Detection of insect's meal in compound feed by Near Infrared spectral imaging

LuisaMandrile[ac]IlariaFusaro[c]GiuseppinaAmato[b]DanielaMarchis[b]GianmarioMartra[a]Andrea MarioRossi[c]

Abstract

Insects have recently emerged as a new protein source for both food and feed. Some studies have already demonstrated that insects' meal can be successfully added to animal feed without threaten animals' growth indices. However, effective and validated tests to individuate insects' meal in feed are strongly needed to meet traceability and safety concerns and to support the European legislation under development. Spectroscopic techniques represent valuable rapid and non-destructive methods that can be applied for in-situ analysis in feed production plants or in farms.

In this work a Fourier Transform Near Infrared spectroscopy imaging (FT NIR) as a potential screening method for the detection and quantification of insects' meal in feed is presented. Discriminant analysis was used for the automatic recognition of insects' meal fragments into the feed matrix. Moreover, the possibility to quantify insect's meal in feed sample was successfully tested. The proposed method is a rapid and green strategy for feed contamination screening analysis.

Keywords

1. Introduction

1.1. Insects as a feed compound

In accordance with the most accurate estimate from Food and Agriculture Organization (FAO), the Earth population will raise 9 billion people in few decades and the food demand is supposed to double within half a century. Moreover, the rapid economic growth of many developing countries will result in an increased need of animal food products within 2050 and much more raw materials for feeding stuffs production will be requested. Nowadays, the most diffused protein source for feedstuff are soybeans intensively cultivated and fished products; however, the increased food demand states the need of new sustainable strategies and resources for livestock nutrition. Since animal feeding represents about the 50% of the costs for the production of final animal food products, the zootechnical growth will strongly depend on the availability of raw materials, which must be convenient both from an economic and an environmental point of view.

In order to encourage a sustainable development of livestock, scientific research is focusing on innovative high protein content products able to preserve the biodiversity and guarantee safe terrestrial and aquatic animal growth. Among new resources for both food and feed, an outstanding interest for insects has recently emerged. Currently, insects are considered as an interesting protein source for animal feed (Premalatha, Abbasi, Abbasi, & Abbasi, 2011) but the use of insects is not allowed as feed material in Europe as well as other Processed Animal Proteins, which are subjected to the "feed ban" in accordance with Commission Regulation 999/2001 put in place to face Transmissible Spongiform Encephalopaties (TSE) crisis at the beginning of the century. Nevertheless, TSE Roadmap II of the European Commission in 2010 drove the partial reintroduction of non-ruminant processed animal proteins (PAPs) for non-ruminant feeding maintaining the prohibition of intra-species recycling (Commission Regulation 56/2013). European Commission seems to be favorable to the use of insects' meal in non-ruminant feed, and forthcoming changes will probably address their use in aquaculture. From a technical point of view, insects' intensive rearing has some interesting advantages: high conversion index, high throughput since they could be in principle grown on food waste, low greenhouse gas and ammonia emission, no space problems concerned, low water consumption and reduced zoonosis transmission risks. Insects have high nutritional values and they are suitable for fish, poultry and swine feed since they are naturally part of their diet and adequate to their digestive apparatus. Some studies have recently demonstrated that insects' meal can be successfully added to compound feed for fish without threaten growth indices (Rumpold & Schluter, 2013). Insects were also substituted to fish and soy meal in broiler feed (Bovera et al., 2015) and laying hens without damaging production rate (Khusro, Andrew, & Nicholas, 2012). Undoubtedly, these remarkable results make insects a very promising protein source. However, it is also out of a doubt that traceability and safety concerns will rise soon in this domain and an efficient infrastructure able to perform routine control activity should be put in place. If insects' meals will join the permitted products' list for feed, validated analytical techniques to determine the presence of insects in compound feed will be necessary. Any possible use of insects' meals in feed is related to the availability of effective and validated tests to individuate and quantify them during routinely controls.

1.2. NIR imaging for feed control

The need of a stable and rapid method for the identification of insects in feedstuffs suggests exploring different strategies able to provide accurate results. Currently, many methods have been reported in literature for the detection of animal origin material in feed (Van Raamsdonk et al., 2007). Most established methods are optical microscopy and polymerase chain reaction(PCR) in accordance with the Regulation (EC) n° 51/2013 provisions. Besides, near-infrared spectroscopy (NIRS) and near-infrared

microscopy (NIRM) ([Baeten et al., 2005](#)) were widely tested as a possible automatic screening method for feed evaluation ([Pavino et al., 2010](#)).

The heterogeneity of the matrix represents one of the most difficulties in the standardization of a methodology for insect detection and quantification in compound feed. Even if some peculiar features can be highlighted when pure insect fragments are observed, a sensitive and selective optical recognition would be difficult and unsuitable for automation.

Spectroscopic techniques represent valuable and rapid non-destructive methods ([Sedman, Ghelter, Enfield, & Ismail, 2010](#)). NIR [spectroscopy](#) is already well established for routine compositional analysis of foodstuffs, such as grains and dairy products ([Williams & Norris, 1987](#)). As it is well known, NIR is a fingerprint technology that can provide complete information about the chemical constituents of the sample. ([Andrés et al., 2007](#), [Wu et al., 2008](#)). By combining spectroscopic techniques with optical microscopy it is possible to develop an individual particle method and reach the sensitivity required for feed evaluation. In general, [hyperspectral](#) imaging integrates conventional digital imaging and traditional spectroscopy into a single system to simultaneously acquire both spatial and spectral information from an object. Spectroscopy detects the analyte of interest based on the spectral signature, and imaging transforms this information into distribution maps for spatial visualization ([Kamruzzaman, Maniko, & Oshita, 2015](#)). It is well known in literature that, very often, it is necessary to establish an initial calibration model using [chemometric](#) analysis to extract interesting information from the 3-dimensional (3D) hypercube that includes 2 spatial dimensions and 1 spectral dimension ([Amigo Martí & Gowen, 2013](#)). Predictive classification models can be calculated and validated through several methods, such as discriminant analysis ([Murray, Aucott, & Pike, 2001](#)). A method based on [Fourier Transform](#) Near Infrared (FT NIR) hyperspectral imaging for insect meal detection and quantification in feed matrix is here presented and the principal advantages and disadvantages of the presented method are addressed in this work. The quantitative method is based on the multivariate recognition of pixels in the spectral image associated to insect fragments by linear discriminant analysis. The aims of this research work are: i) to demonstrate that a multivariate data analysis can provide consistent results even if the matrix is very complex and heterogeneous as feed matrices; ii) to envisage the application of hyperspectral imaging as a promising non-invasive analytical technique for feed evaluation; and iii) propose an effective method for the detection and quantification of insect meal in feed, underlying both the potential and the limitations of the technique.

2. Material and methods

2.1. Reagents and materials

Pure insects' meal samples of two different species (*Tenebrio Molitor* and *Acheta Domesticus*) were provided by the Department of Agricultural, Forest and Food Sciences

of the University of Torino. Generic vegetal feed meals; bovine, swine, poultry PAPs; fish and krill meals were provided by the Veterinary Medical Research Institute for Piemonte, Liguria and the Valle d'Aosta (IZSTO).

2.2. Preparation of calibration standards and test samples

All meal samples are milled in a laboratory porcelain mortar to obtain granulometry below 1 mm and then pressed in a laboratory press (E-Z™ Quick Table Top Press) to prepare thin self-consistent tablets of homogeneous thickness of 1 mm for NIR imaging. Using this sample preparation procedure all fragments are pressed on the flat surface of the tablet, which is a very useful condition for spectroscopic mapping in reflection mode since it permits to overcame focus differences between different grains. Ten tablets of pure meals (vegetal feed, different insect species, PAPs, fish and krill meals) were prepared to collect the spectra of the training set of the LDA classification model. Afterwards, tow series of standards (one per each insect species) for the quantitation experiments were prepared by weighting the components with an analytical balance. 0.1 g of mixed meal containing 0%, 1%, 10%, 15%, 25%, 50% w/w of insect's meal were prepared.

2.3. NIR measurement

The NIR spectra are collected in diffuse reflectance mode by using a iN10 FT-IR microscope (Thermo Fischer Scientific, Waltham, USA) equipped with a cooled MCT detector and a video camera (magnification 15 x). The tablets were placed on a golden slide to ensure complete reflectivity and avoid signal loss. The NIR beam spot size is 100 μm. The spectra were recorded in the spectral range 4000–7000 cm^{-1} with a spectral resolution of 8 cm^{-1}. Acquisition time was 12 s per spectrum for 64 scansions. A flat golden surface is used as a reference for background collection. The same acquisition conditions were obviously adopted both for training set spectra and for subsequent experiments using mixed meals samples.

NIR hyper spectral images of mixed samples were obtained by scanning a 10 mm^2 area. The optimal focus is set once at the beginning of the test in the tablet's central point. The x–y movement of the sample holder under the NIR beam, with a step-size of 200 μm provides the NIR hyper spectral image. Each NIR image is composed by 285 pixels and the best compromise between image resolution and times of analysis (approximately 1 h per map) was defined. The collection time is suitable for routinely analysis because the map collection is totally automatic and no operator supervision in required during collection.

2.4. Data treatment

The NIR spectra of pure meals are used to build the training set for the classification model. Linear Discriminant Analysis (LDA) was used in order to obtain a robust classification model able to distinguish vegetal fragments to animal and insect fragments.

LDA is a probabilistic classification technique which searches for directions (canonical variables) with maximum separation among categories; the first canonical variable is the direction of the maximum ratio between inter-class and intra-class variances. Although the transformation from the original variables to the canonical variables is non-orthogonal, the plots on the first canonical variables are useful display methods, widely applied in classification studies (Oliveri & Simonetti, 2016). An important restriction in the application of LDA concerns the ratio between the number of objects in the training set and the number of variables. From a strictly mathematical point of view, it is sufficient that the number of objects is one more than the number of variables. But, in practice, for a significant result, a total number of objects equal to at least three times, the number of variables is suggested (Casale, Sinelli, Oliveri, Di Egidio, & Lanteri, 2010). For this reason the most informative spectral region between 4975 cm⁻¹ and 4485 cm⁻¹ was considered and a matrix with 339 rows and 127 columns is used for model calculation. The spectra of the training set were at first normalized at 4975 cm⁻¹ to eliminate random scatter effect, diffuse light interference and differences in intensity due to the thickness of the tablet and grains' size. Each animal species is equally represented with about 60 spectra in the training set. Mean centering is used as data preprocessing. A free R-based (Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/) platform for chemometrics distributed by the Chemometrics Group of the Italian Society of Chemistry (http://gruppochemiometria.it/index.php/software) was used for model calculation. The classification performances of the obtained model were evaluated on the basis of accuracy, sensitivity, and selectivity during venetian blinds cross-validation strategy with 10 cancellation segments.

The recognition of insects' fragments in contaminated samples is accomplished by classifying all spectra that compose the NIR map using the classification tool implemented in the R based platform. Wolfram Mathematica® software was then used to refold the map after the classification of each pixel. The pixels (which corresponds to one spectrum) are colored accordingly to the assigned class. The Mahalanobis distance about the center of mass of feed class (Mdclass1) and insect class (Mdclass2) is calculated as in Eq. (1) and the classification is based on the difference between the two calculated distances (ΔMd) (Eq. (2)). If $\Delta Md > 10$ the pixel is assigned to class 1 (feed), if ΔMd < −10 the spectrum is assigned to class 2 (insect), if −10 < ΔMd < 10 the pixels are uncertainly classified, |10| is fixed as an arbitrary threshold.

$$(1) \quad Md = (x-\mu)^T S^{-1}(x-\mu)$$

$$(2) \quad \Delta Md = Mdclass1 - Mdclass2$$

In Eq. (1) $x = (x_1, x_2, \ldots x_n)$ are the observations while $\mu = (\mu_1, \mu_2, \ldots \mu_n)$ represents the observations' mean. S^{-1} is the inverse of the covariance matrix related to the observations.

The map reconstruction allows suspect fragments to be visually individuated in the sample. What is more a rough quantification of insect's meal in compound feed can be performed by counting insect spectra recognized with respect to the total number of spectra collected.

Regression curves of revealed concentration against real concentration are then calculated by means of a Weighted Total Least Squares Regression method. Expanded uncertainty values are calculated using *student t* at 95% of probability for 2 degrees of freedom ($t = 0.816$) and coverage factor $k = 2$.

3. Results and discussion

The spectra of the pure animal meals and vegetal feed are shown in Fig. 1. The assignment of the main bands highlighted in Fig. 1 are reported in details in Table 1 (Socrates, 2001). The spectra shown in Fig. 1 represent the average spectra out of 25 measurements collected on different grains. The spectral variability of different fragments is only related to the intensity of the entire spectrum, due to the different size and morphology of the analyzed object (Shenk, Workman and Westerhaus, 1992). No differences in mutual intensities of the bands, neither in peaks' shape are noticed. The homogeneity composition allows us to consider a unique NIR fingerprint for each meal. The black rectangle in Fig. 1 highlights the spectral region between 4600 cm^{-1} and 4800 cm^{-1} in which the most interesting information is observed. This region can be successfully used as reference signal during screening analysis since the spectral shape in this region represents a very reliable warning bell about the animal contamination in the feed sample. The unique broad band centered at 4700 cm^{-1}, observed in vegetal matrix, is referred to 1st O-H str + 1st O-H def R-OH and to 2nd O-H def + 2nd C-O str of carbohydrates and starch. Whereas, animal proteins and collagen provoke the presence of two different bands, one related to 1st N-H sym str + 1st amide II (4800–4900 cm^{-1}) and the other related to amide combination mode 2nd amide I + 1st amide III (4700–4500 cm^{-1}) (Norris, Barnes, Moore, & Shenk, 1976) which is a common feature for all animal origin components.
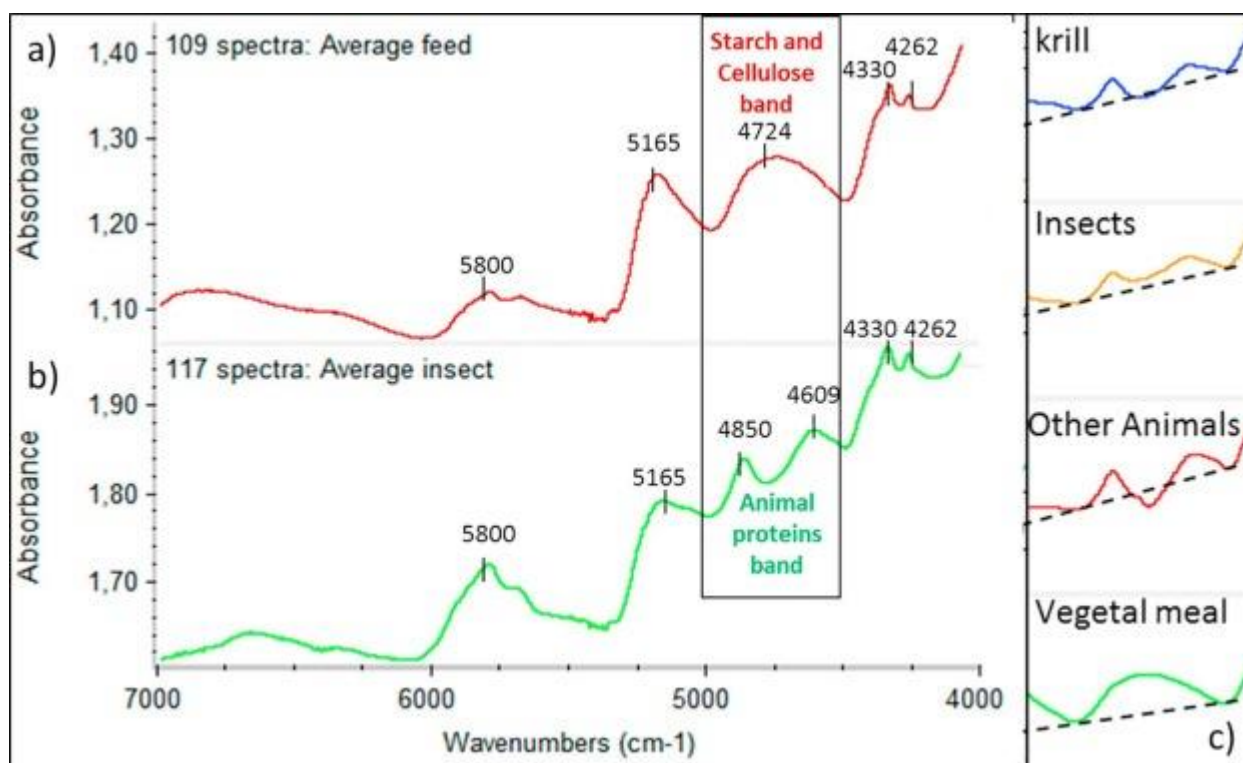
Fig. 1. NIR spectra a) average spectrum of 109 insect's meal spectra b) average spectrum of 117 vegetal compound feed spectra. c) NIR spectra focus between 4150 cm^{-1} and 4500 cm^{-1} of vegetal, animal, insect and krill meals. Each spectrum is an average of at least 100 spectra. The yellow mean spectrum is representative for *Tenebrio molitor, Acheta domesticus* and *Bombix mori.* The red spectrum is representative for bovine, swine, poultry and fish meal. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

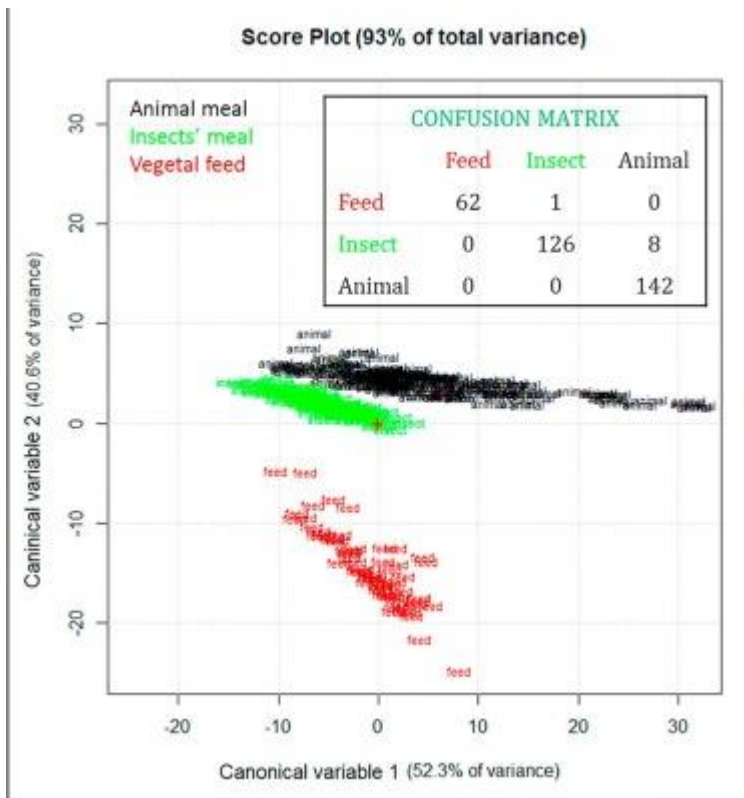Table 1. Assignment of NIR bands observed in plant and insect particles spectra.

Substance	Spectral Range	Assignment
Water	7150–6650 cm^{-1}	$(\nu_{asym} + \nu_{sym})$ H$_2$O
	5200–5100 cm^{-1}	$(\nu_{asym} + \delta)$ H$_2$O
Cellulose	5800–5600 cm^{-1}	2 ν_{asym} CH$_2$
	5000–4600 cm^{-1}	$(\nu + \delta)$OH, δOH, 2 νCO
	4400–4300 cm^{-1}	$(\nu + \delta)$CH, 2δCH
	4262 cm^{-1}	-CH$_x$ stretching combination band
	4330 cm^{-1}	-CH$_x$ stretching combination band
	4720 cm^{-1}	C-O stretching, OH deformation combination band
Starch	4800 cm^{-1}	1st O-H str + 1st O-H def R-OH
	4760 cm^{-1}	2nd O-H def + 2nd C-O str

Substance	Spectral Range	Assignment
Collagen/Protein	6800–6400 cm^{-1}	2 νNH
	5800–5600 cm^{-1}	2 ν_{sym} CH$_2$
	4900 cm^{-1}	$(\nu+\delta)$NH
	4850 cm^{-1}	1st N-H sym str + 1st amide II
	4700–4500 cm^{-1}	2 νCO + (νCN+ δNH)
	4609 cm^{-1}	2nd amide I + 1st amide III CONHR
	4450–4350 cm^{-1}	$(\nu+\delta)$CH$_2$, δCH
Carbonate	4400–4200 cm^{-1}	CO$_3^{2-}$(2 ν_{asym} + δ_{sym})

In order to demonstrate that insects can be selectively recognized by NIR spectroscopy, all probable interfering agents were considered in this study. A spectral comparison between different animal origin contaminants was performed. Clear spectral differences can be noticed in the frequency range from 4000 to 5000 cm^{-1} as shown in Fig. 1c. All the spectra of vegetal material present a unique broad band in this spectral region due to polysaccharides such as starch and cellulose (Zhong & Qin, 2016). The dashes tangents in Fig. 1c underline that the depth of the minimum registered at 4400 cm^{-1} with respect to the two lateral minimum points is a reproducible characteristic for animal species recognition. Farm animals (bovine, swine and poultry) and vertebrate fishes are represented by the red spectrum, whereas the yellow spectrum is representative of *Tenebrio molitor* and *Acheta domesticus*. Krill meal presents intermediate spectral features; this makes it possible to selectively distinguish it from insects' meal, even if krill is an invertebrate animal with exoskeleton rich of chitin as well as terrestrial insects (Clarcke, 1980).

The evident spectral differences were then exploited to set an automatic recognition method using LDA. Consistent statistical separation is obtained between insects, other animals and vegetal feed classes. In particular, the LDA model showed high method accuracy, intended as percentage of correctly classified samples is 97.3%; the sensitivity of the method for insect recognition is 99.2%; the selectivity between insect and other animals 94.6% during venetian blinds cross validation. A graphical display of these results is shown in Fig. 2, in which samples are projected on the first two canonical variables of LDA. The ability of NIR spectroscopy to properly separate the classes corresponding to the different compound of interest is highlighted accordingly to previously published papers (Cozzolino & Murray, 2004).

Score Plot (93% of total variance)

CONFUSION MATRIX

	Feed	Insect	Animal
Feed	62	1	0
Insect	0	126	8
Animal	0	0	142

1. Download high-res image (196KB)
2. Download full-size image

Fig. 2. Projection on the first two canonical variables of LDA. Samples are shown by their class name and color: vegetal feed (red points), insect's meal (green points) and other animal meals (black points). The related confusion matrix is shown in the inset table. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Taking into consideration the high performances of the classification model in cross validation, insects in mixed meals samples recognition was tested in order to further validate the method and to assess its real applicability. Aliquots of 0.1 g of compound feed containing different concentrations of insect's meal were prepared as described in section 2.4 and mapped with the NIR-imaging. Three repetitions of each measurement were performed in order to test method repeatability. After collection, the maps were unfolded and each pixel of the map (which corresponds to one NIR spectrum) was analyzed using the DA classification model previously described. The class assignation was done accordingly to the minor Mahalanobis distance (Md) calculated about the center of mass of each class. Uncertainly classified pixels correspond to spectra that showed $\Delta Md < |10|$, which was chosen as an arbitrary threshold. All repeated measurements are graphically reported in Fig. 3.
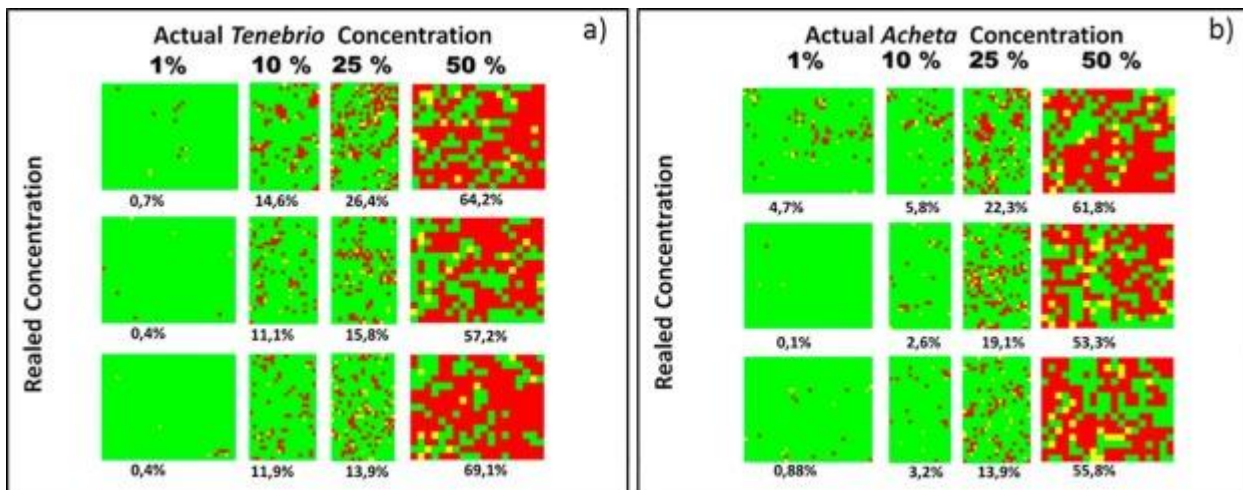
Fig. 3. NIR maps reconstructed on the basis of DA classification of feed sample containing different concentration of *Tenebrio molitor* (a) and *Acheta domesticus* (b) meal; black pixels are assigned to insect class, grey pixels are assigned to feed class, white pixels are uncertainly classified.

The reported results demonstrate that NIR mapping of feed samples, coupled with multivariate classification, is a suitable tool for feed control and a rough quantification can be obtained very rapidly. Since the area of the map assigned to insect class is strictly correlated to the w/w percentage of insect's meal present in the sample, quantitative information can be extracted from this tests. The percentage of spectra assigned to insect class with respect to the total number of spectra collected provides an indication about the abundance of insect meal in the sample. In Fig. 4, the fit of revealed insect's meal concentration vs the real concentration is shown. The best fit is obtained using a quadratic function with χ^2 values close to 1. The correlation study is very straightforward to demonstrate that this method can provide a rough quantitation of the insects' meal amount in feed samples. However, some limitations are inevitably present: it is not easy to classify all the pixels without a doubt because of several reasons. The heterogeneity of the sample, the overlapping fragments and the penetration depth of the NIR beam provoke that, in most cases, the analyzed volume of sample during each spectrum acquisition contains both components (the feed matrix and the insects' meal) contemporarily. Even if, a very good classification model was presented in Fig. 2, the critic aspects of quantitation based on pixels' counting emerge in Fig. 4. The lack of linearity fairly proves that, at the real application level, a very good mathematical model is not always as performing as it was expected. In this case, an intrinsic critical aspect is represented by the fine mixing of the powders which leads to the impossibility to collect the spectra of one particle at a time. Nevertheless, a correlation between real concentration and revealed concentration is achieved (even if not linear) and can be used to have an idea of the analyte concentration

with an average uncertainty of 10%. This finding is considered more than adequate in the present scenario of feed control (Commission Regulation (EU) No 56/(2013)).
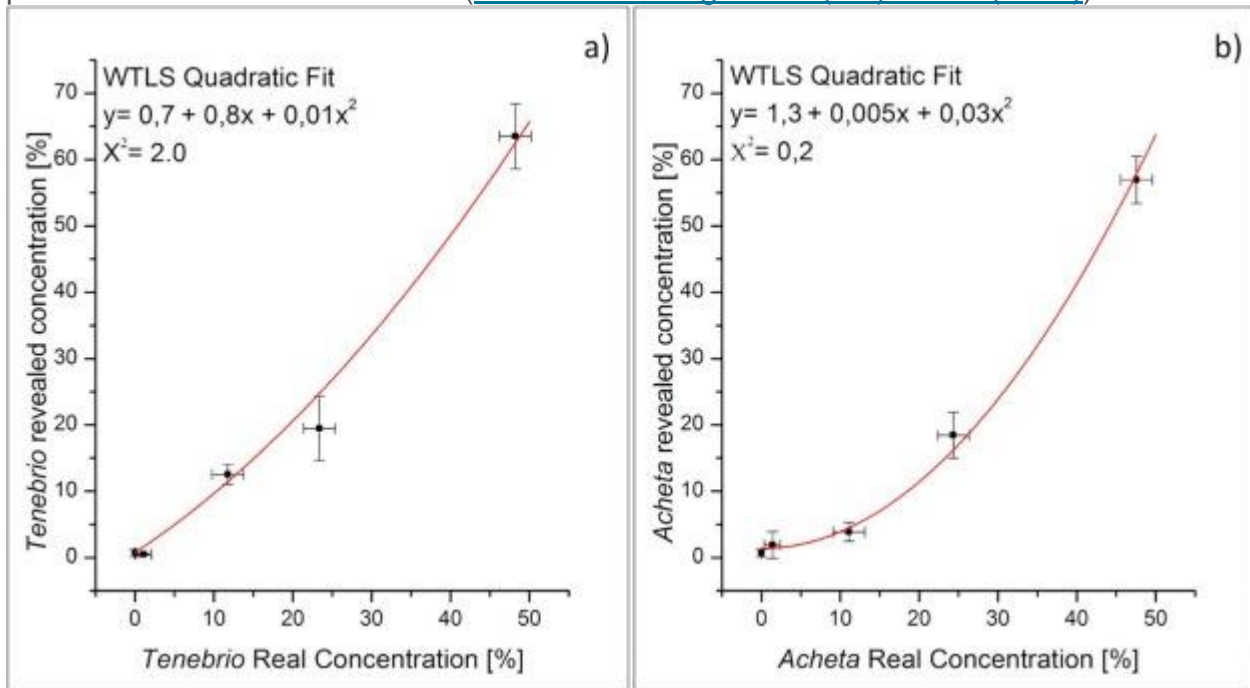
1. Download high-res image (118KB)
2. Download full-size image

Fig. 4. Quadratic fit of a) *Tenebrio* and b) *Acheta* meals concentration in feed calculated by Weighted Total Least Squares Regression.

The NIR images provided a reliable repartition of insect's meal and vegetal feed meal fragments because map collection parameters were optimized considering the grain size of both vegetal and insect meals, which is between 100 μm and 300 μm. The spot size of 100 μm and step size of 200 μm are used in order to maximize the possibility of collecting spectra from one grain at a time. A possibility to further improve this aspect is to prepare very thin tablets (ideally constituted of one monolayer of grains) and to reduce the IR beam spot size, i.e. to enhance the image resolution.

Ideally, the limit of detection (LOD) of the proposed method is 1 insect particle of 100 μm size. When an insect particle is collected by the NIR beam, it is recognized at 99% of probability by the LDA model; therefore, the probability of finding the contaminant fragment in the sample only depends on the probability of laying the contaminant fragments under the NIR microscope. This condition is strongly dependent on the amount of sample analyzed and the number of pixels collected per each image. This probability limit is the same of optical microscopy method, officially accepted for the identification of Processed Animal Proteins in feed. It was proved that 3 maps collection statistically allows the finding of at least 5 insect assigned spectra in a feed sample containing 1% of insect's meal.

4. Conclusions

The set of analyses carried out in the present study allows us to conclude that the combination of NIR spectroscopy with optical microscopy can provide an effective method for the detection of insects' residues in feed. NIR-imaging is attractive, not only for the non-operator dependent results it provides, but also because a short time is required to launch the analysis after system calibration, then the test is completely automatic, a very important aspect for routinely control methodologies. In conclusion, in this work, the suitability of NIR-imaging for the identification and rough quantitation of insect's meal in real samples was demonstrated. LDA classification of the pixels in NIR hyper spectral images of feed samples is proposed as an innovative and powerful control technique. However, the lack of linearity in the correlation study demonstrates that a simple pixels' counting has some limitations at very low concentrations and lead to uncertainty intervals around 10%. This observation suggests that two main issues must be carefully considered during method's set up in the control laboratories: i) grains' size homogeneity, and ii) image resolution, even if these two aspects can be in contrast with the need to minimize the times of analysis. The best compromise should be set in accordance with the accuracy and precision levels desired. However, the possibility to obtain quantitative information about the amount of animal meal in compound feed represents a milestone in feed control domain, since no quantitative methods are in place at the moment for official routinely controls. The proposed method represents a valuable and sustainable control strategy in view of the European legislation developments in the near future.

Acknowledgements

References

J.M. Amigo Martí, A. Gowen**Hyperspectral Imaging and Chemometrics: A Perfect Combination for the Analysis of Food Structure Composition and Quality**
Data Handling in Science and Technology (2013), pp. 343-366

S. Andrés, I. Murray, E.A. Navajas, A.V. Fisher, N.R. Lambe, L. Bünger**Prediction of sensory characteristics of lamb meat samples by near infrared reflectance spectroscopy**
Meat Science, 76 (3) (2007), pp. 509-516

V. Baeten, C. Von Holst, A. Garrido, J. Vancutsem, Michotte A. Renier, P.Dardenne**Detection of banned meat and bone meal in feedstuffs by near-infrared microscopic analysis of the dense sediment fraction**
Analytical and Bioanalytical Chemistry, 382 (2005), pp. 149-157

F. Bovera, G. Piccolo, L. Gasco, S. Marono, R. Loponte, G. Vassalotti**Yellow mealworm larvae (Tenebrio molitor, L.) as possible alternative to soybean meal in broiler diets**
Journal of Animal Science, 94 (2) (2015), pp. 639-647

M. Casale, N. Sinelli, P. Oliveri, V. Di Egidio, S. Lanteri**Chemometrical strategies for feature selection and data compression applied to NIR and MIR spectra of extra virgin olive oils for cultivar identification**

Talanta, 80 (5) (2010), pp. 1832-1837

A. Clarcke**The biochemical composition of krill, Euphausia superba Dana, from South Georgia**

Journal of Experimental Marine Biology and Ecology, 43 (3) (1980), pp. 221-236

Commission Regulation (EU) No 56/2013. URL http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013R0056&qid=1477574827733&from=EN. Accessed 04.08.2017.

D. Cozzolino, I. Murray**Identification of animal meat muscles by visible and near infrared reflectance spectroscopy**

Lebensmittel-Wissenschaft & Technologie, 37 (2004), pp. 447-452

M. Kamruzzaman, Y. Maniko, S. Oshita**Non-invasive analytical technology for the detection of contamination, adulteration, and authenticity of meat, poultry, and fish: A review**

Analytica Chimica Acta, 853 (2015), pp. 19-29

M. Khusro, N.R. Andrew, A. Nicholas**Insects as poultry feed: A scoping study for poultry production systems in Australia**

World Poultry Science Journal, 68 (3) (2012), pp. 435-446

I. Murray, L.S. Aucott, I.H. Pike**Use of discriminant analysis on visible and near infrared reflectance spectra to detect adulteration of fish meal with meat-and-bone meal**

Journal of Near Infrared Spectroscopy, 9 (2001), pp. 297-311

K.H. Norris, R.F. Barnes, J.E. Moore, J.S. Shenk**Predicting forage quality by infrared reflectance spectroscopy**

Journal of Animal Science, 43 (1976), pp. 889-989

P. Oliveri, R. Simonetti**Chemometrics for food authenticity applications**

G. Downey (Ed.), Advances in food authenticity testing, Elsevier Ldt. (2016), pp. 701-728

D. Pavino, S. Squadrone, M. Cocchi, G. Martra, D. Marchis, M.C. Abete**Towards a routine application of vibrational spectroscopy to the detection of bone fragments in feedingstuffs: Use and validation of a NIR scanning microscopy method**

Food Chemistry., 121 (2010), pp. 826-831

M. Premalatha, T. Abbasi, T. Abbasi, S.A. Abbasi**Energy-efficient food production to reduce global warming and ecodegradation: The use of edible insects**

Renewable and Sustainable Energy Reviews, 15 (2011), pp. 4357-4360

B. Rumpold, O. Schluter**Potential and challenges of insects as an innovative source for food and feed production**

Food Science & Emerging Technologies, 17 (2013), pp. 1-11

J. Sedman, A. Ghelter, A. Enfield, A.A. Ismail**Infrared imaging: Principle and practices**

E.C.Y. Li-Chan, P.R. Griffiths, J.M. Chalmers (Eds.), Application of vibrational spectroscopy in food science, Jhon WILEY & Sons Ltd, Barcelona (2010), pp. 110-131

J.S. Shenk, J.J. Workman, M.O. Westerhaus**Application of NIR spectroscopy to agricultural products**

P.A. Burns, E.W. Ciurczak (Eds.), Handbook of near infrared analysis, Marcel Dekker, New York, USA (1992), pp. 383-427

G. Socrates**Infrared and Raman characteristic group frequencies**

John Wiley & Sons, Ldt, New York (2001)

L.W.D. Van Raamsdonk, C. Von Holst, V. Baeten, G. Berben, A.Boix, J. De Jong**New developments in the detection and identification of processed animal proteins in feeds**

Animal Feed Science and Technology, 133 (1–2) (2007), pp. 63-83P. Williams, K. Norris**Near infrared technology in the agricultural and food industry**American Association of Cereal Chemists Inc, St Paul (1987)D. Wu, Y. He, S. Feng, D.W. Sun**Study on infrared spectroscopy technique for fast measurement of protein content in milk powder based on LS-SVM**Journal of Food Engineering, 84 (1) (2008), pp. 124-131J. Zhong, X. Qin**Rapid quantitative analysis of corn starch adulteration in Konjac glucomannan by chemometrics-assisted FT-NIR spectroscopy**Food Analytical Methods, 9 (2016), pp. 61-67