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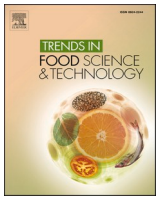

Proteomics for food and feed authentication in the circular food chain

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ABSTRACT

Background: Ensuring consumer trust is critical in the circular economy and the reintroduction of animal proteins into the food chain. Authentication of the tissue and species-specific origin of food and feed samples is crucial for maintaining food and food supply chain safety. Along with analytical methods such as DNA-based methods, microscopy, nuclear magnetic resonance (NMR), proteomic methods can also be implemented for food authentication and safety.

Scopes and approaches: This review focuses on applications of state-of-the-art proteomics methods to safeguard food and feed chains in circular food production systems. Specifically, the utilization of targeted and untargeted proteomics approaches in the safe reintroducing processed animal proteins (PAPs) into the feed supply chain is discussed in a regulatory context. Furthermore, the implementation of proteomics along with DNA-based methods in the authentication of fish and insect species in food and feed products will benefit detection of fraudulent practices. Proteomic techniques such as targeted and untargeted approaches are discussed to tackle authentication challenges and safeguard food safety.

Key findings: We discuss the implementation of proteomic methods in detecting and quantifying prohibited protein material, addressing authentication challenges, and ensuring the integrity of food and feed products. For PAP product species and tissue, origins can be accurately determined through targeted proteomic approaches. Moreover, untargeted proteomics offers the capability of detecting allergens from novel foods such as insects and avoiding potential food fraud. Integrating proteomic methods into routine food and feed analysis workflows shows promise for enhancing regulatory compliance, consumer confidence, and overall food safety in circular food production systems.

1. Introduction

The global population will increase to 8.5 billion by 2030, ultimately increasing the demand for food and further straining natural resources (FAO, 2023). As per the Food and Agricultural Organization of the United Nations (FAO) guidelines, the growing population must have access to nutritious, safe, and affordable food while ensuring reduced carbon footprint is critical (Mc Carthy et al., 2018). Current food supply models rely on finite resources and indicate the need for resource-efficient circular food chains. The sustainable utilization of marine resources, particularly aquaculture, can benefit food security (FAO, 2023). Seafood is a source of essential nutrients, and aquaculture has proliferated, increasing demand for sustainable feed ingredients (FAO, 2023;VKM, 2022).

Transitioning to a circular bio-based economy is vital for the food production industry, efficiently utilizing biological resources in the light of food security (Flynn et al., 2019). Recirculation of by-products as valuable resources can minimize environmental impacts while providing sustainably produced nutritious food (Ghisellini, Cialani, & Ulgiati, 2016; Mirabella, Castellani, & Sala, 2014). Circular food systems can recycle slaughterhouse by-products into feed material by efficiently utilizing resources within the bio-economy (Woodgate et al., 2022). In the European aquaculture sector, the scarcity of protein-rich materials is a food security challenge due to which alternative protein sources such

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as insect proteins, single-cell proteins, soybean, and processed animal proteins (PAP) from food industry by-products were introduced into the food chain (EuropaBio Annual EuropaBio annual Report, 2018). However, in circular food production systems, ensuring safety and traceability is crucial. The outbreak of Bovine Spongiform Encephalopathy (BSE) showed that circular economies can be hazardous if adequate regulations are not in place (Boqvist, Söderqvist, & Vågsholm, 2018; Vågsholm, Arzoomand, & Boqvist, 2020). By-products from the food industry that are reused as feed material are strictly regulated to follow safety standards to uphold consumer safety (Lavelli, 2021). To follow these regulations, it is essential to develop and implement novel analytical methods to address the possible hazards associated with circular food and feed chains within the context of the bioeconomy.

Along with the inclusion of by-products in food production systems, food fraud is another topic in which similar analytical methods are required to be implemented. Food fraud is a global concern across supply chains in the global food markets. Fraudulent practices such as adulteration, substitution, dilution, and use of unauthorized food products violate consumer safety, trust, and rights (Ortea, O'Connor, & Maquet, 2016; Su, Yu, Liang, Wang, & Wang, 2024). Mislabeling food allergens, such as fish, gluten, soybean, egg, or nuts, can have severe consequences for sensitive individuals (Visciano & Schirone, 2021). In addition to misinformation and mislabeling, food adulteration poses significant health hazards. Furthermore, in the global food chain, incidents were recorded indicating potential health risks associated with food fraud. For example, incidents such as melamine-contaminated milk in China affected hundreds of thousands of children (Gossner et al., 2009) and the "horse meat scandal" in Europe, where beef products were adulterated with horse meat contaminated with phenylbutazone, a veterinary steroidal drug which raised food safety concern due to toxicity and carcinogenic effects in humans (Bouzembrak & Marvin, 2016). These incidents raised food safety concerns and highlighted the need for accurate labeling and compliance control. Seafood is highly susceptible to mislabeling and adulteration due to global trade and varying market value; furthermore, there are environmental consequences of seafood fraud as endangered species may be included in the food chain, affecting marine biodiversity (Bouzembrak et al., 2018). Preventing seafood fraud is essential for consumer safety and rights and conserving valuable marine resources.

Due to the complexity of food and feed fraud, the availability of analytical tools for food and feed authenticity and traceability are critical for safeguarding global food chain (Ortea et al., 2016; Saadat, Pandya, Dey, & Rawtani, 2022). The ability to verify the authenticity of food and feed products is often called "food forensics" (Silva, 2018; Saadat et al., 2022). As there is a wide variety of contaminants, adulterants, and hazards, various r molecular tools are required for food forensics to ensure food safety. For example, high-accuracy and cost-effective DNA methods have been used to develop rapid assays to authenticate food products (Toxqui Rodríguez, Vanhollebeke, & Derycke, 2023). Various instrumental methods such as High-Performance Liquid Chromatography (HPLC), trace element analysis, light microscopy, examination of stable isotope ratios (SIR), Nuclear Magnetic Resonance (NMR) spectroscopy, immunoassays, and mass spectrometry can be applied for food authentication purposes (Ortea et al., 2016; Saadat et al., 2022).

In the context of food and feed safety, as proteins are vital components in food and feed materials, analyses of proteins are suitable for regulatory purposes. Proteome analyses can be implemented to develop markers, which can be used to detect the origin of food products or allergens, which help develop rapid assay of food products (Ortea et al., 2016). This review is focused on how proteomics can be implemented for food and feed safety in circular food chains by focusing on aquafeed, seafood, and edible insect species. Circular ingredients are increasingly being used in animal feed, and as food for human consumption. To ensure safety and follow European regulations, analytical methods such as proteomics will help identify prohibited ingredients in food and feed.

2. Proteomics methods

Proteomics is the study of the abundance and identity of proteins in an organism or its tissues. The bottom-up, top-down, and middle-down proteomic approaches are implemented to identify and study proteins. Bottom-up proteomics approach is still more widely used in proteomic studies (Aebersold & Mann, 2003). In bottom-up proteomics, protein samples undergo enzymatic digestion, typically employing proteolytic enzymes like trypsin, followed by separation and identification using high-performance liquid chromatography (HPLC) coupled to high-resolution mass spectrometry (HR-MS) (Raes et al., 2018). Proteomics is often classified as untargeted for biomarker discovery and targeted for biomarker verification. Generally, the aim of untargeted proteomics is the identification and relative quantification of as many proteins as possible based on the generated peptide mass spectra. Using bioinformatic search engines, generated tandem mass spectra (MS/MS) can be matched to relevant proteomic databases to identify the corresponding proteins (Duivesteijn, 2018). Untargeted proteomic approaches are also used to generate peptide spectral libraries. In addition to database searching, de novo sequencing is another technique in untargeted proteomics where the amino acid sequences of peptides are determined directly from the MS/MS data independent of databases. This enables the discovery of short bioactive and novel peptides that can be used to expanding proteomic databases. Targeted proteomics is often used for biomarker candidate verification and absolute quantification assays due to improved selectivity and sensitivity (Sobsey et al., 2019). Typical targeted proteomics methods involve parallel reaction monitoring (PRM) using a HR-MS system or multiple reaction monitoring (MRM) using a triple quadrupole system. When applying PRM or MRM, it is essential to ensure that the targeted peptides are unique for the candidate protein. Table 1 lists the applicability of targeted and untargeted proteomics in food and feed safety by focusing on examples of aquafeed, seafood, and edible insect species.

2.1. Targeted proteomics methods

Food and feed are highly complex and heterogeneous mixes, which undergo a series of steps such as physical and chemical processing. Due to the complex nature of food and feed matrices, the identification of key proteins is challenging (Planque et al., 2016). To ensure uniformity of food and feed samples, efficient homogenization using grinding is required. Robust sample preparation is crucial in targeted proteomics (Fig. 1), where samples are extracted, digested, and purified. During the sample preparation for the targeted proteomics, sample is homogenized, and an extraction buffer with detergents and tris-HCl are added. Extracted samples are further reduced and alkalized using dithiothreitol (DTT) and iodoacetamide (IAA) before enzymatic digestion for 12 h with trypsin. Post-digestion solid phase extraction is performed, and isotopically labeled targeted peptides of interest are added before analyzing samples using MS (M.C. Lecrenier et al., 2021). The peptides are analyzed using LC-MS instruments (Orbitraps, Q-TOFs, triple quadrupole systems) in traditional (PRM or MRM) or advanced targeted acquisition mode. Generally, a chromatographic column of 2.1 × 150 mm C18 with a flowrate of 0.2 mL/min is used with mobile phase A containing water in 0.1% formic acid and mobile phase B acetonitrile in 0.1% formic acid (Lecrenier et al., 2018). For example, in targeted analyses of milk and hemoglobin peptides as described by Lecrenier et al. (2018), elution was carried out as follows: 92% mobile phase A from 0 to 3 min, decreasing to 58% mobile phase A from 3 to 18 min, then to 15% mobile phase A from 18 to 18.10 min, maintained at 15% mobile phase A from 18.10 to 22.50 min, increased back to 92% mobile phase A from 22.50 to 22.60 min and finally held at 92% mobile phase A from 22.60 to 26 min. In this method, a cone flow of 150 L/h and a desolvation flow of 650 L/h of nitrogen were applied. The capillary voltage was set to 3.0 kV, and the collision gas flow was set to 0.20 mL/min. The source and desolvation temperatures were maintained at 150 °C and 350 °C,

Table 1
Proteomics-based methods developed for PAP detection and fish and insect identification.

Category and relevance	Author and year	Technique used	Proteomics approach	Samples Used
Traceability of processed animal proteins	Belghit et al. (2021)	HPLC-MS/MS	Targeted and untargeted	Processed animal proteins – Insect, bovine
	Lecrenier et al. (2021)	HPLC-MS/MS	Targeted	Processed animal proteins – Bovine, pig,
	Steinhilber et al. (2019)	Antibody-based enrichment and HPLC-MS/MS	Targeted	Processed animal proteins - Poultry, pig, bovine, vegetal feed samples
	Niedzwiecka et al. (2019)	Antibody-based enrichment of Hb HPLC -MS/MS	Targeted	Processed animal proteins -Blood products
	Lecrenier et al. (2018)	HPLC-MS/MS	Targeted	Processed animal proteins - blood: bovine and porcine, Milk
	Marchis et al. (2017)	HPLC -MS/MS	Targeted	Processed animal proteins - Bovine, pig, fish, milk
	Marbaix et al. (2016)	HPLC -MS/MS	Untargeted	Processed animal proteins- Bovine, pig, sheep
	Rasinger et al. (2016)	HPLC-MS/MS	Untargeted	Processed animal proteins- Poultry, pig, bovine, sheep
	Lecrenier et al. (2016)	HPLC -MS/MS	Untargeted	Processed animal proteins - Poultry, pork, bovine, fish meal
Authentication of fish species	Chien et al. (2022)	HPLC -MS/MS	Targeted and untargeted	Fish samples – 3 species, processed samples
	Hu et al. (2022)	HPLC -MS/MS	Untargeted	Fish samples – 3 species
	Varunjikar, Moreno-Ibarguen et al. (2022b)	HPLC -MS/MS	Untargeted with spectra library	Fish samples – 7 species, mixed samples
	Gu et al. (2020)	HPLC -MS/MS	Targeted and untargeted	Fish samples – 2 species, processed samples
	Nessen et al. (2016)	HPLC -MS/MS	Untargeted with spectra library	Fish samples – 5 species, processed samples
	Wulff et al. (2013)	HPLC -MS/MS	Untargeted with spectra library	Fish samples – 22 different species
Authentication of feed and food-grade insect species	Bose et al., 2021	HPLC-MS/MS	Targeted and untargeted	Insect samples – 1 insect species
	Varunjikar, Belghit, et al. (2022a)	HPLC-MS/MS	Untargeted with spectra library	Insect samples – 5 insect species
	Stobernack et al. (2022)	HPLC-MS/MS	Targeted and untargeted	Insect samples – 1 insect species
	Bose et al. (2021)	HPLC-MS/MS	Targeted and untargeted	Insect samples – 1 insect species
	Francis et al. (2020)	HPLC-MS/MS	Untargeted	Insect samples - 4 insect species
	Leni, tedeschi, et al. (2020)	HPLC-MS/MS	Targeted	Insect samples - 2 insect species
	Belghit et al. (2019)	HPLC-MS/MS	Untargeted with spectra library	Insect samples – 4 insect species

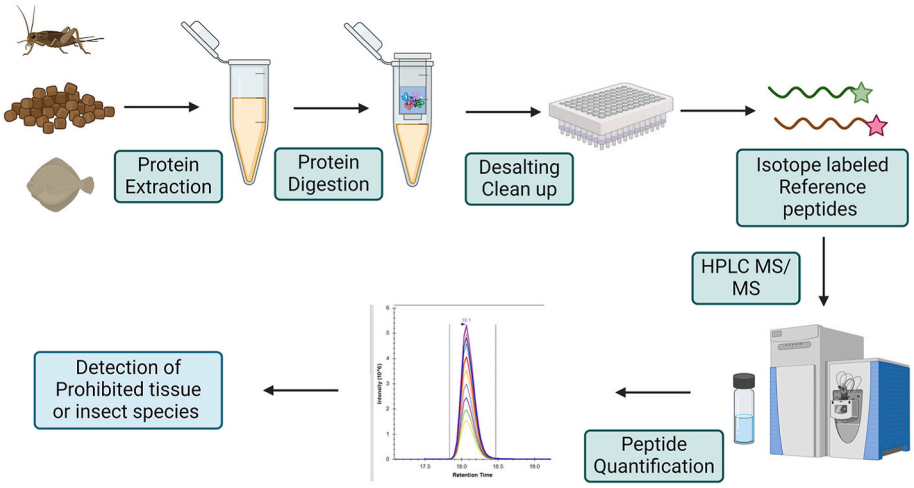

Fig. 1. Workflow for targeted proteomics with multiple reaction monitoring for identification of food and feed samples. Proteins are extracted from samples, digested, desalted, and spiked with isotope-labeled reference peptides, i.e., peptide biomarkers for proteins. Using the UHPLC MS/MS system, peptides are quantified using this workflow feed and, samples are authenticated on species and tissue levels. Generated using app.biorender.com. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

respectively. The cone voltage was fixed at 35 V. Data acquisition and processing were performed using MassLynx software (Lecrenier et al., 2018). The optimization of a targeted method aims at maximizing the peptide signals while minimizing the matrix effects. Matrix effects are defined as the variation observed in detecting or quantifying the target peptide when multiple substances are present in the sample (M.C.

Lecrenier et al., 2021). This effect is due to the co-elution of other molecules with the target peptides and dramatically influences the identification and quantification of analytes (Zhou, Yang, & Wang, 2017). These effects are of significant concern in MS analysis as this affects the sensitivity and the accuracy of the method by decreasing or increasing the signal intensity (Zhou et al., 2017) and background noise (M. C. Lecrenier, Plasman, Cordonnier, & Baeten, 2023). As matrix effects cannot be avoided completely during LC-MS analysis, certain precautions can be taken during sample preparations and HPLC-MS/MS analyses to minimize these (Zhou et al., 2017). Implementation of sample clean-up steps such as solid-phase extraction removes interfering substances, thereby reducing matrix effects. Additionally, dilution strategies are also capable of decreasing matrix components while maintaining measurable levels of targeted peptides, which helps to reduce ion suppression. Amendments to HPLC parameters, such as low flow rate, slow gradient, and mixed column mode, can help to minimize matrix effects (Zhou et al., 2017). While developing methods, validation must be performed on different food samples to ensure the robustness of the method.

Many strategies exist to remove the undesirable compounds that interfere with detecting targeted peptides by mass spectrometry. In the context of PAP detection, various approaches are already proposed, such as immunoaffinity enrichment of the targeted peptides (Niedzwiecka et al., 2019, 2019; Steinhilber et al., 2018b; 2019) or preliminary sedimentation concentrating the ingredients of interest (Lecrenier, 2023). Such measures are adopted to improve the sensitivity of peptide detection during sample analyses.

Different sample preparation strategies can lead to the selection of different markers, as highlighted by an interlaboratory study on the detection of bovine PAPs (M.-C. Lecrenier et al., 2021). Therefore, it is essential to choose the optimal target peptide markers. Food or feed processing methods such as heat or acid treatment can cause post-translational modifications in proteins (Piras, Roncada, Rodrigues, Bonizzi, & Soggiu, 2016). Such modifications result in mass changes that can make the analyte undetectable MS analyses if it is not included in the peptide selection (Raes et al., 2018).

MRM is the targeted proteomics method of choice when the candidate proteins to target are known based on the sample composition or a literature review. Two approaches may be considered for identifying target peptides for an MRM assay. Firstly, an *in-silico* approach which can be performed with freely available software. The *in-silico* approach predicts the peptides generated by the digestion of a protein obtained from a protein sequence database. Freely available software such as Skyline (https://skyline.gs.washington.edu) can be used to create a theoretical list of peptides and their related transitions and design the MRM method. Similarly, a targeted method can be prepared from a preliminary discovery study conducted using an untargeted approach (M. C. Lecrenier et al., 2016). This approach is necessary in cases where the protein composition of the sample is unknown (described in point 1.2).

Targeted proteomics can be further developed into absolute quantification methods, which involve the addition of stable isotope standard peptides (SIS peptides) to the samples. For this, targeted peptides are identified from the protein of interest based on their suitability for the MS analyses. The SIS peptides are synthesized, stable isotope-labeled versions of the target peptides, which differ slightly in mass but have the same chromatographic abilities. The labeled peptides are spiked into the sample in known quantities before LC-MS/MS analysis, allowing them to co-elute with the endogenous peptides. SIS peptides compensate for loss during sample preparation and any changes in retention time during HPLC and monitor the MS ratios that can be altered due to inherent matrix effects. Instead of SIS peptides, synthetic proteins can be added as a linear concatenation of tryptic peptides. These concatemers are cleaved to individual peptides upon digestion with trypsin and can, therefore, be added from the start of the preparation. In this manner, the relative signal intensities of the labeled and endogenous peptides are compared. This comparison allows for precise quantification of the target peptides, accounting for variations in sample preparation and instrument response, thereby enhancing the accuracy and reliability of the targeted MS analysis (Gavage et al., 2020; M. C. Lecrenier et al., 2018).

In complex matrixes such as feed products containing blood meal, blood products, and milk of bovine origine, non-specific signals can be observed despite the high sensitivity and reliability of targeted proteomics (Marchis et al., 2017). In studies conducted on PAP products contaminated with hemoglobin, the lower limit of detection was found to be lower than 0.1% (Lecrenier et al., 2018, Marchi et al., 2017). Although matrix effects are known to affect the limits of detection and quantification, the application of the targeted method could detect 0.015% of blood products in the feed material (Lecrenier et al., 2018, Marchi et al., 2017). The legal limit set by the European Commission, for detection methods used to quantify illicit ingredients in the feed material, a sensitivity of 0.1% (w/w) is required. Most of the targeted proteomics studies are limited to the legal limits of detecting blood products in feed material (Gavage et al., 2020; M. C. Lecrenier et al., 2018; Raes et al., 2018).

In targeted proteomics, quality control is crucial while maintaining the efficiency required for processing a large number of samples as required for food and feed control. Implementing automated sample preparation methods can reduce time and minimize human error, thereby maintaining consistent quality of samples and enhancing throughput (Gavage et al., 2020). Additionally, samples are spiked with internal standards such as SIS peptides to monitor the efficiency of protein extraction and digestion. Using retention time standards in HPLC analyses can help maintain consistent chromatography across samples (Sobsey et al., 2019). Furthermore, in mass spectrometry analyses, scheduling regular calibration of mass spectrometers to maintain accuracy benefits the accurate detection of peptides. Acquiring data in each run including quality control samples while running analyses, can help to monitor instrument performance and data quality. While processing data, automated data processing pipelines for processing data can improve peak detection and quantification.

In addition to quality control measures, the choice of mass spectrometer and data acquisition techniques are crucial for optimal results in food and feed analyses using targeted proteomics. Triple quadrupole mass spectrometers are preferred for high-sensitivity MRM targeted analyses, whereas Q-TOF and Orbitrap mass spectrometers offer high resolution and mass accuracy and are suitable for data-dependent and independent acquisition. A multi-laboratory ring test conducted by Lecrenier et al. (2021) showed that six laboratories using different types of instruments i.e., high-resolution Q-Orbitrap and low-resolution triple quadrupole were able to detect prohibited bovine material in feed samples. Another study used an orbitrap mass spectrometer in parallel reaction monitoring for targeted acquisition to detect banned feed products (Steinhilber et al., 2019). Both orbitrap and triple quadrupole are suitable for food and feed authentication analyses.

2.2. *Untargeted proteomics approaches*

As novel feed and food materials such as insects, algae, and single-cell proteins are being introduced into the food chain, the development of targeted methods for all species and tissues will be time-consuming. Due to the wide variety of protein sources that can potentially be used to adulterate food and feed material, it is challenging to detect non-permitted proteic material using targeted assays that must be developed for specific use (Varunjikar, 2023). Therefore, to overcome analytical challenges associated with targeted methods in the context of feed and food safety, untargeted proteomics can be implemented to detect species and tissue origin of ingredients in complex mixtures (Belghit et al., 2021; Rasinger et al., 2016; Varunjikar, Moreno-Ibarguen et al., 2022b). As discussed above, sample preparation is a critical part of untargeted proteomics; optimization and standardization of extraction

procedures are crucial due to variability between samples (Belghit et al., 2019). In untargeted proteomics, sample preparation involves extraction of proteins using a buffer with detergents followed bottom-up proteomics, by reduction and alkylation using DTT and IAA, respectively, before performing tryptic digestion for 12 h. Proteins are subsequently digested into peptides and solid phase extraction is performed to remove salt and other impurities before performing MS analyses. For example, Lecrenier et al. (2016), described that peptides were analyzed using an ESI-MS/MS instrument coupled with a nano-UPLC system. The digested samples, corresponding to 1 μg of protein, were separated by reverse-phase liquid chromatography on a C18 column with dimensions of 75 μm × 250 mm using a flow rate of 300 nL/min. The mobile phase consisted of two components: mobile phase A, which was 95% water, 5% acetonitrile, and 0.1% formic acid, and mobile phase B, which was 20% water, 80% acetonitrile, and 0.1% formic acid. The digested sample was injected into the system, and the organic content of the mobile phase was linearly increased from 4% B to 30% B over 160 min, followed by an increase from 30% B to 90% B over 25 min. The system then washed with 90% B for 10 min and reconditioned the column with 4% B for 20 min. The column effluent was directed to a spray source. Mass spectrometry spectra were acquired every 0.5 s in the mass-to-charge (m/z) range of 50–2200. The most intense peptide ions, with charges of 2+ to 4+, were sequenced over 3 s. The collision-induced dissociation (CID) energy was automatically adjusted based on the m/z ratio and charge state of the precursor ion. The system operations were controlled by appropriate software (Lecrenier et al., 2016).Untargeted proteomics methods are extremely useful when targeted MRM proteomics assays are unavailable for a given tissue or species (Varunjikar, Belghit, et al., 2022a). In the untargeted spectral library-based approach, reference spectral libraries are compiled using known reference samples for each specific tissue and species. These reference libraries are used for the detection of unknown samples as shown in Fig. 2 (Önder, Shao, Lam, & Brisson, 2014). Additionally, untargeted methods are often used as a screening tool, investigating a complete proteome of an organism or tissue before developing MRM assays (Marbaix et al., 2016; Rasinger et al., 2016). For the detection of peptide biomarkers, protein sequence information is required to analyze untargeted proteomics data; however, due to the lack of genome and proteome information, it is challenging to detect biomarkers for all food and feed-relevant species. Proteomics analysis using spectra library-based methods can be used to identify and differentiate several sources of food and feed protein. This analytical approach has been developed and implemented in regulatory laboratories for food and feed authentication (Belghit et al., 2021; Nessen et al., 2016; Ohana et al., 2016; Varunjikar, Moreno-Ibarguen et al., 2022b; Wulff, Nielsen, Deelder, Jessen, & Palmblad, 2013). The spectral library-based approach has previously been used for tracing blood meal sources in ticks, seafood, and meat authentication, the detection and species and tissue differentiation of processed animal proteins (PAP), the detection of insect species in feed, and the detection of non-permitted feed material in insect feed, and farmed insects (Ohana et al., 2016; Van Der Plas-Duivesteijn et al., 2014; Varunjikar, 2022a; Varunjikar, Moreno-Ibarguen et al., 2022b; Önder, Shao, Kemps, Lam, & Brisson, 2013). However, generating spectral libraries for food- and feed-relevant species or having a centralized database for food and feed samples is a prerequisite for this approach. In contrast to targeted proteomics methods, the untargeted approach can be either qualitative or semi-quantitative, depending on the data acquisition technique used. When DDA is employed, the approach tends to be more qualitative due to limited cycle time, affecting the ability to achieve optimum points for chromatographic peak quantitation (Peterson, Russell, Bailey, Westphall, & Coon, 2012). However, the spectral library-based approach offers higher sensitivity for identifying peptides present in the spectra libraries (Deutsch et al., 2018; Lam, 2011). Data-independent acquisition (DIA) can overcome the limitations of DDA by providing quantitative reproducibility combined with speed and sensitivity. In the future, along with the spectral library-based approach; the spectral library-free approaches implemented in software like DIA-NN can benefit protein detection and ultimately benefit food safety applications.

Biomarker proteins can be quantified using untargeted proteomic methods in complex matrices such as food and feed. However, targeted proteomic methods are preferred for the quantification of peptides due to their accuracy. Several studies have used the untargeted spectral library method for species detection. However, there is not a large body of literature on the limit of detection and quantification (Ohana et al., 2016; Van Der Plas-Duivesteijn et al., 2014; Varunjikar, 2022b; Varunjikar, Moreno-Ibarguen et al., 2022b; Önder et al., 2013). Belghit et al. (2021) showed that feed adulterated with 1% bovine hemoglobin was detected using a spectral library method, which is indicative of the limit of detection, however the actual limit of detection was not measured specifically in the study.

Quality control measures must be implemented while analyzing food and feed samples to ensure the accuracy and reliability of untargeted proteomics. As mentioned before, targeted proteomics and the use of automated sample preparation benefits throughput and quality control. While working with complex matrices such as food and feed, automated homogenization of samples is crucial (Rasinger et al., 2016).

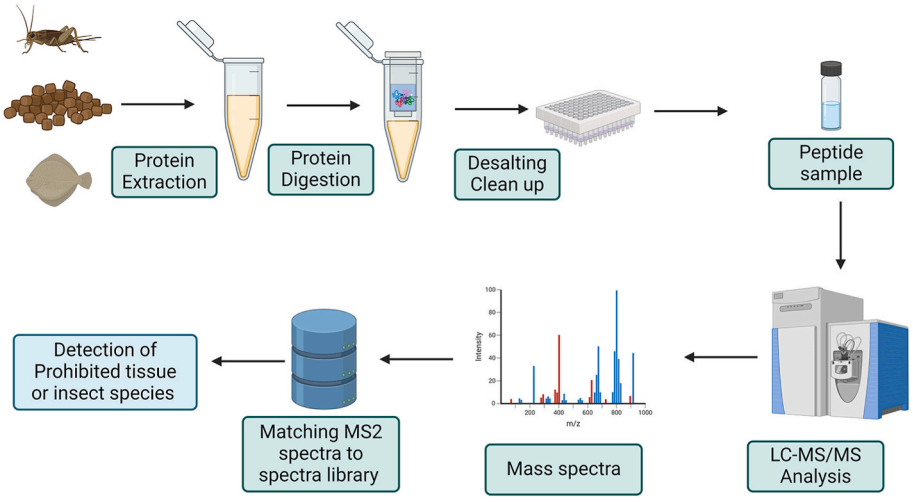

Fig. 2. Untargeted spectra library workflow for food and feed authentication. Proteins are extracted from samples, digested, desalted, and separated using HPLC MS/MS. Collected spectra are matched against the spectra library to detect the tissue and species origin of samples. Generated using app.biorender.com. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Furthermore, spiking samples with known internal standards is recommended to monitor consistent sample preparations. For HPLC-MS/MS analyses, calibration of the retention time, flow rate, and mass spectrometer performance is crucial (Marbaix et al., 2016). Additionally, for untargeted data analyses, the use of a high-quality protein database for peptide identification along with appropriate parameters ensures the accuracy of protein detection. Appropriate quality control measures ensure reproducible results are generated in untargeted proteomics analyses. As described in targeted proteomics, several strategies such as sample clean up, sample dilution, and the use of standardized methods can be employed in untargeted workflow to mitigate matrix effects.

Choice of mass spectrometer and data acquisition techniques are essential in untargeted approach while building spectral libraries for feed and food authentication. High-resolution instruments such as orbitrap mass spectrometers are suitable for data-dependent and independent acquisition, therefore, chosen widely for untargeted analyses. However, previous studies on food and feed material used instruments such as QTOF and ion-trap for building spectral libraries and analyzing samples (Nessen et al., 2016; Varunjikar, Belghit, et al., 2022a). These studies highlight the flexibility of using different mass spectrometers in untargeted proteomics approaches. Orbitrap mass spectrometers are a popular for untargeted proteomics workflow due to their high resolution and mass accuracy. Other instruments, such as QTOF and ion-trap, have also been proven valid for food and feed authentication.

Furthermore, for exploratory proteomics the generation of a proteome database is crucial for searching proteins. In the case of commercially important species, proteome information is limited, or genome information is incomplete. Protein databases are created by translating genomes, using tools such as Expasy Translate tool, into amino acids. For spectra searching, open-source tools such as MaxQuant, Trans-proteomic pipeline, skyline, and Spectronaut can be used. Detected peptides are used for selecting species-specific markers; software-based selection or machine-learning algorithms can be used to identify these peptides, which can benefit food and feed authentication (Marbaix et al., 2016; Rasinger et al., 2016). If the genome is unavailable for the species of interest, direct spectral comparison (Ohana et al., 2016; Rasinger et al., 2016) or DIA-based approaches can be used to authenticate species in food and feed samples.

3. Implementing proteomics for the safe reintroduction of processed animal proteins (PAPs) into the food chain

Animal by-products (ABPs) are defined as "animal-derived materials no longer intended for human consumption and deemed safe for utilization as animal feed" (L. W. D. van Raamsdonk et al., 2019). In the circular economy concept, these materials are valuable feed ingredients for terrestrial livestock and aquaculture. However, due to the risk of transmissible spongiform encephalopathies (TSEs), a complete ban was introduced on the use of ABPs such as processed animal proteins (PAPs) (European Comission 2001/999). Prions were found to be the causative agent of TSE, and PAP utilization in animal feed was identified as the likely cause of bovine spongiform encephalopathy (BSE) transmission observed in 2001 (European Comission 2001/999, L. W. D. van Raamsdonk et al., 2019). A risk assessment conducted by the European Food Safety Authority (EFSA) indicated that the presence of ruminant proteins in animal feed was associated with an increased risk of BSE outbreaks; the risk was considered negligible if ruminant proteins were absent from animal feed (EFSA, 2005). ABPs were categorized into three categories according to their risks to farm animals, the environment, and public health (European Commission 2002/1774). Category 3 materials are ABPs from healthy animals that were fit for human consumption but are no longer intended for human consumption, which includes processed blood, bone, and other body parts (European Comission 2001/999).

In 2013, after meticulous re-evaluations of non-ruminant PAPs, these protein sources were reauthorized for use as feed material in aquaculture (European Commission, 2013/56, EFSA, 2011). In order to control for compliance with the legislation, the real-time polymerase chain reaction (rtPCR) method was developed to detect the presence of ruminant material in feed samples (European Commission, 2013/51; European Commission, 2013/56). The current operational protocol followed by the European Union-Reference Laboratory for Animal Proteins (EURL-AP) for feed analyses is shown in Fig. 3. The official method implemented first for PAP detection includes light microscopy, and if the sample tests positive in light microscope testing, rtPCR is implemented for further testing.

An EFSA assessment in 2018 revealed a fourfold reduction in the risk of BSE compared to 2011 (EFSA, 2018). As a result, the reintroduction of non-ruminant PAPs, specifically pig and poultry-derived, was approved in Europe (European Commission, 2021/1372), and present regulations permit the inclusion of PAPs in aquafeed, poultry, and pig feed (European Commission, 2021/1372). The development of analytical techniques played a pivotal role in facilitating the safe reintegration of non-ruminant PAPs into the food chain in compliance with evolving legislative guidelines. However, the legalization of certain PAP products, especially in terrestrial animal feed, such as collagen, gelatin, bone, and blood meal, poses an analytical challenge since none of the official methods (rtPCR and light microscopy (European Commission, 2013/51)) can determine both species and tissue of origin simultaneously. While the microscopy method can identify different tissues (bone, collagen, muscle), it is limited with regard to species identification. The rtPCR can determine species but not tissue of origin. Given the risks associated with BSE, ruminant PAP products such as blood, meat, bone meal, and other tissues are still prohibited from being used as feed products (van Raamsdonk et al., 2019). However, milk products, collagen, and gelatin from ruminants are accepted as legal feed components; hence, distinguishing between different tissues in feed samples is importance.

Proteomics approaches can significantly contribute to the species and tissue-level differentiation of PAP samples in highly processed feed products (Rasinger et al., 2016). Targeted proteomic approaches, such as selected reaction monitoring (SRM) or multiple reaction monitoring (MRM), offer high specificity and sensitivity for detecting specific proteins (M.-C. Lecrenier et al., 2021; Marbaix et al., 2016; Niedzwiecka et al., 2019; Steinhilber et al., 2018a; Steinhilber et al., 2018b, 2019). The precision offered by the MRM assay is crucial compared to DNA-based methods when it comes to the identification and

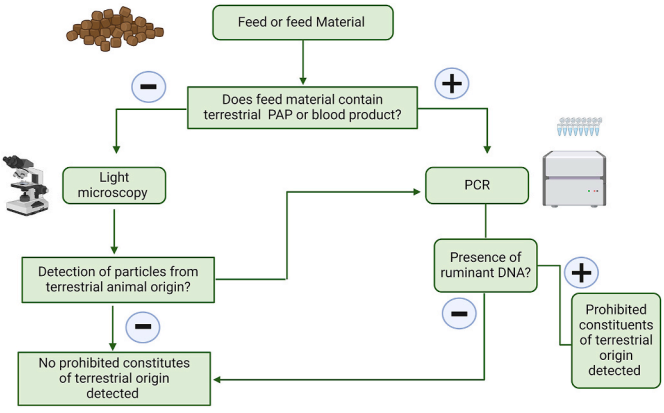

Fig. 3. Standard operational protocol for determination of animal sources in feed material analyses followed by the European Union-Reference Laboratory for Animal Proteins. Plus, sign (+) shows that the results are positive for terrestrial PAP or blood products; minus sign (−) indicates that the results are negative for terrestrial PAP or blood products. Feed material includes products of vegetable or animal origin. *Source: adapted from the European Union-Reference Laboratory for Animal Proteins Standard Operating procedures, 2022.* Generated using app.biorender.com. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

quantification of specific proteins separating prohibited tissue material such as blood or bone from legal ingredients such as milk. DNA-based methods cannot detect tissue origin due to the inherent limitation of the method, and therefore, proteomics methods are suitable for such detection. These targeted proteomic methods enable the differentiation of proteins in highly processed feed samples, including those derived from PAPs. Specific peptide markers such as hemoglobin, casein, beta-lactoglobulin, and collagen have already been demonstrated to distinguish proteins in feed samples (Fumière, Zagon, & Lecrenier, 2022; M.-C. Lecrenier et al., 2021; M. C. Lecrenier et al., 2018; Marbaix et al., 2016; Marchis et al., 2017). Targeted proteomics bridges the regulatory gaps between rtPCR and microscopy by confirming the absence of specific proteins, especially ruminant-derived proteins, in animal feed (Lecrenier et al., 2016, 2018).

A recent inter-laboratory study where six participating laboratories applied their own LC-MS/MS-based proteomic methods for the detection of PAP in samples provided by EURL-AP showed how the targeted methods can be implemented along with official methods to detect prohibited materials in feed (Lecrenier et al., 2021). The sample set analyzed during this study consisted of blank feed or feed adulterated with bovine PAP and milk products at various inclusion levels. An adulteration level of 1% (w/w) from various proteins of bovine origin was successfully detected using this method, and the study showed the potential of targeted LC-MS/MS-based proteomics to address current analytical gaps in the detection and differentiation of PAP (Lecrenier et al., 2021).

Along with the targeted methods, untargeted methods such as spectral library-based methods were implemented for the detection of insect species fed on prohibited PAP materials, such as bovine hemoglobin, to differentiate those from insects fed legal substrates such as milk (Belghit et al., 2021). Insects produced for feed are also considered farmed animals and, thus, are subject to the same feed legislation as other farmed animals (European Commission, 2017/893) (authentication of insect species discussed further in point 4). This work demonstrated that spectral library-based proteomics could monitor and detect non-permitted proteic material along the food chain when applied with other analytical methods. In this study, three types of spectral libraries were built (i.e., insects, bovine milk, and bovine hemoglobin), and feed samples were matched against these libraries to identify prohibited material-fed insect samples (Belghit et al., 2021). Ten samples from five diets were analyzed, and feed material spiked with different concentrations of bovine hemoglobin was analyzed. This study showed that the spectral library method is effective for detecting prohibited PAPs in feed samples or insects fed on illicit PAP samples (Belghit et al., 2021).

In summary, safely reintegrating Processed Animal Proteins (PAPs) into the feed and food chain is crucial to maintaining protein security in Europe (EuropaBio Annual EuropaBio annual Report, 2018). However, for animal and consumer safety and for regulatory compliance, the feed and food must be produced from novel recycled protein sources. Proteomic methods such as targeted and untargeted proteomics can facilitate the safe utilization of non-ruminant PAPs by detecting illicit ingredients in complex feed mixtures. Fig. 4 shows how proteomic methods can be implemented in standard operational protocols to determine animal sources in aquafeed material and other molecular methods. In relation to feed ban relaxation for regulatory purposes, feed samples are analyzed using light microscopy. If the results are positive for terrestrial PAP, further analyses are performed using rt-PCR. Further, if the sample tests positive for bovine material, it is crucial to determine whethe the positive signal is due to the presence of milk, a legal ingredient, or a prohibited ingredient, such as blood or bone. Therefore, mass spectrometry-based methods can be implemented in the regulatory framework to test the tissue origin of PAP samples. Furthermore, proteomic methods can provide additional precision, facilitating the detection and differentiation of specific proteins, especially those derived from prohibited sources. However, "on-spot" testing kits are difficult to develop using proteomics-based approaches due to the analytical complexity of the methods. Devices such as MasSpect pen Technology can be further developed for regulatory use to overcome current limitations in order to monitor the supply chain.

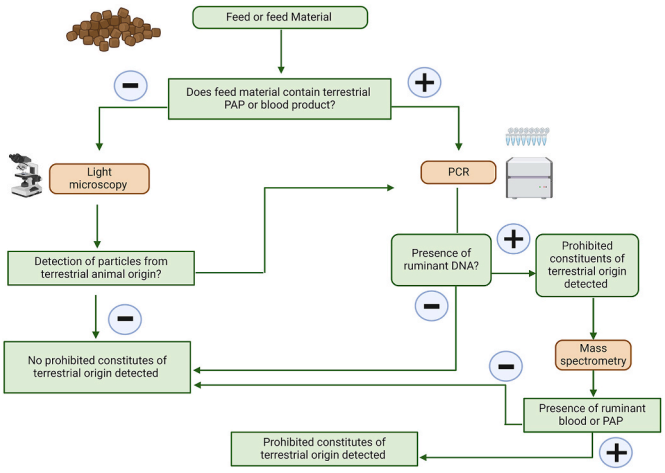

Fig. 4. Standard operational protocol for determination of animal sources in feed material analyses followed by the European Union-Reference Laboratory for Animal Proteins for future. In relation to feed ban relaxation, mass spectrometry-based proteomics can be implemented in the future with official methods i.e., light microscopy and PCR. Plus, sign (+) shows that the results are positive for terrestrial PAP or blood products; minus sign (−) indicates that the results are negative for terrestrial PAP or blood products. *Source: adapted from the European Union-Reference Laboratory for Animal Proteins Standard Operating Procedures, 2022 and workshop of the European Union-Reference Laboratory for Animal proteins Standard Operating Procedures, a future combination of methods.* Generated using app.biorender.com. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Proteomics for authentication of fish species from food and feed

In circular food supply chains, aquaculture practices can circulate by-products into high-quality seafood. Classic aquafeed ingredients such as fishmeal are sourced from capture fisheries and used as feed ingredients, while by-products from farmed fish are prohibited from being used as feed ingredients for the same species; this requires the development of tools such as PCR to identify fish species in feed (L. W. D. D. van Raamsdonk, Prins, van de Rhee, Vliege, & Pinckaers, 2017). Moreover, in the global food market, the mislabeling of fish products has been an issue, with approximately 27% of cases indicating incorrect labeling of seafood products (Bouzembrak et al., 2018; Khaksar et al., 2015). It is essential to specify the origin of seafood, whether it is produced by aquaculture or caught in the wild. As per European regulation, consumers should be properly informed about the contents of the food they consume due to safety reasons (European Commission 2011/1169). Reliable analytical methods are essential to detect fraudulent labeling of seafood products, to ensure consumer safety, and to adhere to the food authenticity regulations.

In recent years, DNA-based methods such as DNA-barcoding, quantitative polymerase chain reaction (qPCR), and shotgun sequencing have been employed for seafood authentication purposes due to their ability to discriminate between closely related fish species (Klapper et al., 2023; Sawyer, Wood, Shanahan, Gout, & McDowell, 2003; Shokralla, Hellberg, Handy, King, & Hajibabaei, 2015; Varunjikar, Moreno-Ibarguen et al., 2022b). For the quantification of mixed seafood (mixture of multiple species) products, combinations of several approaches, such as metabarcoding and next-generation sequencing, were shown to be effective (Klapper et al., 2023; Varunjikar, Moreno-Ibarguen et al., 2022a).

However, when dealing with highly processed, thermally- and acid-treated seafood materials, identifying and quantifying fish species can be challenging for DNA-based methods. Proteomics offers a promising supportive methods for the identification of fish species in both pure and mixed seafood samples (Carrera, Cañas, & Gallardo, 2013; Chien et al., 2022; Hu et al., 2022; Nessen et al., 2016; Varunjikar, Moreno-Ibarguen et al., 2022a; Wulff et al., 2013). Similar to the DNA-based methods, it is important to standardize collection, storage, and processing of samples while analyzing highly processed fish samples using proteomic methods.

Among these methods, targeted proteomic methods often rely on species-specific peptide markers, which are highly effective in detecting of species (Carrera et al., 2013; Hu et al., 2022). Recent studies have shown that peptide biomarkers from proteins such as Collagen alpha-2 (I) chain isoform X1, myosin, light polypeptide 3–1, Collagen alpha-2 (I) chain isoform X1, and Collagen alpha-1(I) chain were effective to identify adulteration of Atlantic salmon and rainbow trout in seafood products (Gu et al., 2020). Previously, for the differentiation of fish species from the Merlucciidae family, parvalbumin fractions were used for the classification of ten commercially valued species (Carrera et al., 2013).

While targeted proteomics methods are known to be effective in species identification and authentication, the detection of specific peptide marker reference proteome from targeted species is required. Reference proteomes are not readily available in the UniProt or NCBI protein databases for detecting species-specific peptide markers in non-model fish species to validate targeted methods. For example, for zebrafish (model organism), more reviewed and unreviewed protein sequences are available in the UniProt database compared to commercially important species such as cod, haddock, salmon, tilapia, and pangasius as shown in Table 2. To validate targeted peptide markers, reviewed proteomic sequences provide a reliable reference for confirming the presence of peptide markers in the test samples (Desiere, 2006). Consequently, databases need to be developed using available genome sequences and translation tools, as mentioned in 1.2. Similarly, it is possible to include sequences from model organisms or higher taxonomic levels, i.e. (teleost relevant taxa), to detect species-specific markers. It is challenging to implement targeted methods with species-specific biomarkers for seafood authentication (Varunjikar, Moreno-Ibarguen et al., 2022b), and, along with established targeted proteomics methods, spectral library-based proteomic methods can be implemented for species identification and quantification of seafood in mixed samples (Nessen et al., 2016; Varunjikar, Moreno-Ibarguen et al., 2022b; Wulff et al., 2013).

Spectral library-based approaches have previously been used to identify fish species from 47 samples (Wulff et al., 2013) and closely related flatfish species in processed and fresh samples (Nessen et al., 2016). Furthermore, the spectral library-based approach was practical for quantifying fish species in mixed samples containing cod, tilapia, and pangasius (Varunjikar, Moreno-Ibarguen et al., 2022b). Generating accurate spectral libraries for fish species is a prerequisite for implementing the spectral library approach in proteomics. The libraries contain high-quality tandem mass spectra to which sample spectra can be matched to quantify and identify given fish species in the mixture. Spectral library-based proteomics provides a helpful tool for seafood authentication due to its analytical flexibility and capability to simultaneously detect and quantify several proteins. However, spectral library methods face challenges in accurately quantifying closely related species in mixed samples, such as cod and haddock (Varunjikar, Moreno-Ibarguen et al., 2022b). The conserved nature of proteins in closely related species, i.e., similarity in protein sequences, reduces the quantification accuracy of the spectral library method (Nessen et al., 2016). In contrast, DNA-based methods such as shotgun DNA sequencing are more accurate when quantifying closely related species in mixed samples (Klapper et al., 2023). Among the proteomic approaches, targeted proteomics methods are capable of separating closely related species, such as Atlantic salmon and rainbow trout, by focusing on selected peptide biomarkers (Gu et al., 2020). The study conducted by Gu et al. (2020) analyzed 37 samples from different regions and batches and commercially available processed food products. The study identified 30 Atlantic salmon samples and five rainbow trout samples by detecting adulteration instances where precision and recovery of the method were measured using relative standard deviation.

Overall, a combination of DNA-based and proteomic methods can be used to authenticate fish species in food and feed products (Varunjikar, Moreno-Ibarguen et al., 2022b). Protein sequences will also become more readily available as genetic information for fish species becomes more abundant and easily accessible, which can benefit species-specific biomarker design and detection for targeted proteomics approaches. Implementing proteogenomic approaches on commercially important fish species will also benefit seafood and feed safety studies.

Table 2
Comparison of reviewed and unreviewed sequences in model and non-model commercial fish species. Compared to non-model species, more reviewed sequences were available for zebrafish (*Danio rerio*). Based on Uniprot (www.uniprot.org) data from 2023.

Organisms (Fish)	Scientific name	Reviewed number of UniProt KB sequences	Unreviewed number of UniProt KB sequences
Zebrafish (model organism)	*Danio rerio*	3601	114570
Atlantic cod	*Gadus morhua*	64	63698
Atlantic haddock	*Melanogrammus aeglefinus*	1	200
Nile tilapia	*Oreochromis niloticus*	22	79640
Northern pike	*Esox lucius*	113	80077
Atlantic salmon	*Salmo salar*	182	89282
Platyfish	*Xiphophorus maculatus*	8	35388
Pangasius	*Pangasianodon hypophthalmus*	0	21535

5. Proteomics for authentication of feed and food-grade insect species

Insects are sustainably cultivated using food by-products and have high contents of protein, fat, and minerals, such that they are considered an ideal ingredient for animal feed (Lock, Arsiwalla, & Waagbø, 2016; Makkar, Tran, Heuzé, & Ankers, 2014; L. W. D. van Raamsdonk et al., 2019). Food and farm by-products can be effectively valorized by cultivating insect species to produce feed and food ingredients (van Huis, 2020). When used as feed ingredients, insects are considered PAPs and are subjected to the same rules and regulations as farmed animals, including the legislation on the prevention of TSE (European Commission, 2021/1372; European Commission, 2017/893; European Commission 2021/1925). Insect species can be used as feed material for pigs, poultry, and aquafeed (European Commission, 2021/1372; European Commission, 2017/893; European Commission 2021/1925). The insect species authorized to be used as feed material in the European Union include (i) black soldier fly (BSF; *Hermetia illucens*), (ii) common housefly (*Musca domestica*), (iii) yellow mealworm (*Tenebrio molitor*), (iv) lesser mealworm (*Alphitobius diaperinus*), (v) house cricket (*Acheta domesticus*), (vi) banded cricket (*Gryllodes sigillatus*), (vii) field cricket (*Gryllus assimilis*), and (viii) silkworm (*Bombyx mori*) (European Commission, 2021/1372; European Commission, 2017/893; European Commission 2021/1925). Due to their high protein, fat, and mineral content, insects can also be food ingredients. Currently, yellow mealworm (*Tenebrio molitor*), migratory locust (*Locusta migratoria*), and house cricket (*Acheta domesticus*) are allowed to be used as novel food ingredients in the European food market (European Commission, 2021/882, European Commission, 2022/188).

Authentication of insect species is crucial to ensure food safety, as illicit insect species can enter the food and feed chain (L. W. D. van Raamsdonk et al., 2019). Analytical methods such as microscopy are still under development for detecting insect species, as the chitin fragments in krill and insects are known to be difficult to discriminate (L. W. D. D. van Raamsdonk et al., 2017). PCR-based reactions have been developed to authenticate legal insect species with a high specificity and sensitivity in feed materials (Zagon, Di Rienzo, Potkura, Lampen, & Braeuning, 2018). For the detection of edible insect species in Europe, such as yellow mealworm (*Tenebrio molitor*), migratory locust (*Locusta migratoria*), and house cricket (*Acheta domesticus*), a multiplexed real-time PCR method has been developed which can authenticate edible insect species (Köppel et al., 2019). The near-infrared spectrometry (NIR) method is based on the profiling of samples by using electromagnetic spectra. This non-invasive method measures light absorbed by the chemical bonds to detect insect species in feed ingredients (L. W. D. van Raamsdonk et al., 2019). The limit of detection for insect PAP using NIR is about 1%, which is above the current acceptable limit of detection. However, the method is widely used by the industry and researchers due to cost-effectiveness, non-invasive nature, and rapid analyses. In addition to these methods, mass spectrometry-based approaches such as proteomics can be used implemented to detect of insect species in food and feed samples for regulatory purposes (Leni, Prandi, et al., 2020).

Due to the limited number of proteins in proteomic databases for insect species, developing targeted proteomic methods for insect authentication has been challenging (Belghit et al., 2019; Bose et al., 2021). However, targeted methods have been developed for the house cricket (*Acheta domesticus*), black soldier fly (BSF; *Hermetia illucens*), lesser mealworm (*Alphitobius diaperinus*), and silkworm (*Bombyx mori*) (Bose et al., 2021; Leni, Prandi, et al., 2020; Stobernack et al., 2022). The method developed by Leni, tedeschi, et al. (2020) demonstrated that the limit of detection (LOD) for insects in aquafeed was 1% (Leni, Prandi, et al., 2020). As more protein sequences become available for insect species of commercial importance, more peptide biomarkers will be detected.

Furthermore, untargeted proteomic approaches were implemented for insect species to detect peptide biomarkers and to create insect spectral libraries (Bose et al., 2021; Francis et al., 2020; Stobernack et al., 2022; Varunjikar, Belghit, et al., 2022a). A spectral library-based approach can be implemented for insect species to overcome the limitation associated with the lack of sequences in genomic and proteomic databases. Previously, this approach has been tested with five insect species, i.e., black soldier fly larvae (*Hermetia illucens*), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), house cricket (*Acheta domesticus*), morio worm (*Zophobas morio*) and for these species spectral libraries were created using two different high-resolution accurate mass instruments (Orbitrap and Q-TOF) (Varunjikar, Belghit, et al., 2022a).

As novel sources of proteins, such as insects, are becoming available in the market, significant concerns are raised due to their allergenic reactions (Pali-Schöll et al., 2019). Along with edible insects, algae spirulina proteins and fungal protein sources such as Quorn also cause allergenic reactions (Hoff, Trüeb, Ballmer-Weber, Vieths, & Wuethrich, 2003; Le, Knulst, & Röckmann, 2014). Several rapid, sensitive, immunochemical, and DNA-based methods have been developed for allergens (Monaci & Visconti, 2010). Mass spectrometry-based proteomic approaches are another valid tool for confirming the presence of allergenic proteins and peptides (Bose et al., 2021; Varunjikar, Belghit, et al., 2022a). Thus, non-target proteomics analyses in insect species can also be used for rapid allergenicity pre-screening of novel food sources. For example, Bose et al. (2021) developed an assay to detect and quantify arginine kinase in house cricket (*Acheta domesticus*) samples and provided evidence for the presence of several other allergens. Several allergens, such as tropomyosin, tropomyosin-2, EF-hand proteins, troponin C, and arginine kinase, were detected in five insect species (Varunjikar, Belghit, et al., 2022a). Similarly, untargeted proteomics has been implemented for the allergenicity assessment of lesser mealworm and black soldier fly larvae (Leni et al., 2020). Risk assessment of insects as novel food products by the European Food Safety Authority (EFSA) took into consideration the proteomic and bioinformatics studies performed on insects studies (EFSA NDA panel, 2021a, EFSA NDA panel, 2021b, EFSA NDA panel, 2022).

By employing proteomics approaches, it is possible to authenticate feed and food-grade insect species from feed and food samples. Targeted and untargeted proteomics methods are being developed to detect legal insect species, and the high accuracy of these methods, as demonstrated by Leni, tedeschi, et al. (2020) in processed food materials, is promising. Therefore, in the future, as the sequence information for economically important insect species becomes available, proteomic methods will improve. A recent study on black soldier fly larvae (*Hermetia illucens*) using a metaproteomic approach shows that insect proteomics is expanding (Bose et al., 2021). However, DNA and NIR-based methods are preferred for feed authentication, due to the ease of analyses compared to proteomic-based methods. Analytical complexity is a major hurdle when applying proteomics for insect feed authentication, but developing technologies can overcome these challenges in future.

6. Conclusions

Proteomics approaches can effectively address challenges in circular food chains. In the future, along with established regulatory methods, i.e., rtPCR and microscopy, mass spectrometry-based proteomic approaches can be used; nevertheless, challenges such as feed material homogeneity and proteome availability in the database still exist. For compliance with feed regulations and accurate detection of tissue and species origin of PAP ingredients, proteomics methods will be implemented in routine analyses. Combining the proteomic method with official methods of EURL-PAP for feed analysis will advance the authenticity testing of feed by continuously updating regulations. Similarly, proteomic approaches such as the spectral libraries and library-independent method offer accuracy in fish and insect species authentication, which can benefit enhancing consumer confidence. Overall, proteomics methods can detect potential feed and food fraud in circular food chains, which can ultimately help in regaining consumer trust.

In conclusion, proteomics methods are crucial for authentication of species and tissue origin of protein ingredients and implementing these methods will benefit food and feed safety. With further advancements such as developing MasSpect Pen technology for spot testing proteomic approaches can effectively help combat food fraud in the circular food chain.

Author contributions

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A.-K. Lundebye: Supervision, writing original draft, writing review and editing.

I. Belghit: Writing original draft, writing review and editing.

R. Ørnsrud: Writing original draft, funding acquisition, writing review and editing.

M. G. H. Berntssen: Writing original draft, funding acquisition, writing review and editing.

M. C. Lecrenier: Writing original draft, writing review and editing.

E. Oveland: Writing original draft, writing review and editing.

N. M. Palmblad: Writing original draft, writing review and editing.

J. D. Rasinger: Supervision, writing original draft, funding acquisition, writing review, and editing.

Declaration of generative AI-assisted technologies in the writing process

During the preparation of this work, authors used Grammarly in order to avoid grammatical errors. After using this tool/service, authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of Competing interest

The author, Dr. J. D. Rasinger, is currently employed with the European Food Safety Authority (EFSA) in the Food Ingredients and Packaging Unit (FIP). However, the present article is published under the sole responsibility of the author J. D. Rasinger and may not be considered as an EFSA scientific output. The positions and opinions presented in this article are those of the author alone and are not intended to/do not necessarily represent the views/any official position of EFSA. The author's main contribution to this article were made before joining EFSA when still employed at the Institute of Marine Research."

The other authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

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References

Aebersold, R., & Mann, M. (2003). Mass spectrometry-based proteomics: Abstract: Nature. *Nature, 422*(6928), 198–207.

Belghit, I., Lock, E. J., Fumière, O., Lecrenier, M. C., Renard, P., Dieu, M., et al. (2019). Species-specific discrimination of insect meals for aquafeeds by direct comparison of tandem mass spectra. *Animals, 9*(5). https://doi.org/10.3390/ani9050222

Belghit, I., Varunjikar, M., Lecrenier, M.-C. C., Steinhilber, A. E., Niedzwiecka, A., Wang, Y. V. V., et al. (2021). Future feed control – tracing banned bovine material in insect meal. *Food Control, 128*(April), Article 108183. https://doi.org/10.1016/j.foodcont.2021.108183

Boqvist, S., Söderqvist, K., & Vågsholm, I. (2018). Food safety challenges and one health within Europe. *Acta Veterinaria Scandinavica, 60*, 1. https://doi.org/10.1186/s13028-017-0355-3

Bose, U., Broadbent, J. A., Juhász, A., Karnaneedi, S., Johnston, E. B., Stockwell, S., et al. (2021). Protein extraction protocols for optimal proteome measurement and arginine kinase quantitation from cricket Acheta domesticus for food safety assessment. *Food Chemistry, 348*, Article 129110. https://doi.org/10.1016/J.FOODCHEM.2021.129110

Bouzembrak, Y., & Marvin, H. J. P. (2016). Prediction of food fraud type using data from Rapid Alert System for Food and Feed (RASFF) and Bayesian network modelling. *Food Control, 61*, 180–187. https://doi.org/10.1016/j.foodcont.2015.09.026

Bouzembrak, Y., Steen, B., Neslo, R., Linge, J., Mojtahed, V., & Marvin, H. J. P. (2018). Development of food fraud media monitoring system based on text mining. *Food Control*. https://doi.org/10.1016/j.foodcont.2018.06.003

Carrera, M., Cañas, B., & Gallardo, J. M. (2013). Proteomics for the assessment of quality and safety of fishery products. *Food Research International, 54*(1), 972–979. https://doi.org/10.1016/j.foodres.2012.10.027

Chien, H. J., Huang, Y. H., Zheng, Y. F., Wang, W. C., Kuo, C. Y., Wei, G. J., et al. (2022). Proteomics for species authentication of cod and corresponding fishery products. *Food Chemistry, 374*, Article 131631. https://doi.org/10.1016/J.FOODCHEM.2021.131631

Desiere, F. (2006). The PeptideAtlas project. *Nucleic Acids Research, 34*(90001), D655–D658. https://doi.org/10.1093/nar/gkj040

Deutsch, E. W., Perez-Riverol, Y., Chalkley, R. J., Wilhelm, M., Tate, S., Sachsenberg, T., et al. (2018). Expanding the use of spectral libraries in proteomics HHS Public Access. *Journal of Proteome Research, 17*(12), 4051–4060. https://doi.org/10.1021/acs.jproteome.8b00485

Duivesteijn, S. J. (2018). Advancing zebrafish models in proteomics. https://hdl.handle.net/1887/66790.

EC. (2021). Commission Regulation (EU) 2021/1372 of 17 August 2021 amending Annex IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards the prohibition to feed non-ruminant farmed animals, other than fur animals, with protein deri. *Official Journal of the European Union, 64*, 1–21.

EFSA. (2005). Opinion of the Scientific Panel on biological hazards (BIOHAZ) on the "Quantitative risk assessment of the animal BSE risk posed by meat and bone meal with respect to the residual BSE risk.". *EFSA Journal, 3*(9). https://doi.org/10.2903/J.EFSA.2005.257

EFSA. (2018). Updated quantitative risk assessment (QRA) of the BSE risk posed by processed animal protein (PAP). *EFSA Journal, 16*(7). https://doi.org/10.2903/J.EFSA.2018.5314

EFSA NDA panel. (2021a). Safety of dried yellow mealworm (Tenebrio molitor larva) as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal, 19*(1), 1–29. https://doi.org/10.2903/j.efsa.2021.6343

EFSA NDA panel. (2021b). Safety of frozen and dried formulations from whole house crickets (Acheta domesticus) as a Novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal, 19*(8). https://doi.org/10.2903/j.efsa.2021.6779

EFSA NDA panel 2022. (2022). Safety of frozen and freeze-dried formulations of the lesser mealworm (Alphitobius diaperinus larva) as a Novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal, Turck, Dominique Bohn, Torsten Castenmiller, Jacqueline De Henauw, Stefaan Hirsch-Ernst, Karen Ildico Maciuk, Alexandre Mangelsdorf, Inge McArdle, Harry J. Naska, Androniki Pelaez, Carmen Pentieva, Kristina Siani, Alfonso Thies, Frank Tsabou, 20*(7). https://doi.org/10.2903/j.efsa.2022.7325

Efsa, Panel, E., Biohaz, H., Andreoletti, O., Budka, H., Buncic, S., et al. (2011). Scientific Opinion on the revision of the quantitative risk assessment (QRA) of the BSE risk posed by processed animal proteins (PAPs). *EFSA Journal, 9*(1), 1947. https://doi.org/10.2903/j.efsa.2011.1947

EuropaBio annual Report. (2018). The EU protein gap a trade policies and GMOs. https://www.gmoinfo.eu/uk/files/353-eu-protein-gap-wcover-06-08.pdf.

European Comission 2001/999. (2001). Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies (31/5/2001). *Official Journal of the European Communities*, 1–69. May.

European Comission. (2021). Commission implementing regulation (EU) 2021/882 of 1 june 2021 authorising the placing on the market of dried Tenebrio molitor larva as a novel food under regulation (EU) 2015/2283 of the European parliament and of the Council, and amending commission im. *Official Journal of the European Union, 64*(L194), 16–20. http://data.europa.eu/eli/reg_impl/2021/882/oj.

European Commission. (2013). Commission Regulation (EU) No 56/2013 of 16 January 2013 amending Annexes I and IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council laying down rules for the prevention, control and eradication of certain transmissible. *Official Journal of the European Union L*, (21), 3–16.

European Commission. (2013). COMMISSION REGULATION (EU) No 51/2013of 16 January 2013amending Regulation (EC) No 152/2009 as regards the methods of analysis for the determination of constituents of animal origin for the official control of feed. *Official Journal of the European Union, 2013*(L 20/33), 33–43. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013R0051&from=EN.

European Commission. (2017). Commission regulation (EU) 2017/893. *Official Journal of the European Union, 2017*(May), 1–25. https://publications.europa.eu/en/publication-detail/-/publication/7d1c640d-62d8-11e7-b2f2-01aa75ed71a1/language-en%0A.

European Commission. (2022). Authorising the placing on the market of frozen, dried and powder forms of Acheta domesticus as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council, and amending Commission Implementing Regulation (EU) 2017/2470. *Official Journal of the European Union, 188*(30), 108–114.

European Commission 2021/1925. (2021). Commission Regulation (Eu) 2021/1925. *Official Journal of the European Union, 1925*(1069), 4–8. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32021R1925&from=EN.

FAO. (2023). Ad-hoc Joint FAO/WHO Expert consultation on risks and benefits of fish consumption. https://www.who.int/publications/m/item/ad-hoc-joint-fao-who-expert-consultation-on-risks-and-benefits-of-fish-consumption.

Flynn, K., Villarreal, B. P., Barranco, A., Belc, N., Björnsdóttir, B., Fusco, V., et al. (2019). An introduction to current food safety needs. *Trends in Food Science & Technology, 84*, 1–3. https://doi.org/10.1016/J.TIFS.2018.09.012

Francis, F., Mazzucchelli, G., Baiwir, D., Debode, F., Berben, G., & Caparros Megido, R. (2020). Proteomics based approach for edible insect fingerprinting in novel food: Differential efficiency according to selected model species. *Food Control, 112*(November 2019), Article 107135. https://doi.org/10.1016/j.foodcont.2020.107135

Fumière, O., Zagon, J., & Lecrenier, M.-C. (2022). Re-Authorization of gelatin and collagen of ruminant origin in non-ruminant feed: A new analytical challenge for the control of the feed ban. *Biotechnology, Agronomy, Society and Environment, 26*(S), 303–308. https://doi.org/10.25518/1780-4507.20059

Gavage, M., Van Vlierberghe, K., Van Poucke, C., De Loose, M., Gevaert, K., Dieu, M., et al. (2020). Comparative study of concatemer efficiency as an isotope-labelled internal standard for allergen quantification. https://doi.org/10.1016/j.foodchem.2020.127413.

Ghisellini, P., Cialani, C., & Ulgiati, S. (2016). A review on circular economy: The expected transition to a balanced interplay of environmental and economic systems. *Journal of Cleaner Production, 114*, 11–32. https://doi.org/10.1016/j.jclepro.2015.09.007

Gossner, C. M. E., Schlundt, J., Embarek, P. B., Hird, S., Lo-Fo-Wong, D., Beltran, J. J. O., et al. (2009). The melamine incident: Implications for international food and feed safety. *Environmental Health Perspectives, 117*(12), 1803–1808. https://doi.org/10.1289/ehp.0900949

Gu, S., Deng, X., Shi, Y., Cai, Y., Huo, Y., Guo, D., et al. (2020). Identification of peptide biomarkers for authentication of Atlantic salmon and rainbow trout with untargeted

and targeted proteomics approaches and quantitative detection of adulteration. *Journal of Chromatography B, 1155*, Article 122194. https://doi.org/10.1016/J.JCHROMB.2020.122194

Hoff, M., Trüeb, R. M., Ballmer-Weber, B. K., Vieths, S., & Wuethrich, B. (2003). Immediate-type hypersensitivity reaction to ingestion of mycoprotein (Quorn) in a patient allergic to molds caused by acidic ribosomal protein P2. *The Journal of Allergy and Clinical Immunology, 111*(5), 1106–1110. https://doi.org/10.1067/MAI.2003.1339

Hu, L., Zhang, H., Hu, Z., Chin, Y., Zhang, X., Chen, J., et al. (2022). Comparative proteomics analysis of three commercial tuna species through SWATH-MS based mass spectrometry and chemometrics. *Food Control, 141*, Article 109162. https://doi.org/10.1016/J.FOODCONT.2022.109162

Silva, C. J. (2018). Food forensics: Using mass spectrometry to detect foodborne protein contaminants, as exemplified by shiga toxin variants and prion strains. *Journal of Agricultural and Food Chemistry, 66*(32), 8435–8450. https://doi.org/10.1021/acs.jafc.8b01517

Khaksar, R., Carlson, T., Schaffner, D. W., Ghorashi, M., Best, D., Jandhyala, S., et al. (2015). Unmasking seafood mislabeling in U.S. Markets: DNA barcoding as a unique technology for food authentication and quality control. *Food Control*. https://doi.org/10.1016/j.foodcont.2015.03.007

Klapper, R., Velasco, A., Döring, M., Schröder, U., Sotelo, C. G., Brinks, E., et al. (2023). A next-generation sequencing approach for the detection of mixed species in canned tuna. *Food Chemistry X, 17*, Article 100560. https://doi.org/10.1016/J.FOCHX.2023.100560

Köppel, R., Schum, R., Habermacher, M., Sester, C., Lucia, Piller, E., et al. (2019). Multiplex real-time PCR for the detection of insect DNA and determination of contents of Tenebrio molitor. *Locusta migratoria and Achaeta domestica in food, 245*, 559–567. https://doi.org/10.1007/s00217-018-03225-5

Lam, H. (2011). Building and searching tandem mass spectral libraries for peptide identification. *Molecular and Cellular Proteomics, 10*(12), 1–10. https://doi.org/10.1074/mcp.R111.008565

Lavelli, V. (2021). Circular food supply chains – impact on value addition and safety. *Trends in Food Science & Technology, 114*, 323–332. https://doi.org/10.1016/J.TIFS.2021.06.008

Le, T. M., Knulst, A. C., & Röckmann, H. (2014). Anaphylaxis to Spirulina confirmed by skin prick test with ingredients of Spirulina tablets. *Food and Chemical Toxicology, 74*, 309–310. https://doi.org/10.1016/J.FCT.2014.10.024

Lecrenier, M. C., Marbaix, H., Dieu, M., Veys, P., Saegerman, C., Raes, M., et al. (2016). Identification of specific bovine blood biomarkers with a non-targeted approach using HPLC ESI tandem mass spectrometry. *Food Chemistry, 213*(1774), 417–424. https://doi.org/10.1016/j.foodchem.2016.06.113

Lecrenier, M.-C., Marien, A., Veys, P., Belghit, I., Dieu, M., Gillard, N., et al. (2021). Inter-laboratory study on the detection of bovine processed animal protein in feed by LC-MS/MS-based proteomics. *Food Control, 125*(November 2020), Article 107944. https://doi.org/10.1016/j.foodcont.2021.107944

Lecrenier, M. C., Planque, M., Dieu, M., Veys, P., Saegerman, C., Gillard, N., et al. (2018). A mass spectrometry method for sensitive, specific and simultaneous detection of bovine blood meal, blood products and milk products in compound feed. *Food Chemistry, 245*(September), 981–988. https://doi.org/10.1016/j.foodchem.2017.11.074

Lecrenier, M. C., Plasman, L., Cordonnier, A., & Baeten, V. (2023). Preliminary feed sedimentation step for the sensitive and specific detection of processed animal proteins by mass spectrometry-based proteomics. *Journal of Agricultural and Food Chemistry, 71*(42), 15774–15784. https://doi.org/10.1021/ACS.JAFC.3C03253/SUPPL_FILE/JF3C03253_SI_001.PDF

Leni, G., Prandi, B., Varani, M., Faccini, A., Caligiani, A., & Sforza, S. (2020). Peptide fingerprinting of Hermetia illucens and Alphitobius diaperinus: Identification of insect species-specific marker peptides for authentication in food and feed. *Food Chemistry, 320*(November 2019), Article 126681. https://doi.org/10.1016/J.FOODCHEM.2020.126681

Leni, G., tedeschi, tullia, faccini, A., pratesi, federico, folli, claudia, puxeddu, ilaria, et al. (2020). Shotgun proteomics, in-silico evaluation and immunoblotting assays for allergenicity assessment of lesser mealworm, black soldier fly and their protein hydrolysates. https://doi.org/10.1038/s41598-020-57863-5.

Lock, E. R., Arsiwalla, T., & Waagbø, R. (2016). Insect larvae meal as an alternative source of nutrients in the diet of Atlantic salmon (Salmo salar) postsmolt. *Aquaculture Nutrition, 22*(6), 1202–1213. https://doi.org/10.1111/anu.12343

Makkar, H. P. S., Tran, G., Heuzé, V., & Ankers, P. (2014). State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology, 197*, 1–33. https://doi.org/10.1016/J.ANIFEEDSCI.2014.07.008

Marbaix, H., Budinger, D., Dieu, M., Fumière, O., Gillard, N., Delahaut, P., et al. (2016). Identification of proteins and peptide biomarkers for detecting banned processed animal proteins (PAPs) in meat and bone meal by mass spectrometry. *Journal of Agricultural and Food Chemistry, 64*(11), 2405–2414. https://doi.org/10.1021/acs.jafc.6b00064

Marchis, D., Altomare, A., Gili, M., Ostorero, F., Khadjavi, A., Corona, C., et al. (2017). LC-MS/MS identification of species-specific muscle peptides in processed animal proteins. *Journal of Agricultural and Food Chemistry, 65*(48), 10638–10650. https://doi.org/10.1021/acs.jafc.7b04639

Mc Carthy, U., Uysal, I., Badia-Melis, R., Mercier, S., O'Donnell, C., & Ktenioudaki, A. (2018). Global food security – issues, challenges and technological solutions. *Trends in Food Science & Technology, 77*, 11–20. https://doi.org/10.1016/J.TIFS.2018.05.002

Mirabella, N., Castellani, V., & Sala, S. (2014). Current options for the valorization of food manufacturing waste: A review. *Journal of Cleaner Production, 65*, 28–41. https://doi.org/10.1016/J.JCLEPRO.2013.10.051

Monaci, L., & Visconti, A. (2010). Immunochemical and DNA-based methods in food allergen analysis and quality assurance perspectives. *Trends in Food Science & Technology, 21*(6), 272–283. https://doi.org/10.1016/J.TIFS.2010.02.003

Nessen, M. A., van der Zwaan, D. J., Grevers, S., Dalebout, H., Staats, M., Kok, E., et al. (2016). Authentication of closely related fish and derived fish products using tandem mass spectrometry and spectral library matching. *Journal of Agricultural and Food Chemistry, 64*(18), 3669–3677. https://doi.org/10.1021/acs.jafc.5b05322

Niedzwiecka, A., Boucharef, L., Hahn, S., Zarske, M., Steinhilber, A., Poetz, O., et al. (2019). A novel antibody-based enrichment and mass spectrometry approach for the detection of species-specific blood peptides in feed matrices. *Food Control, 98*(November 2018), 141–149. https://doi.org/10.1016/j.foodcont.2018.11.036

Ohana, D., Dalebout, H., Marissen, R. J., Wulff, T., Bergquist, J., Deelder, A. M., et al. (2016). Identification of meat products by shotgun spectral matching. *Food Chemistry, 203*, 28–34. https://doi.org/10.1016/j.foodchem.2016.01.138

Önder, Ö., Shao, W., Kemps, B. D., Lam, H., & Brisson, D. (2013). Identifying sources of tick blood meals using unidentified tandem mass spectral libraries. *Nature Communications, 4*, 1–10. https://doi.org/10.1038/ncomms2730

Önder, Ö., Shao, W., Lam, H., & Brisson, D. (2014). Tracking the sources of blood meals of parasitic arthropods using shotgun proteomics and unidentified tandem mass spectral libraries. *Nature Protocols, 9*(4), 842–850. https://doi.org/10.1038/nprot.2014.048

Ortea, I., O'Connor, G., & Maquet, A. (2016). Review on proteomics for food authentication. *Journal of Proteomics, 147*, 212–225. https://doi.org/10.1016/J.JPROT.2016.06.033

Pali-Schöll, I., Verhoeckx, K., Mafra, I., Bavaro, S. L., Clare Mills, E. N., & Monaci, L. (2019). Allergenic and novel food proteins: State of the art and challenges in the allergenicity assessment. *Trends in Food Science & Technology, 84*, 45–48. https://doi.org/10.1016/J.TIFS.2018.03.007

Peterson, A. C., Russell, J. D., Bailey, D. J., Westphall, M. S., & Coon, J. J. (2012). Parallel reaction monitoring for high resolution and high mass accuracy quantitative. *Targeted Proteomics*. https://doi.org/10.1074/mcp.O112.020131

Piras, C., Roncada, P., Rodrigues, P. M., Bonizzi, L., & Soggiu, A. (2016). Proteomics in food: Quality, safety, microbes, and allergens. *Proteomics, 16*(5), 799–815. https://doi.org/10.1002/pmic.201500369

Planque, M., Arnould, T., Dieu, M., Delahaut, P., Renard, P., & Gillard, N. (2016). Advances in ultra-high performance liquid chromatography coupled to tandem mass spectrometry for sensitive detection of several food allergens in complex and processed foodstuffs. *Journal of Chromatography A, 1464*, 115–123. https://doi.org/10.1016/J.CHROMA.2016.08.033

Raes, M., Planque, M., Marbaix, H., Gillard, N., Lecrenier, M.-C., Dieu, M., et al. (2018). Mass spectrometry based approaches for analysing proteins in processed food and feed. *Clinical Oncology, 3*(1485), 2–5.

Rasinger, J. D., Marbaix, H., Dieu, M., Fumière, O., Mauro, S., Palmblad, M., et al. (2016). Species and tissues specific differentiation of processed animal proteins in aquafeeds using proteomics tools. *Journal of Proteomics, 147*, 125–131. https://doi.org/10.1016/j.jprot.2016.05.036

REGULATION (EU) No 2013/51. (2010). COMMISSION REGULATION (EU) No 51/2013. *Machinery, 66*(March 2000), 1–27.

Saadat, S., Pandya, H., Dey, A., & Rawtani, D. (2022). Food forensics: Techniques for authenticity determination of food products. *Forensic Science International, 333*, Article 111243. https://doi.org/10.1016/J.FORSCIINT.2022.111243

Sawyer, J., Wood, C., Shanahan, D., Gout, S., & McDowell, D. (2003). Real-time PCR for quantitative meat species testing. *Food Control, 14*(8), 579–583. https://doi.org/10.1016/S0956-7135(02)00148-2

Shokralla, S., Hellberg, R. S., Handy, S. M., King, I., & Hajibabaei, M. (2015). A DNA mini-barcoding system for authentication of processed fish products. *Scientific Reports, 5*, 1–11. https://doi.org/10.1038/srep15894

Sobsey, C. A., Ibrahim, S., Richard, V. R., Gaspar, V., Mitsa, G., Lacasse, V., et al. (2019). Targeted and untargeted proteomics approaches in biomarker development. https://doi.org/10.1002/pmic.201900029.

Steinhilber, A. E., Schmidt, F. F., Naboulsi, W., Planatscher, H., Niedzwiecka, A., Zagon, J., et al. (2018a). Mass spectrometry-based immunoassay for the quantification of banned ruminant processed animal proteins in vegetal feeds. *Analytical Chemistry, 90*(6), 4135–4143. https://doi.org/10.1021/acs.analchem.8b00120

Steinhilber, A. E., Schmidt, F. F., Naboulsi, W., Planatscher, H., Niedzwiecka, A., Zagon, J., et al. (2018b). Species differentiation and quantification of processed animal proteins and blood products in fish feed using an 8-plex mass spectrometry-based immunoassay. *Journal of Agricultural and Food Chemistry, 66*(39), 10327–10335. https://doi.org/10.1021/acs.jafc.8b03934

Steinhilber, A. E., Schmidt, F. F., Naboulsi, W., Planatscher, H., Niedzwiecka, A., Zagon, J., et al. (2019). Application of mass spectrometry-based immunoassays for the species- A nd tissue-specific quantification of banned processed animal proteins in feeds [Research-article]. *Analytical Chemistry, 91*(6), 3902–3911. https://doi.org/10.1021/acs.analchem.8b04652

Stobernack, T., Zarske, M., Niedzwiecka, A., Zagon, J., Steinhilber, A., Poetz, O., et al. (2022). LC-MS-based detection of silkworm pupae in feed with and without prior immunoaffinity enrichment. *Journal of Insects as Food and Feed*, 1–12. https://doi.org/10.3920/JIFF2022.0098

Su, G., Yu, C., Liang, S., Wang, W., & Wang, H. (2024). Multi-omics in food safety and authenticity in terms of food components. *Food Chemistry, 437*, Article 137943. https://doi.org/10.1016/j.foodchem.2023.137943

Toxqui Rodríguez, M. del S., Vanhollebeke, J., & Derycke, S. (2023). Evaluation of DNA metabarcoding using Oxford Nanopore sequencing for authentication of mixed seafood products. *Food Control, 145*, Article 109388. https://doi.org/10.1016/j.foodcont.2022.109388

Vågsholm, I., Arzoomand, N. S., & Boqvist, S. (2020). Food security, safety, and sustainability—getting the trade-offs right. *Frontiers in Sustainable Food Systems, 4*, 16. https://doi.org/10.3389/fsufs.2020.00016

Van Der Plas-Duivesteijn, S. J., Mohammed, Y., Dalebout, H., Meijer, A., Botermans, A., Hoogendijk, J. L., et al. (2014). Identifying proteins in zebrafish embryos using spectral libraries generated from dissected adult organs and tissues. *Journal of Proteome Research, 13*(3), 1537–1544. https://doi.org/10.1021/pr4010585

van Huis, A. (2020). Insects as food and feed, a new emerging agricultural sector: A review. *Journal of Insects as Food and Feed, 6*(1), 27–44. https://doi.org/10.3920/JIFF2019.0017

van Raamsdonk, L. W. D., Prins, T. W., Meijer, N., Scholtens, I. M. J., Bremer, M. G. E. G., & de Jong, J. (2019). Bridging legal requirements and analytical methods: A review of monitoring opportunities of animal proteins in feed. *Food Additives & Contaminants: Part A, 36*(1), 46–73. https://doi.org/10.1080/19440049.2018.1543956

van Raamsdonk, L. W. D. D., Prins, T. W., van de Rhee, N., Vliege, J. J. M. M., & Pinckaers, V. G. Z. Z. (2017). Microscopic recognition and identification of fish meal in compound feeds. *Food Additives & Contaminants: Part A, 34*(8), 1364–1376. https://doi.org/10.1080/19440049.2017.1283711

Varunjikar. (2023). *Proteomic tools for food and feed authentication*. University of Bergen. https://hdl.handle.net/11250/3053481.

Varunjikar, M. S., Belghit, I., Gjerde, J., Palmblad, M., Oveland, E., & Rasinger, J. D. (2022a). Shotgun proteomics approaches for authentication, biological analyses, and allergen detection in feed and food-grade insect species. *Food Control, 137*(December 2021), Article 108888. https://doi.org/10.1016/J.FOODCONT.2022.108888

Varunjikar, M. S., Moreno-Ibarguen, C., Andrade-Martinez, J. S., Tung, H.-S., Belghit, I., Palmblad, M., et al. (2022b). Comparing novel shotgun DNA sequencing and state-of-the-art proteomics approaches for authentication of fish species in mixed samples. *Food Control, 131*, Article 108417. https://doi.org/10.1016/j.foodcont.2021.108417

Visciano, P., & Schirone, M. (2021). Food frauds: Global incidents and misleading situations. *Trends in Food Science & Technology, 114*, 424–442. https://doi.org/10.1016/J.TIFS.2021.06.010

Vkm, K. M., Andersen, L. F., Berstad, P., Bukhvalova, B., Monica, C., Dahl, L., et al. (2022). *Christine louise parr; kirst*.

Woodgate, S. L., Wan, A. H. L., Hartnett, F., Wilkinson, R. G., Simon, |, & Davies, J. (2022). The utilisation of European processed animal proteins as safe, sustainable and circular ingredients for global aquafeeds. https://doi.org/10.1111/raq.12663.

Wulff, T., Nielsen, M. E., Deelder, A. M., Jessen, F., & Palmblad, M. (2013). Authentication of fish products by large-scale comparison of tandem mass spectra. *Journal of Proteome Research, 12*(11), 5253–5259. https://doi.org/10.1021/pr4006525

Zagon, J., Di Rienzo, V., Potkura, J., Lampen, A., & Braeuning, A. (2018). A real-time PCR method for the detection of black soldier fly (Hermetia illucens) in feedstuff. https://doi.org/10.1016/j.foodcont.2018.04.032.

Zhou, W., Yang, S., & Wang, P. G. (2017). *Matrix effects and application of matrix effect factor* (Vol. 9, pp. 1839–1844). https://doi.org/10.4155/BIO-2017-0214, 23.

European Commission 2002/1774 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption: Vol. 248 (pp. 1–89). 1083 https://op.europa.eu/s/wA0X.

European Commission 2011/1169 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.