MAIN PAPER

Near infrared spectroscopy in animal science production: principles and applications

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ABSTRACT

Near infrared (NIR) is one of the techniques belonging to vibrational spectroscopy. Its radiation (750 to 2500nm) interacts with organic matter, and the absorption spectrum is rich in chemical and physical information of organic molecules. In order to extract valuable information on the chemical properties of samples, it is necessary to mathematically process spectral data by chemometric tools. The most important part in the development of an NIR method is building the predicting model generally called calibration. NIR spectroscopy has several advantages over other analytical techniques: rapidity of analysis, no use of chemicals, minimal or no samples preparation, easily applicable in different work environments (on/in/at line applications). On the other hand, NIR spectroscopy has some disadvantages: low ability to predict compounds at low concentration (<0.1%), necessity of accurate analysis as reference, development of calibration models required high trained personnel, need of a large and up-to-date calibration data set (often difficult to obtain), difficulties to transfer calibration among instruments, initial high financial investments. In the feed industry, NIR spectroscopy is used for: feed composition, digestibility (*in vivo, in vitro, in situ*), traceability assessment (to avoid possible frauds). As far as animal products are concerned, NIR spectroscopy has been used to determine the main composition of meat, milk, fish, cheese, eggs. Furthermore, it was also used to predict some physical properties (tenderness, WHC (Water Holding Capacity), drip loss, colour and pH in meat; coagulation ability in milk; freshness, flavour and other sensorial parameters in cheese). Interesting applications of NIR spectroscopy regard issues like: determination of animal products' authenticity and the detection of adulteration (in order to prevent frauds), discrimination PDO (P*rotected Designation of Origin)* and PGI (*Protected Geographical Indication*) from other non traditional products, detect handling aspects (freezing, thawing or fresh). There is a growing interest in the evaluation of animal products' quality directly on-line to have a continuous control of the production process. Furthermore, new portable instruments are becoming now available, which will allow to easily monitor some processes at the factory (i.e. ripening and ageing of sausages and cheeses).

Key words: NIR spectroscopy, Feed, Animal products, Quality, Traceability.

RIASSUNTO

LA SPETTROSCOPIA NEL VICINO INFRAROSSO (NIRS) NELLE PRODUZIONI ANIMALI: PRINCIPI E APPLICAZIONI

Le analisi chimiche convenzionali sono state impiegate a lungo come unico metodo di riferimento per le determinazioni analitiche dei prodotti agro-alimentari. A partire dalla metà del secolo scorso, una nuova

tecnica ha assunto sempre maggiore interesse per i controlli di routine: il NIRS (Near Infrared Spectroscopy).
Il vantaggio principale di questa tecnologia è la sua capacità di fornire dei risultati in modo molto rapido e accurato. Essendo, però, un sistema analitico secondario, la tecnologia NIR richiede una calibrazione aggiornata e rappresentativa, creata sulla base di consuete analisi di laboratorio. Nell' ambito delle scienze animali, il NIR è stato ampiamente impiegato nel settore "feed" per: (I) determinare le componenti nutrizionali principali dei foraggi, insilati e mangimi; (II) stimare la degradabilità della sostanza secca e la digeribilità in vivo, in vitro ed in situ; (III) monitorare le fermentazioni degli insilati; (IV) applicare gli strumenti on\at\in line; (V) garantire la tracciabilità delle materie prime e degli alimenti forniti agli animali.
Per quanto riguarda il settore "food", la tecnologia NIR ha trovato ampio utilizzo per: (I) determinare i componenti principali di latte, carne, pesce, uova, prodotti caseari; (II) rilevare possibili adulterazioni e frodi alimentari; (III) determinare alcune proprietà reologiche e sensoriali dei prodotti; (IV) discriminare prodotti ottenuti in particolari zone di produzione; (V) discriminare pesci e molluschi allevati o pescati, di origine o alimentati secondo regimi alimentari diversi. Le potenzialità di questa tecnologia sono davvero ampie, molte delle quali ancora inesplorate. Oggi, il principale obiettivo della ricerca è quello di poter utilizzare la spettroscopia NIR come tecnica d'analisi ufficialmente riconosciuta e accettata. Per raggiungere questo obiettivo c'è ancora molta "strada da percorrere" e necessita lo sviluppo di strumenti sempre meno ingombranti (talvolta anche portatili), dotati di sonde in grado di analizzare prodotti in situ, e progettando strumenti facilmente incorporabili alle linee di produzione e alle macchine di lavorazione.

Parole chiave: Spettroscopia NIR, Alimenti zootecnici, Prodotti di origine animale, Qualità, Tracciabilità.

Introduction

Applications of NIR spectroscopy as analytical tool in animal production are receiving a growing interest and attention. Starting in 60s, subsequently Karl Norris' work, it was possible to observe an major development of NIR in different areas of animal production. Nowadays this technology has established a fundamental role in the improvement of the production system and it is perceived by many operators as an accurate and easy to use analytical tool.

This improvement in NIR utilization was possible thanks to the extensive use of computers, the development of instruments and of appropriate chemometric procedures so that daily, new applications of spectroscopic techniques are being demonstrated and published. NIR spectroscopy facilitates real-time measurements at all stages of production from raw material analysis to ingredients and finished product verification (Woodcock *et al*., 2008). In animal production, NIR is used all along the supply chain of animal products aiming to (I) evaluate the quality of cereals and forages just harvested on the fields; (II) determine the main nutritive value of feeds provide to different kind of animals to maximise their growth and performances, reducing excretions; (III) monitoring the silage evolution during fermentation; (IV) analyse the main composition of meat, milk, dairy products, eggs and all of the animal products; (V) identification of possible food frauds and discriminate products according to their origins; (VI) analyse manure aiming to reduce its impact on environment; (VII) use of NIR spectroscopy on/in/at line to allow a continuous control of different productive processes (harvesting machines, feed dispensers, fermentation batches, meat and dairy product processing, and milking). The almost instantaneous acquisition of the analytical results consent to continuously monitor production processes and correct, readjust the input factor optimizing production systems (Baeten and Dardenne, 2002). The versatility of the instruments available on the market makes it possible

to monitor and analyse all the steps of the animal supply chain (Cen and He, 2007).

Theory, instruments and principles of application

Near Infrared (NIR) spectroscopy is a type of vibrational spectroscopy that employs photon energy (*hv*) in the energy range of $2.65x10^{-19}$ to $7.96x10^{-20}$J, which correspond to the wavelength range of 750 to 2500nm (wavenumbers: 13,300 to 4000cm^{-1}) (Pasquini, 2003). The basic principles of NIR spectroscopy involve the production, recording and interpretation of spectra arising from the interaction of electromagnetic radiation with the organic matter (Manley *et al.*, 2008). NIR radiation interacting with a sample may be absorbed, transmitted or reflected. Thus, there are different mode of measurements in NIR spectroscopy fitting different applications. In practise the common modes are transmittance, interactance, transflectance, diffuse transmittance, and diffuse reflectance, with the last two being the most frequently used (Huang *et al*., 2008). The possible mode of measurements to choose depends on the physical characteristics of sample like if solid or liquid, transparent or opaque and by the size of the particles.

Recording the response of certain molecular bonds (for example, O-H; N-H; C-H) to NIR radiation, generates a spectrum that may be characteristic of a sample and may act as a "fingerprint" (Woodcock *et al*., 2008). This spectrum is rich in chemical and physical information about organic molecules, and may therefore yield valuable information about the composition of a product (Katsumoto *et al*., 2001). By carrying all of this information, spectra allow us to evaluate, explore and select a population of samples before we determine what we should measure by classical technique. This is an important research characteristic of spectroscopic techniques (Murray, 1999).

A NIR spectrometer instrument mainly consists of light source, beam splitter system (wavelength selector), sample holder, optical detector, and data processing analysed system (Manley *et al.*, 2008). Each of these main constituents can have different origins and properties so that it is possible to classify the instruments according to the characteristics of the parts which compose the instrument itself.

NIR radiation sources can be thermal or non-thermal. In the thermal group are included (I) the quartz halogen lamps and (II) the Nernst filament. In the second group (I) light-emitting diodes (LED); (II) laser diodes and (III) lasers are the main source of light, which consist of discharge lamps.

According to the wavelength selection, spectrophotometers can be distinguished in (I) discrete-wavelength spectrophotometers and (II) continuous spectrum NIR instruments. The first one irradiates a sample with only a few wavelengths selected using filters or light-emitting diodes (LEDs). The second one may include a diffraction grating or an interferometer.

Infrared detectors can be differentiated according to their spectral response, their speed of response and the minimum amount of radiant power that they can detect. In NIR applications, photon detectors are the main used. The detection devices most widely-used for NIR analysis can be divided into *single*- and *multi-channel* detectors. Single-channel detectors comprise lead-salt semiconductors. Lead sulphide (PbS) is used over the range 1100-2500nm, indium gallium arsenide (InGaAs) over 800-1700nm (extended range up to 2500nm), and silicon detectors over 400-1100nm. Multi-channel detectors comprise diode arrays or charge-coupled devices (CCDs).

NIR instrumentation has evolved dramatically in response to the need for

speed in analyses and flexibility in adapting to different samples (Blanco and Villarroya, 2002).

Instrument selection must be guided by end application. For instance, low cost instruments, based on filters and LEDs, suffice for many dedicated laboratory and routine in field applications (Pasquini, 2003).

Chemometric and spectral data treatment

The data acquired from a sample by an NIR spectrometer contains spectral information related to the sample composition. Different chemical entities have absorption at specific wavelengths which can be used to define the chemical composition of different substances. However, absorption peaks of different chemical molecules overlaps in several parts of the spectral region. The absorption signal is often weak compared to other phenomena that intervene in the interaction between light and particle like all the scattering effects. For these reasons, it is necessary to mathematically process spectral data in order to extract valuable information on the chemical properties of samples.

Chemometric is the chemical discipline that uses mathematical and statistical methods to design or select optimal procedures and experiments and to provide maximum chemical information by analyzing chemical data (Massart *et al*., 1988). Chemometric is a vast discipline, which requires much more than a paper's paragraph. Nevertheless it is not the aim of this work to enter on the details of chemometrics, but it can be useful to have a short summary of which are the main methods used to extract information from NIR.

The most important part in the development of an NIR method is building the predicting model generally called calibration. The calibration is simply a regression model that will allow the prediction of chemical composition based on spectral data. The whole process in the development of the predicting model involve several steps: (I) spectral data acquisition; (II) use of suitable reference methods to determined analyte concentration on a certain number of samples; (III) data pre-processing to reduce scattering effect of particle size and enhance spectral-analyte relationship; (IV) development of the mathematical relationship between NIR absorption and concentrate of analytes; (V) validate the models using another set of samples not used in the calibration set (Cen and He, 2007).

Common pre-processing methods include: smoothing techniques, normalisation, derivatives, multiple scatter correction (MSC), standard normal variate (SNV) and de-trending. Pre-processing techniques more recently developed include orthogonal signal correction (OSC), direct signal correction (DOSC) and orthogonal wavelet correction (OWAVEC) (Leardi, 2008)

Many approaches have been proposed for the development of calibration. In the 60s and 70s with the use of a discrete number of wavelength (filter instruments) the method of choice was multiple linear regression (SMLR). In 80s, with the introduction of monochromators and recording of full range spectra, it was possible to use more sophisticated approaches like principal component regression (PCR) partial least squares (PLS). With the increase in the size of calibration databases there was the need of new calibration methods able to handle non linear relationship like Local calibration and artificial neural network (Berzaghi *et al*., 2001).

In chemometrics methods, qualitative analysis is also an important issue in NIR analysis, which could be attributed to the

problem of pattern recognition. It refers to technique in which knowledge about the category of individual of samples is used for classification. The classification model is developed on a training set of samples with known categories. The model performance is evaluated by the use of a validation set by unknown samples. Pattern recognition methods are numerous, such as linear discriminant analysis (LDA), principal component analysis (PCA), K-nearest neighbours (KNN), cluster analysis (CA), discriminant partial least square (DPLS), soft independent modelling of class anthology (SIMCA), artificial neural network (ANN) and support vector machine (SVM). (Cen and He, 2007).

Since the ultimate goal of a calibration model is to accurately predict unknown samples (Geladi, 2002), it is of critically importance that the calibration data set represent the possible sources of variation that will be encountered in the unknown samples. For this reason, it becomes very important to select the samples that provides the largest information for the development of the calibration data set. The work by Shenk and Westerhaus (1991) has revolutionized the process of samples selection by using the global and neighbour Mahalanobis distances that enable the identification of the most relevant samples for calibration.

Internal validation involves validation of a calibration using the same sample set as that used for calibration development. The two validation methods normally used are independent or external validation and cross-validation. External validation requires a separate large and representative set of test objects in order to give relevant and reliable estimates of the future prediction ability of the model. Cross validation is a very reliable validation method; it seeks to validate the calibration model on an independent test data set but it does not use sample for testing only.

Advantages and disadvatages of NIRS application

Near-Infrared spectroscopy has several advantages over other analytical techniques: (I) the spectral measurements is really rapid one sample can be scanned in less than 1min; (II) less expensive because there isn't any use of chemical reagents and a single operator can analyze a large number of samples; (III) several scans can be made on the same object, which permits to obtain a more representative sample composition and a more accurate result of analysis; (IV) sample requires minimal (drying and grinding) or no preparation; (V) several constituents of the same sample can be measured at the same time; (VI) easily applicable in different environments (like industry, laboratory, harvesters, etc.); (VII) measurements can also be carried out on/in/at line; (VIII) the opportunity to use optical probes makes it possible to analyse the sample *in-situ*; (IX) the availability of portable instruments permits to obtain spectra directly in the field, useful to follow process like ripening.

On the other hand, NIR spectroscopy has some disadvantages to take into account: (I) low sensitivity of the signal which can limit the determination of substances with concentration below 0.1%; (II) it is a secondary analytical method, so it requires an accurate chemical an physical analysis as reference samples; (III) development of calibration models require high trained personnel; (IV) accurate and robust calibration require a large data set incorporating large variation, which is often difficult to obtain; (V) it requires a continuous maintenance of the calibration data set; (VI) with some hardware it is difficult to transfer calibration between instruments of

the same manufacture or between different manufactures (Manley *et al*, 2008); (VII) although NIR technique has low measuring cost, the initial high financial investment for the instrumentation represents a important obstacle for the purchase.

Applications

NIRS and feed

In the years following the work of Norris *et al*. (1976), there was a large number of reports on the use of NIR spectroscopy to predict many aspects of forage composition. The major part of these works aimed to estimate the chemical fractions of feed, such as crude protein (CP), dry matter (DM) and fiber fractions. NIR analysis has been so successful in these fields that it has been recognised by the AOAC as an official method of analysis for these parameters in forages. This analytical technique is also considered in proficiency tests at the same level of the traditional wet chemistry methods (www.foragetesting.org). Nutritive value of a forage depends not only on its chemical composition, but also on its digestive utilization. This is the reason why several authors used NIR as a tool to predict *in vivo*, *in vitro* or in *situ digestibility* of forages and feed (Givens and Deaville, 1999, Andrés *et al.*, 2005 and Lovett *et al.*, 2004).

In all these applications, both in chemical prediction and digestibility evaluations of feed, NIR spectroscopy has a role in reducing costs, time required to obtain the answer, allowing a greater number of samples that may be analyzed (Garcia and Cozzolino, 2006).

An other important topic, which was heavily discussed during these years in the feed field, was the physical properties requested to sample's to be scanned by NIR spectroscopy. Most of the researches on NIR for forage characterisation has used dried and ground samples. More recently, work using fresh-ungrounded forages and intact feed has taken place. The potential of NIR to predict nutritional traits using fresh-intact samples is increasingly recognised, as it both reduces the cost of analysis and eliminates potential impacts of sample preparation (drying and grinding) upon the estimation of some nutrients. In addition, the effect of particle comminution and the presence of residual moisture were also investigated, in order to determine the optimum sample preparation procedures to predict a variety of silage and feed biological parameters from oven dried samples (Lovett *et al.*, 2005 and Cozzolino *et al.*, 2006). Brimmer and Hall (2001) suggest that the main difficulty in measuring the NIR reflectance spectrum of ungrounded samples (flakes, pellets, tablets and whole forages) is that sample presentation is highly heterogeneous, giving rise to considerable variations in the spectral baseline. In order to improve the reproducibility of collected NIR spectra, these authors recommend the use of a large analysis area (60cm^2 or more). In fact, De la Haba *et al*. (2008) demonstrated that NIR equation developed for fine milled products may be used in the same instrument for the analysis of non-milled products using a larger optical windows' cells.

Furthermore, fast, portable and low cost diode array instrumentation can be used to analyse samples through the production process, thus allowing instant decisions on compound feed processing. As instruments become more rugged to work in harsh environment there will new opportunity for application directly at production line (Fernández-Ahumada *et al*., 2008a) or at the farm. This technique would enable feed manufacturers to make any corrections required during mixing and preparation of lots that must correspond to composition labels (Fernández-Ahumada *et al.*,

2008). Fernández-Ahumada *et al.*, (2008) discovered that NIR equations developed with samples of finished intact compound feeds can be applied to samples taken at the mixing stage (ground product). This finding is of great economical importance. These results are encouraging, although a number of technical and scientific problems remain to be solved before NIR spectroscopy can be implemented as a tool for PAT (Process Analytical Technology) in the feed processing industry.

In recent years, the growth of research into traceability of feeds has been associated with the Bovine Spongiform Encephalopathy (BSE) crisis and Genetically Modified Organisms (GMO) disputes, and there is a need for the improved detection of feed ingredients to address the problem of feed and food safety. The BSE and dioxin crises highlighted the need to include, on the label, more detailed qualitative and quantitative information regarding the composition of compound feedingstuffs (Pérez-Marín *et al*., 2004). Many methods including NIR spectroscopy have been developed for traceability of feeds as, for instance, detecting meat and bone meal (MBM) in compound feeds (Fernandez Pierna *et al*., 2004 de la Roza-Delgado *et al*., 2007 and De la Haba *et al*., 2007), quantifying animal-origin fats in fat blends (Garrido-Varo *et al*., 2008), determining the botanical composition of a complex mixture of forage (Li *et al.*, 2007), discriminating fish bones from other animal bones in the sediment fraction of compounds feeds (De la Haba *et al*., 2007a) and predicting the inclusion rate of the main ingredients provided approximate indications on the feed formula in milled (Xiccato *et al*., 2003) and non-milled feeds for several animal species (Pérez-Marin *et al.,* 2004).

NIR spectroscopy has the main advantage to be really flexible, and this permits to use this technology all along the supply chain of feed production. In Table 1 are summarized some applications of NIR spectroscopy on feed field.

Meat and meat products

It is well known that all the meat supplied to the markets must undergo to high quality controls, at the levels of slaughtering, meat cutting, and distribution (Monin, 1998), in order to guarantee its safety and to satisfy consumers' requests (Andrés *et al.*, 2008). Since NIR is a rapid method, its use by the industry offers a suitable tool to implement frequent quality control during the entire meat processing chain.

In contrast to conventional methods, NIR spectroscopy enables rapid, simple and simultaneous assessment of numerous meat properties (Prevolnik *et al.*, 2004) but, on the other hand, NIR is less accurate if compared to other specific analytical technique. Regarding the applications of reflectance spectroscopy, it has been used previously in meat analyses and, recently, numerous studies and applications have been developed for the prediction of meat properties, particularly chemical composition, estimation of tenderness, WHC (Water Holding Capacity), drip loss, colour and pH (Monin, 1998, Ripoll *et al*., 2008).

Appearance is one of the main traits determining purchase decision and acceptability of meat. It relies on three main factors: colour, amount of visible fat (marbling) and wetness (exudation).

As far as colour is concerned, Andrés *et al.* (2008) found that NIR demonstrates good ability in predicting luminosity (L*), but poor results were obtained for a* and b* value, probably because these last two parameters change rapidly according to the ambient oxygen. To predict colour it could be really useful to use VIS-NIR technique, because the range of visible spectra (400 to

Table 1. Some applications of NIR spectroscopy on the feed field.

Objective of the trial	Measurement modes	Parameters	Scatter correction	derivative		$R^{2(1)}$		$SECV^{(2)}$		References
Chemical composition of wet whole maize silage	Reflectance (400 to 2500 nm)	DM[3] (g Kg^{-1})	SNDV[8]	2nd		0.85		27.4		Cozzolino et al., 2006
		CP[4] (g Kg^{-1} DM)	SNDV	2nd		0.91		6.5		
		OMD[5] (g Kg^{-1} DM)	SNDV	2nd		0,53		30		
		ADF[6] (g Kg^{-1} DM)	Raw	-		0.86		22.1		
		NDF[7] (g Kg^{-1} DM)	MSC[9]	-		0,60		67.1		
		pH	SNDV	2nd		0,51		0.18		
Fermentation parameters in grass and corn silage	Reflectance (400 to 2500 nm)					Grass	Corn	Grass	Corn	Sørensen, 2004
		NH_3N[10] (DM),%	SNVD	2nd	Dry Wet	0.89 0.79	0.77 0.72	0.023 0.028	0.007 0.008	
		Lac[11] (DM), %	SNVD	2nd	Dry Wet	0.96 0.93	0.95 0.91	0.71 0.84	0.36 0.47	
		Hac[12] (DM), %	SNVD	2nd	Dry Wet	0.68 0.74	0.72 0.84	0.97 0.91	0.52 0.41	
		pH	SNVD	2nd	Dry Wet	0.92 0.91	0.78 0.62	0.11 0.13	0.063 0.080	
		EtOH[13] (DM), %	SNVD	2nd	Wet	0.89	0.92	0.22	0.11	
Predict biological parameters of maize silage	Reflectance (1100 to 2500 nm)	In vitro digestibility				Calibration	Validation			Lovett et al., 2004
		DOMD[14]	SNVD	1st		0.741	0.439	12		
		In vitro gas product.								
		DOMD	SNVD	1st		0.753	0.619	12		
		A(ml)(15)	SNVD	1st		0.175	0.084	9		
		µt1/2 (16)	SNVD	1st		0.695	0.622	0.0032		
		µt12 (17)	SNVD	1st		0.610	0.538	0.0025		
		Lag phase	SNVD	1st		0.763	0.686	0.087		
		T1/2(18) asymptote	SNVD	1st		0.727	0.581	0.539		
		In situ degradability						24		
		'a' fraction[19]	SNVD	1st		0.795	0.037	64		
		'b' fraction[20]	SNVD	1st		0.113	0.045	79		
		'a + b' fraction[21]	SNVD	1st		0.024	-0.086	0.0094		
		'c' fraction[22]	SNVD	1st		0.070	-0.221	24		
		Solubility	SNVD	1st		0.586	0.545			

[1]Coefficient of correlation; [2]Standard Error of Cross Validation; [3]Dry matter; [4]Crude protein; [5]In vitro organic matter digestibility; [6]Acid Detergent Fibre; [7]Neutral Detergent Fibre; [8]Standard Normal Variate and Detrend; [9]Multiplicative Scatter Correction; [10]NH_3 nitrogen; [11]Lactic acid; [12]Acetic acid; [13]Ethanol; [14]Digestible Organic Matter in dry Matter; [15]Asymptote; [16]Fractional rate of fermentation; [17]Fractional rate of fermentation at 12h; [18]Time of half asymptote; [19]Immediately soluble; [20]Potentially degradable [21]Extend of degradable fraction; [22]The fractional rate of disappearance of the 'b' fraction.

780nm) contains much more information about this parameter in comparison to NIR spectra region.

pH is one of the most commonly measured parameters in meat, as it affects technological processing ability, as well as most sensory traits. Thus early prediction of ultimate pH (pHu) would be of interest to identify problems of DFD (dark, firm and dry) and PSE (pale, soft and exudative) beef carcasses at the end of the slaughter line. Several authors obtained contrasting results about the ability of NIR in predicting pH. In fact, Prieto *et al*., (2008) reported unsatisfactory predicting models for the estimation of pH value of oxen and young cattle meat samples, while Andrés *et al*., (2008) found that this parameter could be accurately predicted by NIR (R^2=0,97 and RPD=3.17).

The same contradictory results were also obtained for the measurement of tenderness/ toughness in meat. Warner-Bratzler (WBSF) is the instrumental technique that usually yields the best correlation with sensory panel scores for meat toughness but, as many other analytical techniques, it requires long time for analysis and trained specialists. Hildrum *et al*. (1994) showed that sensory hardness and tenderness can be predicted by NIR spectroscopy. Ripoll *et al*., 2008 also demonstrated the ability to create a good calibration for WBSF measured in cooked-minced meat, even if the meat structure was destroyed by mincing samples.

Strong correlation with tenderness has the amount of collagen in meat, but poor prediction results were obtaining using NIR as analytical technique. This lack of predictability is probably due to the fact that the NIR spectrum of collagen does not differ much from myofibrillar proteins, which are present in muscles at 10 times higher concentrations (Prieto *et al*., 2006).

Hoving-Bolink *et al*. (2005) tried to use NIR to measure early post mortem pork meat to predict ultimate drip loss and intra-muscular fat on three different location (*M. longissimus thoracis, M. longissimus lumborum and M. semimembranosus*). Results indicated that it was possible to predict intra-muscular fat content (confirming Savenije *et al*., 2006), but for drip loss no correlation was achieved. Nevertheless, from other studies, it was clear that post rigor drip loss during storage can be predicted adequately by NIR (Geesink *et al*., 2003 & Brøndum *et al*., 2000). The content of intramuscular fat of meat and its fatty acid (FA) composition, is an important quality factor that influences technological properties like shelf-life (lipid and pigment oxidation) and flavour (Sierra *et al*., 2008). Furthermore, there is a growing consumers attention to the fat composition of meat and meat products, as nutritional guidelines are recommending lower daily fat intake. Intramuscular fat content and composition depend on many factors (animal genetic, feeding regime, age) and rapid and economical method of analysis could be implemented to identify meat products with better nutritional characteristics. Sierra *et al*.'s results (2008) showed that NIT (850-1050nm) technology could be used as a rapid and easy method to determine the main FAs in beef. Similar results were also obtained from Berzaghi *et al.* (2005) analysing the fatty acid profile in breast meat of laying hen, but lower predicting accuracy was reported for FAs in present at low concentrations. Fatty acid content could also be accurately determined in goose fatty liver (Molette *et al*., 2001), obtaining predictions of oleic acid with an R^2 of 0.988 facilitated by the high fat content of this product. García-Olmo *et al.* (2000) also reported successful application of NIR in the determination of FA profile of Iberian pig fat with small prediction errors

(less than 0.3%) and great R^2 values (above 0.95) for C16:0, 18:0, 18:1 and 18:2.

Prieto *et al*., (2008) observed poor performances of NIR for prediction of WHC (press, drip and cooking losses) when tested in oxen and young cattle samples (R^2<0.6, RPD<1.3). It is well known that NIRS can not predict directly WHC of meat samples. However, good correlations between WHC and the wavelengths related to protein and intramuscular fat could be expected, since these chemical components are closely related to WHC.

Interesting application of NIR was obtained also analysing processed meat. In fact, it was also used to study the feasibility of applying quality controls to sausages by performing a proximate analysis (fat, moisture and protein) on the finished product (intact and homogenized) (Gaitán-Jurado *et al*., 2008). Speaking about processed meat analysis, it could be really useful to use on line NIR technology. On-line analysis of chemical composition of meat during grinding have been reported on pilot scale batches of ground beef (Isaksson *et al*., 1996 and Tøgersen *et al.*, 1999). Furthermore an application of on-line analysis of meat inside an industrial mixer has also been reported (Schwarze, 1996).

Industries can receive for sure important advantages if this technology is used properly and this explains the growing number of trials that has been conducted in this field.

Other important issues in the food industry are the determination of meat authenticity and the detection of adulteration. These topics are attracting and increasing amount of attention (McElhinney and Downey, 1999, Cozzolino and Murray, 2004). With meat and meat products major authenticity issues concern the substitution of high value raw materials with cheaper ones.

NIR could be used as an authentication tool in order to prevent frauds and to detect handling aspects (freezing, thawing). These applications can have interesting advantages for industries which could rapidly evaluate the meat just received (Alomar *et al.,* 2003). These authors demonstrated that NIR was able to correctly classify the 78% of meat samples according to breed and muscle they belong to.

The discriminant ability of NIR was also demonstrated by Berzaghi *et al.* (2005). They were able to discriminate 100% of hen's freeze-dried breast meat according to the presence\absence polyunsaturated FA in the diet provided to animals. In table 2 are listed some applications of NIR spectroscopy applied to meat and meat products.

Fish

Fish is a kind of foodstuff which is highly perishable and many of its properties can change during storage (moisture, oil, protein and volatile nitrogen from protein breakdown). These fish quality changes can have different intensity according to fish species, fish processing systems and seasonal variations. Nevertheless, the major responsible of this fish's flesh evolution is the modality (as instance temperature) and the period of storage, which causes different deteriorative changes in proteins, lipids and organoleptic properties.

Fish quality has traditionally been evaluated through sensory assessments, but these modalities are subjective and a trained taste panel is needed to carry out this evaluation (Cozzolino *et al.*, 2002). This is costly, and it is not readily available in all situations and locations (Nilsen *et al*., 2002).

The lack of simple, reliable and non-destructive methods for the determination of carcass composition in fish and fish by-products, has been one of the main obstacles for the development of quality control in

Table 2. Some applications of NIR spectroscopy on meat and meat products field.

Objective of the trial	Measurement modes	Parameters	Scatter correction	derivative	$R^{2(1)}$		SECV(2)		References
Predicting chemical, instrumental and sensory quality of beef	Reflectance (408 to 2492. 8 nm)				Calibration	Prediction			Ripoll *et al.*, 2008
		Fat	SNVD(5)	1st	0.759	0.748	0.229		
		Moisture	None	1st	0.717	0.861	0.220		
		Protein	MSC(6)	1st	0.158	0.110	1.047		
		Myoglobine	None	1st	0.914	0.894	0.287		
		WHC(3)	None	1st	0.892	0.864	1.502		
		WBSF(4)	SNDV	1st	0.743	0.743	0.663		
		Tenderness	SNDV	1st	0.981	0.979	0.370		
		Juiciness	SNDV	1st	0.588	0.538	0.645		
		Overall appraisal	None	1st	0.589	0.564	0.456		
Estimate physical parameters of adult steers and young cattle	Reflectance (1100 to 2500 nm)				Steers	Young cattle	Steers	Young cattle	Prieto *et al.*, 2008
		pH	MSC or Log (1/R) or None	1st or 2nd	0.410	0.472	0.06	0.08	
		L* colour			0.585	0.869	1.50	1.56	
		a* colour			0.008	0.707	1.58	1.15	
		b* colour			0.345	0.901	1.46	1.08	
		press loss (%)			0.476	0.576	2.08	2.51	
		drip loss (%)			0.258	0.195	0.36	0.55	
		cooking loss (%)			0.138	0.001	1.61	2.45	
Prediction of fatty acid composition of beef in intramuscular fat	Transmittance (850 to 1050 nm)				Calibration	Validation			Sierra *et al.*, 2008
		SFA(7)	SNDV	2nd	0.859	0.837	0.182		
		BFA(8)	SNDV	2nd	0.747	0.701	2.907		
		MUFA(9)	SNDV	2nd	0.866	0.852	0.140		
		PUFA(10)	None	-	0.252	0.244	0.033		
		CLA(11)	None	-	0.587	0.586	1.613		
Quantitative analysis of pork dry-cured sausages	Reflectance (400 to 1700 nm)		Different treatment according to the sample presentation		Calibration*	Validation*			Gaitán-Jurado *et al.*, 2008
		Fat		None, 1st or 2nd	0.99	0.99	1.88		
		Moisture			0.98	0.98	1.13		
		Protein			0.97	0.96	0.95		
Prediction of sensory characteristics of lamb meat samples	Reflectance (400 to 2498)				Calibration	Validation			Andrés *et al.*, 2007
		Flavour	SNDV and MSC	1st and 2nd	0.343	0.271	0.465		
		Abnormal flavour			0.139	0.048	0.436		
		Overall liking			0.272	0.243	0.481		
		Intramuscular fat (%)			0.841	0.794	0.409		

(1)Coefficient of correlation; (2) Standard Error of Cross Validation; (3) Water Holding Capacity; (4) Warner-Bratzler Shear Force; (5) Standard Normal Variate and Detrend; (6) Multiplicative Scatter Correction; (7) Saturated Fatty Acids; (8) Branched Fatty Acids; (9) Monounsaturated Fatty Acids; (10) Polyunsaturated Fatty Acids; (11) Conjugated Linoleic Acids.

Calibration and Validation=higher value obtained using different scan modes.*

the fish industry. Recently, new technology (like NIR) partially solved these problems, permitting to industries to improve and increase the assessment of fish qualities.

Cozzolino *et al*. (2002) demonstrated that NIRS is a simple and easy technique that can be used to monitor the quality of raw fish but, on the other hand, NIR does not completely replace all reference analytical methods (as, for instance, oil quality assessment) and it is important to maintain skill in reference analysis by lab staff.

This is a limitation for the application of NIR, because the industry would like to get more specific chemical information related to the freshness of the sample, which still requires to be checked periodically.

Fasolato *et al.* (2008a) carried out an experiment for the assessment of quality-parameters and authentication in sole (*Solea vulgaris*) by NIR with the aim to evaluate the performance of this technique in predicting activity water (a_w), pH, moisture, expressible drips (ED%) and total volatile nitrogen (TVN). NIR showed high precision in predicting ED%, a_w and moisture, but it evidenced very low correlations between spectra and TVN.

Other types of raw fish were analysed successfully by NIR: rainbow trout (Lin *et al*., 2006) sea bass (*Dicentrarchus labrax L.*) (Xiccato *et al.*, 2004) Atlantic salmon (Huang *et al*., 2003) cod (Bechmann and Jørgensen, 1998 and Bøknæs *et al*., 2002) swordfish cutlet (*Xiphias gladius*) (Fasolato *et al*., 2008)

Given the perishable nature of fish, extension of its shelf life is a requirement of normal trading (Uddin *et al*., 2005). Freezing is one of the most used preservation modality since it is effective for protecting against microbiological deterioration of fish meat but this treatment can have more or less impact on fish's physicochemical and organoleptic properties.

This is the reason why frozen fish usually have a much lower market price than fresh one, therefore, the substitution of frozen-thawed for fresh fish is a significant authenticity issue (Uddin *et al*., 2005). The substitution and sale of defrosted products labelled as fresh is one of the commercial frauds more difficult to "unmask" and also one of the most frequent one (Fasolato *et al*., 2008)

Many trials' results revealed that NIR can be a strategic tool to use in this kind of problematic (Table 5). In the case of the study regarding swordfish cutlet, NIR was able to discriminate correctly more than 90% of the samples according to their storage treatment (Fasolato *et al*., 2008). Good discriminate results were also obtained in a trial conducted on smoked salmons: NIR spectroscopy was able to completely discriminate fresh from thawed salmon fillets (stored for 2-4 months in freezer). A little bit less accurate, but still really interesting, was the discrimination between fresh and thawed salmon after one month of freezer storage (Fasolato *et al*., 2008).

NIR spectroscopy was also used to discriminate sea bass fish (*Dicentrarcus labrax L.*) according to its origin (Xiccato *et al*., 2004) (Table 4). The different origin (Greece, Italy, Turkey, etc.), rearing systems and feeding regimes used for sea bass production may affect the comprehensive flesh quality. Consequently the prices of this fish could appreciably change according to these factors. The authors of the trial demonstrated that NIR spectroscopy was able to discriminate properly the origin and the farming conditions of fish, but samples had to be first freeze-dried in order to obtain proper discrimination of different groups.

Providing information about the preserving method and origin of a fish product is of enormous importance for the perception of its quality by the final

Table 3. Some applications of NIR spectroscopy for fish analysis.

Objective of the trial	Measurement modes	Parameters	Scatter correction	derivative	$R^{2(1)}$		SECV[(2)]	References
					Calibration	Validation		
Prediction of chemical characteristics of minced raw fish	Reflectance (1100 to 2500)	Moisture			0.99	0.99	3.86	Cozzolino *et al.*, 2002
		TVN[(3)]	SNVD[(4)]	1st	0.96	0.83	3.51	
		Oil			0.99	0.96	8.01	
		temperature			0.98	0.92	1.07	
Determination of water and protein in surimi	Transmittance (900 to 1100)	Crude protein	MSC & SG[(5)]	2nd	0.982		0.13	Uddin *et al.*, 2006
		Water	SG	2nd	0.978		0.38	
					Calibration*	Validation*		
Chemical composition of European sea bass	Reflectance (1100 to 2500 nm)	Water			0.97	0.97	2.76	Xiccato *et al.*, 2004
		Ether extract		2nd	0.98	0.97	2.81	
		Crude protein			0.81	0.68	0.55	
		Gross energy			0.98	0.96	1.20	

[(1)]Coefficient of correlation; [(2)]Standard Error of Cross Validation; [(3)]Total Volatile Nitrogen; [(4)]Standard Normal Variate and Detrending; [(5)]Savitzky-Golay.
Calibration and Validation=higher value obtained using different sample preparation.*

Table 4. Performance of four-cluster analysis of fresh and freeze-dried minced filled of sea bass (Dicentrarcus labrax L.)(Xiccato et al., 2004).

	Correctly classified (%)	Classified in two or more cluster (%)	Wrongly classified (%)	Not classified (%)
Fresh minced fillet				
Extensive	65	16	4	15
Semi-intensive	58	25	2	15
Intensive	37	43	2	18
Sea cages	45	38	1	6
Freeze dried fillet				
Extensive	83	0	0	17
Semi-intensive	80	0	2	18
Intensive	74	0	2	24
Sea cages	83	0	1	16

consumer. For the benefit of the consumer and prevention of unfair competition in the trade of fishery products, correct labelling of froze-thawed fish or fillets is desirable. Consequently, control of labelling is possible only if there is any rapid and reliable methods that allow food control authorities to distinguish between fresh and frozen-thawed fish or fillets (Uddin *et al*., 2005) and distinguish fish according to the origin.

Table 5. Discrimination between fresh and defrost sole (*Solea vulgaris*) (Fasolato *et al.*, 2008a) and swordfish cutlet (Xiphias gladius) (Fasolato *et al.*, 2008).

	Product: sole		Product: Swordfish cutlet	
NIR attribution	Fresh	Thawed	Fresh	Thawed
Fresh	103	7	82	5
Thawed	3	28	8	55
Total samples	106	35	90	60
Mistakes	3	7	8	5
Good assigned %	98	80	91.1	91.6

NIR seems to be a really useful and promising technique in this sense. In table 3 are reported some application of NIR spectroscopy for fish analysis.

Milk and dairy products

The major components of milk can be analysed off-line in few minutes using an infrared (IR) spectroscopy instrument such as Milko-scan (Foss Analytical A/S). Although these type of instruments have practically replaced any wet chemistry determination of routine samples, there is a growing interest in the evaluation of milk quality on-line so that have a rapid device to control the product during the milking stage (Saranwong and Kavano, 2008).

Tsenkova and contributors published many papers about the application of NIR spectroscopy to evaluate and monitor many properties of milk quality: protein, fat, lactose content (Tsenkova *et al*., 2000), Somatic Cell Count (SCC) determination (Tsenkova *et al*., 2001a and 2001b) disease diagnosis and pathogen identification (Tsenkova *et al*., 2006). These parameters (especially fat, protein and SCC) have economic importance because, in most countries, milk trade is based on these factors. SCC is a recognized indicator of cow's health, as it reflects the level of infection and resultant inflammation in the mammary gland of dairy cows, associated with mastitis. Cow health is becoming a growing issue in intensive dairy farm and early detection of potential problem is the key to avoid great economical losses. As milk composition reflects the metabolism of the animal, applications like the determination of ketones body (De Roos *et al*., 2007) by FT-NIR spectroscopy would be able to identify nutritional problems becoming an important management tool. Saranwong and Kavano (2008) applied NIR spectroscopy also to evaluate the contamination of milk through the determination of the aerobic bacteria count.

In Italy the major part of produced milk (around 70%) is transformed to cheese. Therefore it is really important to know the milk ability to coagulate and also in to have some initial knowledge about all the phases of the process, from the beginning to the end. Klandar *et al*., (2007) compared different analytical methods to assess this property and they demonstrated that NIR appear to be suitable as a non destructive, non-intrusive and on-line method to evaluate the rennet-induced milk coagulation. These results confirmed what Laporte *et al*. (1998) reported more than ten years ago monitoring the rennet coagulation in cow's milk using a near-infrared optic probe. In fact they found that NIR technology provides a better estimate of the coagulation profile than the coagulometer (thermal probe). Giangiacomo *et al*., (1998) utilized NIR, fluorimetric

Table 6. Some applications of NIR spectroscopy on milk and dairy products.

Objective of the trial	Measurement modes	Parameters	Scatter correction	derivative	$R^{2(1)}$		SECV[2]	References
					Calibration	Validation		
Analysis of fat, protein and casein in cow's milk	Transmission (1100 to 2500)	Fat	SNV	2nd	1	1	0.12	Laporte and Pasquin, 1999
		Crude protein	SNV[3]	2nd	0.98	0.95	0.11	
		True protein	SNV	2nd	0.99	0.91	0.08	
		Casein	SNV	2nd	0.98	0.96	0.09	
Milk quality assessment in milking robot	Transmittance (600-1050 nm)	Fat	None	-	0.96	0.95	0.25	Kawasaki *et al.*, 2008
		Lactose	None	-	0.82	0.83	0.26	
		Protein	None	-	0.78	0.72	0.15	
		SCC[4]	None	-	0.64	0.68	0.28	
		MUN [5]	None	-	0.31	0.53	1.50	
					Calibration	Test set		
Disease diagnosis and pathogen identification	Transflectance (1100 to 2500 nm)	SCC	SG[7] polynomial filter		0.72	0.77	0.368	Tsenkova *et al.*, 2006
		AEC [6]		1st	0.73	0.76	0.491	
Monitoring rennet coagulation in cow's milk	Reflectance (1100-2500 nm)	Percent of coagulation	None and SNVD [8]	1st	0.94	0.87	0.22	Laporte *et al.*, 1998
		Appearance and texture				Validation		
		Adhesivity				0.72	0.25	
		Friability				0.49	0.25	
		Elasticity				0.68	0.20	
		Firmness				0.82	0.25	
Prediction of sensory attributes of European Emmental cheese	Diffuse reflection (1000 to 2500 nm)	*Olfacto-gustatory characteristics*						Karoui *et al.*, 2006a
		Aroma intensity				0.60	0.20	
		Odour intensity				0.32	0.28	
		Bitterness				0.63	0.14	
		Saltiness				0.53	0.20	
		Acidity				0.63	0.17	
		Sweetness				0.71	0.11	

[1]Coefficient of correlation; [2]Standard Error of Cross Validation; [3]Standard Normal Variate; [4]Somatic Cell Count; [5]Milk Urea Nitrogen; [6]Absolute Electrical Conductivity; [7]Savitzky-Golay; [8]Standard Normal Variate and Detrend.

and colorimetric methods to monitor the primary clotting phase on reconstituted skim milk powder added with liquid calf rennet solution and to detect eventually the relationship between water and the other constituents during the phases changes. The authors found interesting results by plotting selected NIR 2nd derivative absorbance values against time which permitted to obtain a trend of coagulation.

Nowadays, in the cheese manufacturing industry, in which a high degree of automation is needed, cheese making indices (coagulation and syneresis, predict curd moisture, fat loses and curd yield) could be predicted on line. This is what it was accomplished by Fagan *et al*. (2008) demonstrating the excellent potential of an on-line optical light backscatter sensor to monitor curd syneresis. Čurda and Kukačková (2004) used NIR technique for assessment of dry matter (DM), fat, crude protein, pH and rheological properties (as penetration) of processes cheeses. They obtained rather high correlation coefficients of calibration for all measured quantities (R^2>0.98). Penetration and pH could be also estimated, but with a lower accuracy. Karoui *et al*. (2006) confirmed that NIR can be used for the determination of fat, total nitrogen, water soluble nitrogen and non protein nitrogen with varying degrees of accuracy in Emmental cheeses, but it failed to determine NaCl and pH parameters.

Cheese ripening is a common preservation technique for this kind of foodstuff. Different product can have different degrees of ripening and this has an heavy effect on texture, colours and flavour development on the final product. Proteolysis and lipolysis are the principal and most complex biochemical events occurring during the maturation of cheese, while during cheese ripening, caseins are converted into water-soluble nitrogenous compounds, such as peptides and amino acids. González-Martín *et al*. (2009) applied NIR spectroscopy for the determination of these peptides in different kinds of cheeses (cow's, ewe's and goat's) with different ripening times using a remote reflectance fibre-optic probe. The obtained results with the NIR method were comparable with those of the chromatographic method (RP-HPLC) and they showed that it is possible to rapidly identify and quantify the peptides in unknown cheese produced with milk of different species.

As far as fresh cheeses are concerned, "freshness" is associated with low acidity, limited proteolysis, and no bitter taste. Cattaneo *et al*., 2005 utilised FT-NIR and FT-IR spectroscopy to evaluate the shelf-life period in which "freshness" is maintained. Discriminant analysis, in this case, was able to correctly separate "fresh" samples (<6 days) from the other ones stored for more than 6 days. Lower accuracy was obtained for the discrimination of samples stored for more than 6 days. Cattaneo *et al*. (2008) suggested further investigations in order to differentiate the type of cheese during the whole shelf life period.

In many countries of European union, some dairy products are produced following precise techniques and recipes, which reflect gastronomic and historical traditions of circumscribed geographical areas. These artisan food products are therefore differentiated from other similar products and may be labelled according to the specific conditions which characterize their origin and the processing technologies used. These are referred to as products with *Protected Designation of Origin* (PDO) and *Protected Geographical Indication* (PGI), and are often associated with high production costs; they may, consequently be highly priced. This makes these products prone to adulteration with cheaper alternatives, for economic reasons (Manley *et al*., 2008).

It seems indispensable in these cases to find out an analytical technique able to discriminate between conventional food and product obtained in specific geographical area. Karoui *et al*. (2005) evaluated the potential of combined infrared and fluorescence spectroscopies to determinate the geographical origin of Emmental cheeses. A good classification of cheeses was observed for 89% and 86,8% of the calibration and validation spectral data set, respectively. These results showed that spectroscopic techniques may provide useful fingerprints and allow the identification of Emmental cheeses according to the geographic origin and production conditions.

The same authors tried also to predict some sensory attributes of European Emmental (Karoui *et al*. 2006a). Aroma intensity and flavour are among the most important properties for the consumers and numerous studies have been performed in attempts to find correlation between sensory qualities and objective instrumental measurements. Cattaneo *et al*. (2008) compared sensory scores and near infrared absorption for characterising Bitto (an Italian PDO cheese). The aim of this trial was to study the relationship between sensory scores and NIR data in verifying which attributes can be related to specific absorption bands in the whole NIR region. The preliminary results of this study, proved that FT-NIR can be an useful tool for the prediction of cheese sensory characteristics. Karoui *et al*. (2006a) evaluated four appearance-texture attributes (adhesivity, friability, elasticity and firmness) and six olfacto-gustatory attributes (aroma intensity, odour intensity, bitterness, saltiness, acidity and sweetness) and demonstrated the feasibility of NIR spectroscopy to predict some of these sensory attributes (e.g. adhesivity, firmness and sweetness) of Emmental cheeses originating from different European regions showing coefficient of prediction with a R^2 higher than 0.70. Lower correlations were obtained for the other parameters, probably because the samples number was limited and data variability was quite low.

An other important dairy product for human nutrition is yogurt. This foodstuff contains high content of nutrients, can be digested and assimilated more easily than fresh milk. These characteristics make of yogurt a really popular food all over the world. NIR spectroscopy was also used to analyse the major components of this product. The most important parameters taken into account by NIR calibration were sugar content and acidity (Shao *et al.*, 2007) which have the greatest influence on the sensorial acceptance of yogurt from consumers. The obtained results were quite encouraging with a correlation coefficient of 0.92 and 0.91 for sugar content and acidity respectively.

Recently NIR spectroscopy has been used also for developing calibration models on goat milk (Dračková *et al*., 2008) obtaining good model fitting (R^2>0.87) for all of the main quality traits (proteins, fat, lactose, total solids, non-fatty solids). Less accurate models were obtained for freezing point and titratable acidity.

Furthermore, Castillo *et al.* (2000) used NIR spectroscopy to monitor the whole process of goat milk curd formation. This study had the objective to develop a device able to suggest to the cheese maker, the optimal cutting time of the curd which strongly effects the final quality of goat cheese.

Table 6 lists some results carried out on milk and dairy products.

Eggs

In the sector of egg, the application of NIR for the determination of egg freshness is rather limited. Only few studies have been published about the potential of this

technique to determine the egg freshness. The first publication on measuring eggs by NIR addressed an early stage of Norris' work (Norris K.H., 1996). Subsequently, Bamelis *et al*. (2003) showed significant variations of the spectra with the storage duration and Kemps *et al.* (2006) reported correlation coefficient higher than 80% for the prediction of the albumen quality in terms of Haugh unit, an expression relating the thick albumen height and the egg mass. Recently, a predictive model of thick albumen height was obtained with a determination coefficient of 0.82 by means of diffuse reflectance Fourier Transform (FT-NIR) spectroscopy (Berardinelli *et al*., 2005).

After laying, several changes occur in the internal constituents of shell eggs during storage. These modification are mainly related to gaseous exchanges with the ambient through the shell pores (water and CO_2) and the osmotic exchanges between the albumen and yolk through the vitelline membrane.

The rate at which these changes occur during storage depends, mostly, on duration, environmental temperature and relative humidity, and hen age and strain. Giunchi *et al.* (2008) demonstrated that diffuse reflectance FT-NIR spectroscopy, conducted by means of fiber optic probe in direct contact with different points of the shell, appeared able to discriminate shell eggs during storage: they were able to classify egg samples according to the days of storage with an overall rate of 100%. The cluster analysis, they carried on, seemed to group the spectral data in distinct clusters characterized by different days of storage. Furthermore, standard errors of prediction (SEP) and coefficient of correlations obtained for the thick albumen height, Haugh unit and air cell height indicated that the model

Table 7. An application of NIR spectroscopy on freeze-dried eggs.

Objective of the trial	Measurements mode	Parameters	Scatter correction	deri-va-tive	$R^{2(1)}$		SECV[2]	References
					Calibration	Validation		
Prediction of chemical freeze dried egg yolk's composition	Reflectance (1100 to 2498 nm)	Dry matter	SNVD[7]	1st	0.81	0.76	0.44	Dalle Zotte *et al.*, 2006
		Crude protein	MSC[8]	-	0.75	0.71	0.75	
		Lipids	SNVD	1st	0.85	0.84	0.51	
		Ash	MSC	-	0.06	0.05	0.60	
		SFA[3]	SNVD	1st	0.60	0.54	0.75	
		MUFA[4]	DT[9]	1st	0.61	0.57	0.69	
		PUFA[5]	DT	1st	0.75	0.73	0.59	
		pH	SNVD	1st	0.21	0.17	0.12	
		Lightness (L*)	MSC	2nd	0.19	0.14	1.58	
		Redness (a*)	MSC	2nd	0.56	0.51	0.94	
		Yellowness (b*)	MSC	2nd	0.15	0.13	2.36	
		Chroma (C*)	MSC	2nd	0.15	0.13	2.32	
		Hue (H°)	MSC	2nd	0.56	0.48	0.11	
		TBARS (µg MDA/g)[6]	MSC	2nd	0.70	0.60	1.73	

[1]Coefficient of correlation; [2]Standard Error of Cross Validation; [3]Saturate Fatty Acids; [4]Monounsaturated fatty Acids; [5]Polyunsaturated Fatty Acids; [6]Thiobarbituric Acid-Reactive Substances; [7]Standard Normal Variate and Detrending; [8]Multiplicative Scatter Correction; [9]Detrending.

was suitable for a rough screening of eggs.

Egg white and egg yolk are also extensively utilised as ingredients because of their unique functional properties, such as gelling, foaming, emulsifying. Really common is the use of freeze dried eggs in foodstuffs production. The freeze-drying is an useful technique to preserve perishable food. Dalle Zotte *et al.* (2006) obtained interesting results using NIR reflectance spectroscopy in the prediction of chemical composition of freeze-dried egg yolk (Table 7) and they were able to discriminate eggs obtained by hens fed with different n-3 PUFA (polyunsaturated fatty acids) feeding source (marine origin, extruded linseeds, ground linseed).

The application of NIR spectroscopy in egg industry seems to be really interesting, since it is able to monitor a vast number of eggs in a short time providing information about storage history and chemical content. This last advantage is particularly useful for foodstuff processing, where eggs are often used for their functional properties, therefore knowing possible variation in egg composition help to formulate proper food recipes.

Conclusions

NIR spectroscopy, combined with appropriate chemometric tools, offers a fast and accurate method for feed and food analysis and has the potential to be used on a wide range of products.

Undoubtedly, NIR spectroscopy is playing and will play a central role in many industries and productive sectors to elevate food production chains to higher quality standard requirement.

In animal production NIR analysis is considered as a suitable tool to implement frequent controls during the entire production chain (from harvest to feed formulation in the case of feed industries, from slaughter house to retailing for meat and meat products field, and from milking to cheese ripening in the case of dairy sector).

NIR spectroscopy has also a potential role in increasing consumer confidence by confirming integrity of food, particularly in the areas of authenticity of both raw ingredients and final products.

Although NIR spectroscopy is daily used by a wide number of industries operating in feed and food fields, the true potential of this technology is yet to be fully recognised or understood so that many applications have still to be discovered. The challenges for the future of this technology will be the development of application which can be manage by personal with little training and to bring NIR spectroscopy directly into the production process with rugged and low cost instruments.

REFERENCES

Alomar, D., Gallo, C., Castañeda, M., Fuchslocher, R., 2003. Chemical and discriminant analysis of bovine meat by near infrared reflectance spectroscopy (NIRS). Meat Sci 63: 441-450.

Andrés S., Murray, I., Calleja, A., Javier Giráldez, F., 2005. Nutritive evaluation of forages by near infrared reflectance spectroscopy. J Near Infrared Spec 13: 301-311.

Andrés, S., Murray, I., Navajas, E. A., Fisher, A. V., Lambe, N. R., Bünger, L., 2007. Prediction of sensory characteristics of lamb meat samples by near infared reflectance spectroscopy. Meat Sci 76: 509-516.

Andrés, S., Silva, A., Soares-Pereire, A. L., Martins, C., Bruno-Soares, A. M., Murray, I., 2008. The use of visible and near infrared reflectance spectroscopy to predict beef *M. longissimus thoracis et lumborum* quality attributes. Meat Sci 78: 217-224.

Baeten, V., Dardenne, P., 2002. Spectroscopy: Developments in instrumentation and analysis. Grasas y Aceites 53 (1): 45-63.

Bamelins, F. R., Kemps, B. J., Mertens, K., Govaerts, T., De Ketelaere, B., Kamers, B., Kokou, T., Decuypere, E. M., De Baerdemaeker, J. G., 2003. Non destructive internal egg freshness assessment using VIS-NIR spectroscopy. Proc. Xth European Symposium on the quality of eggs and egg products, France, Saint-Brieuc Ploufragan, pp. 91-97.

Bechmann, I. E., Jørgensen, B. M., 1998. Rapid assessment of quality parameters for frozen cod using near infrared spectroscopy. Lebensm-Wiss U.-Technol 31: 648-652.

Berardinelli, A., Giunchi, A., Guarnieri, A., Pezzi, F., Ragni, L., 2005. Shell egg albumen height assessment by FT-NIR spectroscopy. Transaction of the ASAE 48 (4): 1426-1428.

Berzaghi, P., Flinn, P. C., Dardenne P., Lagerholm, M., Shenk, J. S., Westerhaus, M. O., Cowe, I. A., 2001. Comparison of linear and non-linear NIR calibration methods using large forage databases. Proceedings of the 10th International NIRS Conference Ed. By A. M. C. Davies and R. K. Cho.

Berzaghi, P., Dalle Zotte, A., Jansson, L. M., Andrighetto, I., 2005. Near-infrared reflectance spectroscopy as a method to predict chemical composition of breast meat and discriminate between different n-3 feeding sources. Poultry Sci 84: 128-136.

Blanco, M., Villarroya, I., 2002. NIR spectroscopy: a rapid- response analytical tool. Trends in analytical chem 21 (4): 240-250.

Bøknæs, N., Jensen, K. N., Andersen C. M., Martens H., 2002. Freshness assessment of thawed and chilled cod fillets packed in modified atmosphere using near-infrared spectroscopy. Lebensm-Wiss. U.-Technol 35: 628-634.

Brimmer, P. J., Hall, J. W., 2001. Method development and implementation of near-infrared spectroscopy in industrial manufacturing support laboratories. In: Williams, P. K., Norris, K. (Eds.), Near-Infrared Technology in the Agricultural and Food Industries, second ed. American Association of Cereal Chemists Inc., St. Paul, Minnesota, USA, pp. 187–198.

Brøndum, J., Munck, L., Henckel, P., Karlsson, A., Tornberg, E., Engelsen, S. B., 2000. Prediction of water-holding capacity and composition of porcine meat by comparative spectroscopy. Meat Sci 55: 177–185.

Castillo, M., Payne, F. A., Hicks, C. L., Lopez, M. B., 2000. Predicting cutting and clotting time of coagulating goat's milk using diffuse reflectance: effect of pH, temperature and enzyme concentration. Int Dairy J 10: 551-562.

Cattaneo, T. M. P., Giardina, C, Sinelli, N., Riva, M., Giangiacomo, R., 2005. Application of FT-NIR and FT-IR spectroscopy to study the shelf-life of Crescenza cheese. Int Dairy J 15: 693-700.

Cattaneo, T. M. P., Tornelli, C., Erini, S., Panarelli, E. V., 2008. Relationship between sensory scores and near infrared absorptions in characterising Bitto, an Italian protected denomination origin cheese. J Near Infrared Spec 16: 173-178.

Cen, H., He, Y., 2007. Theory and application of near infrared reflectance spectroscopy in determination of food quality. Trends Food Sci Tech 18: 72-83.

Cozzolino, D., Murray, I., Scaife, J. R., 2002. Near infrared reflectance spectroscopy in the prediction of chemical characteristics of minced raw fish. Aquacultur Nutr 8: 1-6.

Cozzolino D., Murray I., 2004. Identification of animal meat muscles by visible and near infrared reflectance spectroscopy. Lebensm.-Wiss. U.-Technol 37: 447-452.

Cozzolino, D., Fassio, A., Fernández, E., Restaino, E., La Manna, A., 2006. Measurement of chemical composition in wet whole maize silage by visible and near infrared reflectance spectroscopy. Anim Feed Sci Tech 129: 329-336.

Čurda, L., Kukačková, O., 2004. NIR spectroscopy: a useful tool for rapid monitoring of processed cheeses manufacture. J Food Eng 61: 557-560.

Dalle Zotte, A., Berzaghi, P., Jansson, L. M., Andrighetto, I., 2006. The use of near-infrared reflectance spectroscopy (NIRS) in the prediction of chemical composition of freeze dried egg yolk and discriminant between different n-3 PUFA feeling source. Anim Feed Sci Tech 128: 108-121.

De la Haba, M. J., Garrido-Varo, A., Pérez-Marín, D. C., Guerrero, J. E., 2007. Near infrared analysis as a first-line screening technique for identifying animal species in rendered animal by-product meals. J Near Infrared Spec 15: 237-245.

De la Haba, M. J., Fernández Pierna, J. A., Fumière, O., Garrido-Varo, A., Guerrero, J. E., Pérez-Marín, D. C., Dardenne, P., Baeten, V., 2007a. Discrimination of fish bones from other animal bones in the sedimented fraction of compound feeds by near infrared microscopy. J Near Infared Spec 15: 81-88.

De la Haba, M. J., Garrido-Varo, A., Núñez, N., Pérez-Marín, D. C., Guerrero, J. E., 2008. Using calibrations developed for fine milled meat and bone meal on spectra measured on non-milled samples. J Near Infrared Spec 6:275-279.

De la Roza-Delgado , B., Soldado, A., Martínez-Fernández, A., Vicente, F., Garrido-Varo, A., Pérez-Marín, D., De la Haba, M. J., Guerrero-Ginel, J. E., 2007. Application of near-infrared microscopy (NIRM) for the detection of meat and bone meals in animal feeds: A tool for food and feed safety. Food Chem 105: 1164-1170.

De Roos, A. P. W., Van Den Bijgaart, H. J. C. M., Horlyk, J., De Jong, G., 2007. Screening for subclinical ketosis in dairy cattle by Fourier transform infrared spectrometry. J Dairy Sci 90: 1761-1766.

Dračková, M., Hadra, L., Janštová, B., Navrátilová, P., Přidalová, H., Vorlová, L., 2008. Analysis of goat milk by Near-Infrared spectroscopy. Acta vet Brno 77: 415-422.

Fasolato, L., Ferlito, J. C., Mirisola, M., Tepedino, G., Tanzi, E., Barbuti, S., 2008b. Pros and cons of smoked salmon. Eurofishmarket 10: 18-53.

Fasolato, L., Manfrin, A., Corrain, C., Perezzani, A., Arcangeli, G., Rosteghin, M., Novelli, E., Lopparelli, R. M., Balzan, S., Mirisola, M., Serva, L., Segato, S., Bianchi, E., 2008a. Assessment of quality-parameters and authentication in sole (*Solea vulgaris*) by NIRS (Near infrared reflectance spectroscopy). Industrie alimentari-XLVIII 355-362.

Fasolato, L., Mirisola, M., Tepedino, G., Balzan, S., Arcangeli, G., Rosteghin, M., Corrain, C., Manfrin, A., Berzaghi, P., 2008. No more defrost for fresh. Eurofishmarket 1: 22-45.

Fernández-Ahumada, E., Garrido-Varo, A., Guerrero-Ginel, J. E., Pérez-Marín, D., Fearn, T., 2008a. Taking NIR calibrations of feed compounds from the laboratory to the process: calibration transfer between predispersive and postdispersive instruments. J Agric Food Chem. 56: 10135-10141.

Fernández-Ahumada, E., Guerrero-Ginel, J. E., Pérez-Marín, D., Garrido-Varo, A., 2008. Near infrared spectroscopy for control of the compound-feed manufacturing process: mixing stage. J Near Infrared Spec 16: 285-290.

Fernández Pierna, J. A., Baeten, V., Michotte Renier, A., Cogdill, R. P., Dardenne, P., 2004. Combination of support vector machines (SVM) and near-infrared (NIR) imaging spectroscopy for the detection of meat and bone meal (MBM) in compound feeds. J Chemometr 18: 341-349.

Gaitán-Jurado, A. J., Ortiz-Somovilla, V., España-España, F., Pérez-Aparicio, J., De Pedro-Sanz E. J., 2008. Quantitative analysis of pork dry-cured sausages to quality control by NIR spectroscopy. Meat Sci 78: 391-399.

Geesink, G. H., Schreutelkamp, F. H., Frankhuizen, R., Vedder, H. W., Faber, N. M., Kranen, R. W., Gerritzen, M.A., 2003. Prediction of pork quality attributes from near infrared reflectance spectra. Meat Sci, 65: 661–668.

Garcia, J., Cozzolino, D., 2006. Use of Near infrared reflectance (NIR) spectroscopy to predict chemical composition of forages in broad-based calibration model. Agricultura técnica 66 (1): 41-47.

García-Olmo, J., De Pedro, E., Garrido-Varo, A. Jimenez, A., Salas, J., Santolalla, M., 2000. Fatty acids analysis of Iberian pig fat by near infrared spectroscopy (NIRS). CIHEAM- Options Mediterraneennes: 191-195.

Garrido-Varo, A., Pérez-Marín, D., Bautista-Cruz, J., Guerrero-Ginel, J. E., 2008. Near infrared spectroscopy for quantification of animal-origin fats in fat blends. J Near Infrared Spec 16: 281-283.

Gelaldi, P., 2002. Some recent trends in the calibration literature. Chemometr Intell Lab, 60: 211-214.

Giangiacomo, R., Lizzano, R., Barzaghi, S., Cattaneo, T.M.P., 1998. NIR and other luminometric methods to monitor the primary clotting phase of milk. J Near Infrared Spec 6 (1): 205-212.

Giunchi A., Bardinelli, A., Ragni, L., Fabbri, A., Silanghi, F. A., 2008. Non-destructive assessment of shell eggs using FT-NIR spectroscopy. J Food Eng 89: 142-148.

Givens, D. I., Deaville, E. R, 1999. The current and future role of near infrared reflectance spectroscopy in animal nutrition: a review. Aust J Agric Res 50: 1131-1145.

González-Martín, I., Hernández-Hierro, J. M., Vivar-Quintana, A., Revilla, I., González-Pérez, 2009. The application of near infrared spectroscopy technology and a remote reflectance fibre-optic probe for the determination of peptides in cheeses (cow's, ewe's and goat's) with different ripening times. Food Chem 114: 1564-1569.

Hildrum, K. I., Nilsen, B. N., Mielnik, M., Naes, T., 1994. Prediction of sensory characteristics of beef by near infrared spectroscopy. Meat Sci 38 (1): 67-80.

Huang, Y., Cavinato, A. G., Mayes, D. M., Kangas, L. J., Bledsoe, G. E., Rasco, B. A., 2003. Nondestructive determination of moisture ad sodium chloride in cured Atlantic salmon (*Salmo salar)* (Teijin) using short-wavelength near-infrared Spectroscopy (SW-NIR). J Food Sci 68(2): 482-486.

Huang, H., Yu, H., Xu, H., Ying, Y., 2008. Near infrared spectroscopy for on/in line monitoring of quality in foods and beverages: A review. J Food Eng 87: 303-313.

Hoving-Bolink, A. H., Vedder, H. W., Merks, J. W. M., De Klein, W. J. H., Reimert, H. G. M., Frankhuizen, R., van den Broek, W. H. A. M., Lambooij, E., 2005. Perspective of NIRS measurements early post mortem for prediction of pork quality. Meat Sci 69: 417-423.

Isaksson, T., Nilsen, B. N., Tøgersen, G., Hammond, R. P., Hildrum, K. I., 1996. On-line, proximate analysis of ground meat batches directly at a meat grinder outlet. Meat Sci 43: 245-253.

Karoui, R., Dufour, É., Pillonel, L., Schaller, E., Picque, D., Cattenoz, T., Mouazen, A. M., Schaller, E., De Baerdemaeker, J., Bosset J. O., 2005. The potential of combined infrared and fluorescence spectroscopies as a method of determination of the geographic origin of Emmental cheeses. Int Dairy J 15: 287-298.

Karoui, R., Mouazen, A. M., Dufour, É., Pillonel, L., Schaller, E., De Baerdemaeker, J., Bosset J. O., 2006. Chemical characterisation of European Emmental cheeses by near infrared spectroscopy using chemometric tools. Int Dairy J 16: 1211-1217.

Karoui, R., Pillonel, L., Schaller, E., Bosset J. O., De Baerdemaeker, J., 2006a. Prediction of sensory attributes of European Emmental cheese using near-infrared spectroscopy: a feasibility study. Food Chem 101: 1121-1129.

Katsumoto, Y., Jang, J. H., Berry, R. J. and Ozaki, Y., 2001. Modern pre-treatment methods in NIR spectroscopy. Near Infrared Analysis 2: 29-36.

Kawasaki, M., Kawamura, S., Tsukahara, M., Morita, S., Komiya, M., Natsuga, M., 2008. Near infrared spectroscopic system for on-line milk quality assessment in a milking robot. Computers and electronics in agriculture 63: 22-27.

Kemps, B. J., Bamelins, F. R., De Ketelaere, B., Mertens, K., Tona, K., Decuypere, E. M., De Baerdemaeker, J. G., 2006. Visible transmission spectroscopy for the assessment of egg freshness. J Sci food agr 86: 1399-1406.

Klandar, A. H., Lagaude, A., Chevalier-Lucia, D., 2007. Assessment of the rennet coagulation of skim milk: A comparison of methods. Int Dairy J 17: 1151-1160.

Laporte, M. F., Martel, R., Pasquin, P., 1998. The near-infrared optic probe for monitoring rennet coagulation in cow's milk. Int Dairy J 8: 659-666.

Laporte, M. F., Pasquin, P., 1999. Near-Infrared analysis of fat, protein, and casein in cow's milk. J Agri Food Chem 47: 2600-2605.

Leardi, R., 2008. Chemometric methods in food authentication. In: modern techniques for food authentication Da-Wen Sun 1st ed., Amsterdam; Boston: Elsevier/Academic Press. Pp 585-616.

Li, H., Lv, X., Wang, J., Li, J., Yang, H., Qin, Y., 2007.

Quantitative determination of soybean meal content in compound feeds: comparison of near-infrared spectroscopy and real-time PCR. Anal Bioanal Chem 389: 2313-2322.

Lin, M., Mousavi, M., Al-Holi, M., Cavinato, A. G., Rasco, B.A., 2006. Rapid near infared spectroscopic method for the detection of spoilage in rainbow trout (Oncorhynchus mykiss) fillet. J Food Sci 71(1): S18-S23

Lovett, D. K, Deaville, E. R, Mould, F., Givens, D. I., Owen, E., 2004. Using near infrared reflectance spectroscopy (NIRS) to predict the biological parameters of maize silage. Anim Feed Sci Tech 115: 179-187.

Lovett, D. K., Deaville, E. R., Givens, D. I., Finlay, M., Owen, E., 2005. Near infrared reflectance spectroscopy (NIRS) to predict biological parameter of maize silage: effects of particle comminution, oven drying temperature and the presence of residual moisture. Anim Feed Sci Tech 129: 323-332.

Manley, M., Downey, G., Baeten, V., 2008. Spectroscopic technique: Near-Infrared (NIR) spectroscopy. In: Modern techniques for food authentication. Da-Wen Sun 1st ed., Amsterdam; Boston: Elsevier/Academic Press. Pp 65-115.

Massart, D. L., Vandeginste, B. G. M., Buydens, L. M. C., *et al,* 1988. Chemometrics: A textbook, Vol.2. Amsterdam: Elsevier.

McElhinney, J., Downey, G., 1999. Chemometric processing of visible and near infrared reflectance spectra for species identification in selected raw homogenised meat. J Near Infrared Spec 7: 145-154.

Molette, C. , Berzaghi, P., Dalle Zotte, A., Remignon, H., Babile, R., 2001. The Use of Near-Infrared Reflectance Spectroscopy in the Prediction of the Chemical Composition of Goose Fatty Liver. Poultry Sci 80: 1625-1629.

Monin, G., 1998. Recent methods for predicting quality of whole meat. Meat Sci 49: S231-S243.

Murray, I., 1999. NIR spectroscopy of food: single things, dabble things and spectra. NIR news 10: 10-12.

Nilsen, H., Esaiassen, M., Heia, K., Sigernes, F., 2002. Visible/near infrared spectroscopy. A new tool for the evaluation of fish frshness? J Food Sci 67(5): 1821-1826.

Norris K. H., Barnes R F., Moore J. E., Shenk J. S., 1976. Predicting forage quality by near infrared reflectance spectroscopy. J Anim Sci 43 (4): 889-897.

Norris, K. H., 1996. History of NIR. J Near Infrared Spec 4: 31-37

Pasquini C., 2003. Near infrared spectroscopy: fundamental, practical aspects and analytical application. J Braz Chem Soc, 14 (2): 198-219.

Pérez-Marín, D., Garrido-Varo, A., Guerrero-Ginel, J. E., Gómez-Cabrera, A., 2004. Near-infrared reflectance spectroscopy (NIRS) for the mandatory labelling of compound feedingstuffs: chemical composition and open-declaration. Anim Feed Sci Tech 116: 333-349.

Prevolnik, M., Candek-Potokar, M., Skorjanc, D., 2004. Ability of NIR spectroscopy to predict meat chemical composition and quality – a review. Czech J Anim Sci 49, 500–510.

Prieto, N., Andrés, S., Giráldez, F. J., Mantecón, A. R., Lavín, P., 2006. Potential use of near infrared reflectance spectroscopy (NIRS) for the estimation of chemical composition of oxen meat samples. Meat Sci 74: 487-496.

Prieto, N., Andrés, S., Giráldez, F. J., Mantecón, A. R., Lavín, P., 2008. Ability of near infrared reflectance spectroscopy (NIRS) to estimate physical parameters of adult steers (oxen) and young cattle meat samples. Meat Sci 79: 692-699.

Ripoll, G., Albertí, P, Panea, B., Olleta, J. L., Sañudo, C, 2008. Near-infrared reflectance spectroscopy for predicting chemical, instrumental and sensory quality of beef. Meat Sci 80: 697-702.

Sarawong, S., Kawano, S., 2008. System design for non-destructive near infrared analysis of chemical components and total aerobic bacteria count of raw milk. J Near Infrared Spec 16: 389-398.

Savenije, B. , Geesink G. H., Van Der Palen, J. G. P., Hemke G., 2006. Prediction of pork quality using visible/near-infrared reflectance spectroscopy. Meat Sci 73: 181-184.

Shao, Y., He., Y., Feng, S., 2007. Measurement of

yogurt internal quality through using Vis/NIR spectroscopy. Food Res Int 40: 835-841.

Shenk, J. S., Westerhaus, M. O., 1991. Population definition, sample selection, and calibration procedures for near infrared Reflectance spectroscopy. Crop Sci 31: 469-474.

Sierra, V., Aldai, N., Castro, P., Osoro, K., Coto-Montes, A., Oliván, M., 2008. Prediction of the fatty acid composition of beef by near infrared transmittance spectroscopy. Meat Sci 78: 248-255.

Sørensen, L. K., 2004. Prediction of fermentation parameters in grass and corn silage by near infrared spectroscopy. J Dairy Sci 87: 3826-3835.

Stuth, J., Jama, A., Tolleson, D., 2003. Direct and indirect means of predicting forage quality through near infrared reflectance spectroscopy. Field Crop Res 84: 45-56.

Schwarze, H., 1996. Report n. 96-10-1. Third European Symposium on Near Infrared (NIR) Spectroscopy: Continuous fat analysis in the meat industry, pp. 43-49.

Tøgersen, G., Isaksson, T., Nilsen, B. N., Bakker, E. A., Hildrum, K. I., 1999. On-line NIR analysis of fat, water and protein in industrial scale ground meat batches. Meat Sci 51: 97-102.

Tsenkova, R., Atanassova, S., Toyoda, K., Ozaki, Y., Itoh, K., Fearn, T., 1999. Near-infrared spectroscopy for dairy management: measurement of unhomogenized milk composition. J Dairy Sci 82: 2344-2351.

Tsenkova, R., Atanassova, S, Itoh, K., Ozaki, Y., Toyoda, K, 2000. Near infrared spectroscopy for biomonitoring. Cow milk composition measurements in a spectral region from 1,100 to 2,400 nanometers. J Anim Sci 78: 515-522.

Tsenkova, R., Atanassova, S, Ozaki, Y., Toyoda, K, Itoh, K., 2001. Near-infrared spectroscopy for biomonitoring: influence of somatic cell count on cow's milk composition analysis. Int Dairy J 11: 779-783.

Tsenkova, R., Atanassova, S, Kawano, S., Toyoda, K, 2001a. Somatic cell count determination in cow's milk by near-infrared spectroscopy: a new diagnostic tool. J Anim Sci 79: 2550-2557.

Tsenkova, R., Atanassova, S, Morita, H., Ikuta, K, Toyoda, K., Iordanova, I. K., Hakogi, E., 2006. Near infrared spectra of cows'milk for milk quality evaluation: disease diagnosis and pathogen identification. J Near Infrared Spec 14: 363-370.

Uddin, M., Okazaki, E., Turza, S., yumico, Y., tanaka, M., Fukuda, Y., 2005. Non-destructive Visible/NIR spectroscopy for differentiation of fresh and frozen-thawed fish. J Food Sci 70 (8): C506-C510.

Uddin, M., Okazaki, E., Fukushima, H., Turza, S., Yumiko, Y., Fukuda, Y., 2006. Nondestructive determination of water and protein in surimi by near-infared spectroscopy. Food Chem 96: 491-495.

Xiccato, G., Trocino, A., De Boever, J. L., Maertens, L., Carabaño, R., Pascual, J. J., Perez, J. M., Gidenne, T., Falcao-E-Cunha, L., 2003. Prediction of chemical composition, nutritive value and ingredient composition of European compound feeds for rabbits by near infrared reflectance spectroscopy (NIRS). Anim Feed Sci Tech 104: 153-168.

Xiccato, G., Trocino, A., Tulli, F., Ribaldi, E., 2004. Prediction of chemical composition and origin identification of European sea bass (*Dicentrarcus labrax* L.) by near infrared reflectance spectroscopy (NIRS). Food Chem 86: 275-281.

Woodcock, T., Downey, G., O'Donnel, C. P., 2008. Better quality food and beverages: the role of near infrared spectroscopy. J. Near Infrared Spec. 16: 1-29.

Websites: www.foragetesting.org