overtones in the 5900–5500 cm^{-1} range and bending vibrations within lipid chains at 4350–4250 cm^{-1}.

Raman spectroscopy

Raman spectroscopy has the potential to play a key role in quality control by monitoring insect powders and insect-based products, detecting changes in chemical composition that indicate spoilage, contamination, or degradation. It can also be effective in identifying additives, preservatives, and contaminants in insect products, ensuring compliance with food safety regulations. As a non-destructive method, Raman spectroscopy

Table 9. Main absorption bands of insects.

IR Range	Wavenumber (cm^{-1})	Functional group	Structure
MIR	3500–3000	O-H	Chitin
	3000–2800	C-H	Lipid
	1740	C = O	Lipid
	1700–1500	N-H, C = O	Proteins, Chitin
	1300–1200	C-O	Chitin, Lipids
	1200–900	C-H	Carbohydrates/polysac
NIR	8600–8200	C-H	Lipid
	7250–7100	C-H	Chitin
	6700–6300	N-H	Protein
	5900–5500	C-H	Lipid
	5100–4350	N-H	Protein
	4350–4250	C-H	Lipid
	4250–4100	C-H	Chitin

Table 10. Recent research activity of Raman spectroscopy combined with chemometrics in insects.

Application	Instrument	Results
Determine the presence of resilin in *Schistocerca gregaria* (*23*)	Confocal Raman (HORIBA Jobin Yvon)	PCA
Identify the effects of enzymatic hydrolysis on *H. illucens* protein concentrate (*24*)	Microscopy Raman (DXR2)	PCA
Discriminate and classify mosquito species (*25*)	Raman microscopy (Technos®)	Linear Discriminant Analysis (LDA) Accuracy = 86%; Quadratic Discriminant Analysis (QDA) Accuracy = 89%; Logistic Regression (LR), Accuracy = 85%; Quadratic Support Vector Machine (QSVM), Accuracy = 93%

Note: These bands are based on the results of several investigations (*6*, *7*, *16*, *19*, *21*).

allows for the analysis of insect samples without altering their properties, making it particularly suitable for quality assessments. Portable Raman spectrometers enable on-site analysis in farm settings. Additionally, the technique can also be used to investigate the biochemical composition of insects, their metabolic processes, and the effects of rearing conditions. However, Raman spectroscopy has been explored for only a limited number of applications (Table 10). This technique can be crucial for species identification, distinguishing between insect species based on their unique Raman spectral signatures, ensuring product authenticity, and preventing mislabeling.

Omucheni et al. (*123*) used Raman spectroscopy in combination with machine learning techniques to enable the rapid and non-destructive identification of two mosquito species, *Anopheles gambiae* and *Anopheles arabiensis*, which are morphologically indistinguishable and traditionally differentiated through molecular methods. This approach offers several advantages over conventional PCR assays, including reduced sample preparation time and the ability to reuse samples. The study applied several machine learning algorithms for species classification, including linear discriminant analysis (LDA), Logistic Regression (LR), quadratic discriminant analysis (QDA), and quadratic support vector machine (QSVM). The Raman bands most relevant for mosquito species identification were predominantly centered around 1400, 1590 cm^{-1}, and 2067cm^{-1} linked to the presence of melanin pigment present in the mosquito cuticle. Regarding model performance, the cross-validation accuracy was 86% for LDA, 85% for LR, 89% for QDA, and 93% for QSVM. When applied to the test data set, the accuracies were 79% for LDA, 82% for LR, 81% for QDA, and 93% for QSVM. These results suggested that the QSVM model outperformed the others in both cross-validation and test scenarios, demonstrating its superior ability to classify the mosquito species accurately.

Batish et al. (*124*) evaluated the impact of three commercial enzymes, alcalase, papain, and pepsin, on the hydrolysis of *H. illucens* larvae protein. Raman spectroscopy was used in this study to analyze the structural changes in the protein of *H. illucens* larvae after enzymatic hydrolysis. The technique provided secondary structural information about proteins by examining specific regions (amide I and amide II) that indicate changes in the protein structure, such as C = O stretching and N–H bending. The Raman spectral data showed that enzymatic hydrolysis produced structural changes in the α-helices and β-sheets of the protein following enzymatic hydrolysis.

Woodrow et al. (*125*) used Raman spectroscopy for the non-invasive identification and analysis of resilin, an elastic protein found in insect structures, particularly in the desert locust (*Schistocerca gregaria*). PCA was applied to analyze the variance in the Raman spectra, discriminating between different types of cuticles. The technique allowed for the differentiation between resilin-rich and low-resilin cuticular regions based on their unique spectral signatures, all while preserving the integrity of the samples. The study also identified key Raman bands associated with tyrosine, dityrosine, and chitin skeletal modes, providing valuable insights into the molecular structure and composition of the cuticle.

Conclusions and outlooks

As the demand for insects continues to grow due to their sustainability and nutritional value, the need for efficient, non-destructive, and cost-effective analytical methods is increasing. Vibrational spectroscopy can

be a powerful tool for ensuring the authenticity, quality, and compliance of insect-based food products with its ability to provide rapid, on-siteanalyses. IR spectroscopy, including MIR and NIR techniques, has already been tested to successfully predict protein, amino acids, and lipid content in insect powders and a variety of food products. Combining IR spectral data with traditional machine learning algorithms such as PLSR, PCA, and SIMCA has been studied to differentiate insect species and detect adulteration in insect powders. Additionally, vibrational spectroscopy allows monitoring the growth of live insect larvae. Real-time analysis of larvae during the rearing process helps to optimize feeding and environmental conditions. Raman spectroscopy, although having fewer applications than IR techniques, is a promising method for detecting structural changes in insect proteins, as well as identifying species-specific spectral signatures. Raman spectroscopy can be used to detect biochemical composition differences of insects in response to different rearing conditions. This potential application may provide useful data for optimizing insect farming practices.

Overall, the integration of vibrational spectroscopy into the insect food industry demonstrates benefits, including quality control, real-time monitoring capabilities, and improved production efficiency. The continued development of portable and miniaturized spectroscopic devices further supports the implementation of these techniques in field applications, offering convenience and cost-effectiveness for insect farmers and insect food manufacturers. Future research should focus on optimizing spectroscopic techniques for specific insect species and developing standardized protocols for quality assessment across the insect industry, while addressing challenges such as calibration transfer and real-world deployment constraints for miniaturized devices.

Author contributions

CRediT: **Carmen Mendez-Sanchez:** Conceptualization, Methodology, Writing – original draft; **Mayreli Ortiz:** Resources, Supervision, Writing – review & editing; **Carme Güell:** Resources, Writing – review & editing; **Montserrat Ferrando:** Resources, Writing – review & editing; **Daniel Cozzolino:** Supervision, Writing – review & editing; **Silvia De Lamo Castellvi:** Conceptualization, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing.

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