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Analytical Methods

Raman spectroscopy a promising technique for quality assessment of meat and fish: A review

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Abstract

Raman spectroscopy data have been compared to different traditional methodologies such as protein solubility, apparent viscosity, water holding capacity, instrumental texture methods, dimethylamine content, peroxide values, and fatty acid composition commonly used to determine quality in fish and meat muscle treated under different conditions of handling, processing and storage through the changes of proteins, water and lipids of muscle food. It has been shown that Raman spectroscopy data are related to the results obtained with these traditional quality methods and could be used to evaluate muscle food quality. In addition, Raman spectroscopy provides structural information about the changes of proteins, water and lipids of muscle food that occur during the deterioration. Raman spectroscopy can also be used for determining muscle food identification. Besides, this spectroscopy technique has several advantages compared to traditional methods since it is a direct and non-invasive technique which requires small portions of sample.

Keywords: Muscle food; Fish; Meat; Raman spectroscopy; Quality assessment; Authenticity

Contents

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1. Introduction

In the last decade, the interest in establishing the most adequate methods to assess the quality in food has increased. These interests are associated with new technological advances, the increasing interest in quality of consumer goods, the increasing of R&D laboratories in the industry and the establishment of more regulations and standards for food in general and for meat and fish in particular. All these conditions helped to make the industry more quality conscious and so, it also resulted in the steady growth of quality control (Grunert, Bredahl, & Bunsø, 2004; Müller & Steinhart, 2007; Röhr, Lüddecke, Drusch, Müller, & Alvensleben, 2005). Therefore, the establishing of an adequate measurement of food quality poses an important problem not only for the industry (producer and manufacturer), but also to the interests of distributors, retailers, consumers and authorities. Also the trend today is that consumers have become more exigent for information about the products they purchase. Consumers are not only interested in the composition or quality of the products, but also in their origin and how they have been handled, processed and stored and there are also questions of authenticity when the product is processed like filleted fish or pasties meat, where distinguishing the origin of the products or determining their species may be difficult. All these circumstances could be controlled by the traceability in the production chain, however, some of the analytical methods used are expensive and time consuming (Anklam & Battaglia, 2001; Grunert et al., 2004; Monin, 1998; Müller & Steinhart, 2007; Röhr et al., 2005).

The term quality is often used in different senses and meanings. Quality may be defined as the totality of features and characteristic of a product that bear on its ability to satisfy a given need. There are many other definitions of quality in the scientific literature. Botta (1995) listed some of them and presented their main qualities with respect to seafood: safety, nutritional quality, availability, convenience and integrity, and freshness quality. This quality concept could be applied to muscle food in general including meat and meat products. Other authors also include a wide range of other aspects of quality such as value for money, legal value, technological value, socio-ecological value and even psychological, political, and ecological qualities according to their particular expertise and interests (Bremner, 2000). In this respect, the concept of food quality should be based on the manufacturer's, the consumer's, the surveillance and legislative bodies' different demands (Anklam & Battaglia, 2001; Grunert et al., 2004; Röhr et al., 2005). Therefore, there are both an objective and a subjective interpretation of the quality. The objective interpretation is related to the material properties which can be described and measured objectively. For example, it has been established that when the concept of meat quality is analyzed it is composed of a number of factors like color, tenderness, taste and others (Monin, 1998; Ólafsdóttir et al., 1997). On the other hand, there are other subjective definitions depending on the consumer's opinion and appreciation. In the case of the processor, the concept of poor quality for him may mean too small or too large a meat, too firm or too soft, but most often, quality is synonymous with freshness or the degree of microbiological spoilage which the muscle food has undergone. It may also involve safety aspects such as being free from harmful bacteria, parasites or chemical compounds. Once the meat or fish has been processed, quality will relate to other characteristics: nutritional aspects, such as good balance in their amino acid composition, sanitary aspects, such as the numbers and types of bacteria, occurrence of parasites and presence of preservatives, processing aspects such as size, texture, etc. and finally those aspects involved in consumer acceptance, mainly organoleptic attributes such as color, odor, flavour, etc. (Anklam & Battaglia, 2001; Grunert et al., 2004; Röhr et al., 2005).

The loss of these qualities in muscle food like meat and fish is very fast since this muscle is highly perishable due to the influence of numerous *ante-* and *post-mortem* factors, and unless it is preserved in some way it easy becomes inedible. There have been many processes and technologies applied to prolong the shelf life of these products, but they all created new problems as several days may pass until the obvious signs of spoilage appear. The behaviour of the food products during the deterioration or lost of quality is very complex and it is a consequence of the variation in main components of muscle food such as proteins, water, lipids, etc. In this respect, for example during processes such as freezing and frozen storage, it is accepted that myofibrillar proteins suffer denaturation and/or aggregation due to various factors: partial dehydration by freezing of water, concentration of solutes in the tissue, formation of formaldehyde, or interactions of proteins with lipids or their oxidation products (Sikorski & Kolakowska, 1994). These processes depend on other factors too, such as storage time and temperature. The denaturation and/or aggregation of myofibrillar proteins during frozen storage are accompanied by loss of muscle food quality which is determined by methods that measure changes occurring in texture and functional properties of myofibrillar proteins. There exist several traditional methods to determine functionality of proteins such as solubility, apparent viscosity, extractability, water holding capacity (WHC), etc. Various methods have been developed to measure textural modifications such as texture profile analysis, Kramer shear-compression cell method, puncture test, Warner–Bratzler cell method, stress-relaxation test, tensile test, etc. (Bourne, 2002). Other methods have been also used to determine quality of fish muscle such as dimethylamine content (DMA) which is produced in certain fish species (gadoids) by the degradation of trimethylamine-*N*-oxide (TMAO) to dimethylamine (DMA) and formaldehyde and they have been related to denaturalization and aggregation of myofibrillar proteins and modifications of muscle texture during frozen storage.

Other related changes in proteins of meat and fish are those that can be observed in the water and which also lead to the loss of quality. In this respect, the WHC is regarded as an essential quality parameter which has been related to important textural attributes such as juiciness. The WHC is an important quality method in fish, and especially in meat, for several reasons. Meat is sold by weight, thus any water loss is economically undesirable. Also, the WHC influences the appearance of fresh meat whilst on sale and might affect the sensory properties of cooked meat, as high cooking loss has been reported to make meat perceivably less juicy. It has been also shown that there is a certain loss of WHC during frozen storage and therefore a loss of fish muscle quality. Moreover, it is well known that lipid content and fatty acid composition are also factors that influence the quality of meat and fish muscle. For example, pork meat is susceptible to oxidative deterioration, depending on the degree of unsaturation in the pork lipids. Products with high degree of unsaturation are more susceptible to lipid oxidation and hence off-flavour development with the subsequent rejection by the consumer. Hence the importance of controlling the lipids content to avoid problems during storage and processing of muscle foods such as detrimental changes in flavour, texture and odour, collectively known as organoleptic changes, and rancidity (Ólafsdóttir et al., 1997). Many techniques are available for assessing the extent of lipid oxidation in muscle food. These methods have traditionally been divided into those that measure primary changes such as peroxide values, which determine the formation of hydroperoxides, and those that measure secondary changes such as TBA test, which determine formation of malonaldehyde (Ruiz-Capillas & Moral, 2001). Therefore, the assessment of muscle food quality is based on the measurement of all these changes in the main muscle components (proteins, water, and lipids) with different methods.

In this sense, important efforts have been made in order to improve the measurement of these modifications in meat and fish by effect of processing and storage. It is essential to controls not only for the raw materials but also for the final products. Some control mechanisms and plans – e.g. hazard analysis critical control point plans (HACCP) – have been established, as mandated by the government. These require stipulation of specific objective parameters of quality. However, in order to establish standards and uniformity in measurement and assessment of quality, it is necessary to have objective methods for measuring specific quality attributes. In this way, there exist sensory, microbiological, physical, chemical and biochemical objective methods commonly used to evaluate the changes which are associated to the loss of quality of muscle food (meat and fish) (Monin, 1998; Ólafsdóttir et al., 1997). However, these methods have some disadvantages, they normally increase costs, they are time consuming, the information they provide is limited, toxic substances for the preparation of samples are used, application *in situ* is not possible, they are destructive, etc. The current tendency of investigations in this field is directed to look for methods that ideally fulfil a series of requirements like speed, non-destructive, portable and of application *on line*. In this way, spectroscopic methods have gained importance in the evaluation of food quality since they could solve some of the problems presented by the traditional methods used to evaluate muscle food quality, since spectroscopic techniques have the property of being direct, non-destructive or non-invasive (Li-Chan, 1996).

For that reason, an interest objective of this revision is to analyse if the Raman spectroscopy could be individually applied for evaluation of muscle food quality in substitution of traditional methods by evaluating the changes that originate in the components of muscle food (fish and meat) due to the effect of different processes of manufactures such as handling, processing and storage, which are responsible for the loss of quality of the product. Ultimately, the purpose is to evaluate the possibilities of Raman spectroscopy as a potential tool for the assessment of muscle food quality systems in order to arrange one effective quality assurance system.

2. Traditional methods for muscle food quality assessment

Traditionally, the evaluation of quality in muscle food has focused on the evaluation of the possible presence of microorganisms of public health significance and on offering an impression of the hygienic quality of the fish and meat during handling, processing and storage. Microbial methods are extensively used to assure that muscle food complies with mandatory standards or guidelines for a number of pathogenic or other microorganisms, e.g. in relation to international trade. However, microbiological data will generally not give any information about eating quality and freshness. Traditional microbial methods have several drawbacks. They are laborious, time consuming, costly and require a complex process of sample preparation. Some rapid microbiological methods have been developed during the last decade and some of these automated procedures could be used. However, in the majority of rapid methods large numbers of samples are needed. Therefore, it is recommended that such analyses be limited in number and extent.

Sensory analysis is also another important and common method to evaluate quality of fish and meat muscle since the consumer is the ultimate judge of quality of a product (Lee & O'Mahony, 2005). Sensory evaluation is defined as the scientific discipline used to evoke, measure, analyse and interpret reactions to characteristics of food as perceived through the senses of sight, smell, taste, touch and hearing. It is important to notify that sensory evaluation has a subjective connotation, however, although this evaluation could be performed scientifically under carefully controlled conditions so that the subjective effects may be reduced. In this sense, the sensory methods used to evaluate muscle food quality have evolved throughout the years. The use of structured scaling for muscle food quality assessment

and profiling is now common for a detailed description of one or more sensory attributes. In this respect, in the meat industry schemes are developing and are beginning to embrace all the quality aspects that are important to meat quality: appearance, colour, tenderness, juiciness and aroma or flavour (Wood, Holder, & Main, 1998). In the case of fish, the most commonly used method for fish quality sensory assessment is the EU-scheme (Council Regulations (EC) No. 2406/96, 1996). However, this only uses general parameters and it has been criticised for being too broad and not being able to distinguish more than three quality levels. Therefore, new improved quality grading systems that are both rapid and objective such as the quality index method (QIM) have been developed for various fish species (Herrero, Huidobro, & Careche, 2003; Martinsdottir, Luten, Schelvis-Smit, & Hyldig, 2003). However, the sensory analysis, like the microbial methods, is time consuming, expensive, and can only be performed by well-trained experts, and apart from that, it is not possible to use sensory panels as a routine quality assurance method to determine muscle food quality. Therefore, instrumental methods as an alternative to sensory and microbial analysis for determining meat and fish quality are necessary, in order to facilitate and improve quality determination for the processor and the inspector/consumer. The objective of methods like biochemical and physico-chemical methods for the evaluation of muscle food quality is related to the ability to set quantitative standards. The establishing of tolerance levels of chemical spoilage indicators would eliminate the need of basing decisions regarding product quality on personal opinions. Of course, in most cases sensory methods are useful for identifying products of very good or poor quality. Thus, biochemical and physico-chemical methods may best be used in resolving issues regarding products of marginal quality. In addition, biochemical/chemical indicators have been used to replace more time consuming microbiological methods. Such objective methods should, however, correlate with sensory quality evaluations and the chemical compound to be measured should increase or decrease with the level of microbial spoilage or autolysis. Nevertheless, most of this chemical and physico-biochemical methods are also time consuming and destructive, and it is not possible to apply *on line* during handling, processing and storage. This is the main reason why in the last few years great efforts have been made in order to improve the methodology for the measurement of the objective quality of fish and meat, in order to find some methods that solve all this disadvantages in the traditional methodology and may become sufficiently robust, rapid, and non-destructive or non-invasive for quality assessment of muscle food. In this respect, the use of spectroscopic techniques have several advantages compared to the traditional methods due to the fact that they are direct, non-destructive or non-invasive and their application *in situ* is possible (Li-Chan, 1996). That is the reason why they have increased their importance in the determination of food quality.

3. Raman spectroscopy to determine structure of muscle food components

There are different spectroscopic methods, such as circular dichroism, fluorescence spectroscopy, nuclear magnetic resonance, Raman and infrared spectroscopy to provide structural information of biological samples (McKelvy et al., 1996; Pelton & McLean, 2000; Schweitzer-Stenner, 2006). Among them, Raman spectroscopy is very valuable for determining structural information of solid samples and aqueous solutions. This spectroscopic technique is also often used in chemistry to identify substances or to study changes in chemical bonding. Raman spectroscopy requires a well-developed protocol for calibrating both the wavelength and the intensity. A calibration procedure ensures such long-term stability and provides reliable relative intensities which are valuable for quantitative analysis. Raman frequency standards such as sulphur have been used to calibrate the frequency. It has been shown that an internal patron could be employed for calibrating the intensity. Actually, it has been developed commercial calibration accessories which makes wavelength and intensity calibrations fast, simple, and accurate. Raman spectra exhibit well-resolved bands of fundamental vibrational transitions, thus providing a high content of molecular structure information of several compounds. Raman spectroscopy provides information mainly about secondary and tertiary structure of proteins (Herrero, in press; Li-Chan, Nakai, & Hirotsuka, 1994; Pelton & McLean, 2000; Tuma, 2005) and has proven to be a powerful technique for investigating the structure of water and lipids (Colaianni & Nielsen, 1995; Maeda & Kitano, 1995; Ozaki, Cho, Ikegaya, Muraishi, & Kawauchi, 1992). In this sense, Raman spectroscopy can be used in food analysis since it has several advantages such as being a non-destructive method and not requiring any pre-treatment of samples, further to this, small portions of sample are required, it provides information about different food compounds at the same time and not only does it offer qualitative and quantitative analysis of food components but also it allows their structural analysis.

3.1. Raman spectroscopy and structure of proteins

The spectral assignments of proteins Raman bands are usually based on model compounds such as amino acids or short peptides. Raman spectroscopy can provide information on the secondary and tertiary structure of proteins in solid samples (Li-Chan et al., 1994) like muscle food. The most useful Raman bands for determining the secondary structure of proteins are amide I (1645–1685 cm^{-1}) and amide III bands (1200–1350 cm^{-1}) (Fig. 1) since they are composed of contributions from α-helix, β-sheet, turns and random coil structures (Herrero, in press; Li-Chan, 1996; Li-Chan et al., 1994; Pelton & McLean, 2000; Schweitzer-Stenner, 2006; Tuma, 2005). Generally speaking, proteins with high α-helical content show an amide I

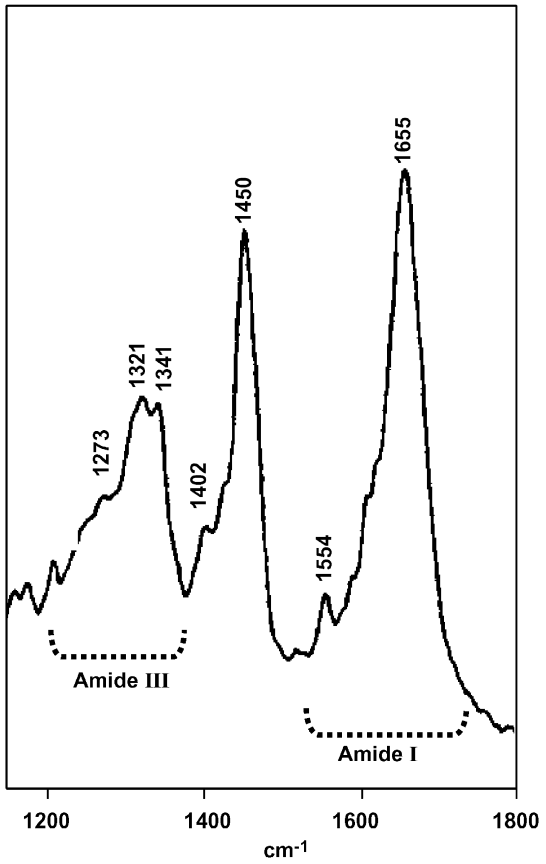

Fig. 1. Typical Raman spectra in the 1200–1800 cm^{-1} region of fish muscle (hake). Adapted to Careche et al. (1999).

band centred around 1650–1658 cm^{-1} while those with predominantly β-sheets structures show the band at 1665–1680 cm^{-1}, and a high proportion of random coil or disorder structure are attributable to proteins with an amide I band centred at 1660–1665 cm^{-1}. Estimates of protein secondary structure percentages can be made from the amide I profile. The intensity of the amide III band is difficult to interpret by the fact that vibrational spectroscopy of proteins produces a complex pattern of bands in the range 1225–1350 cm^{-1}. This is due to the fact that frequency ranges that are characteristics of β-sheet (1230–1245 cm^{-1}) and random coil (1240–1255 cm^{-1}) overlap to some extent. Another way of looking at the secondary structure of protein, and confirming the protein structure elucidated by amide I band, is by using the C—C stretching vibrations near 890–1060 cm^{-1}, which are characteristic of α-helices (890–945 cm^{-1}) and β-sheets (1020–1060 cm^{-1}) structure.

The information about proteins tertiary structure is provided by local environments such as tryptophan (544, 577, 760, 879, 1014, 1340, 1363, 1553 and 1582 cm^{-1}), tyrosil doublet (855 and 828 cm^{-1}), and aliphatic hydrophobic residues assigned at 1450 cm^{-1} (CH_2 and CH_3 bending vibrations) (Fig. 1) and at 2930 cm^{-1} (C—H stretching,) bands (Fig. 2a) in the Raman spectra provide information about tertiary structure of proteins (Herrero, in press; Li-Chan, 1996; Li-Chan et al., 1994).

3.2. Raman spectroscopy and structure of water

Raman spectroscopy can be used for determining the structure of water in solutions of biological macromolecules and complex biological systems, including muscle fibres. A water broad Raman band between 3100 and 3500 cm^{-1} which is attributable to OH stretching motions has been described (Fig. 2a) (Maeda & Kitano, 1995). Also, a Raman band in the low-frequency range (below 600 cm^{-1}) related to intermolecular water and protein librations and restricted translational motions involving the bending and stretching vibrations of the O(N)—H···O(N) units has been described. This spectral range below 600 cm^{-1} shows intermolecular fluctuation bands which are due to interactions of hydrogen bonded to water and protein molecules (Colaianni & Nielsen, 1995; Maeda & Kitano, 1995).

3.3. Raman spectroscopy and structure of lipids

Fatty acids in lipids and their degree of saturation can influence the intensity and location of bands in the Raman spectra of the lipids (Baeten, Meurens, Morales, & Aparicio, 1996; Li-Chan, 1996; Ozaki et al., 1992). Raman bands observed in fats and oils near 1750, 1660, 1470, 1443, 1306, and 1269 cm^{-1} are assigned undoubtedly to the C=O stretching modes, C=C stretching modes, CH_2 scissoring modes, CH_2 twisting modes, and CH in plane deformation modes of lipids. The frequencies of the C=C stretching bands of unsaturated fatty acids are very sensitive to the configuration around the C=C bond, *trans* and *cis* unsaturated fatty acids give the C=C stretching band in the 1670–1680 and 1650–1665 cm^{-1} regions, respectively. It has been demonstrated that the iodine value, which is a number widely used to indicate the unsaturation level of fat-containing food products, can be estimated directly by measuring the Raman spectra of the food. In particular, it has been noted that oils with high iodine values provide an intense C=C stretching band (1660 cm^{-1}). Some authors has also suggested that a comparison in the 1500–1700 cm^{-1} region gives a practical guide for the examination of the unsaturation level of fat-containing food products (Li-Chan, 1996; Ozaki et al., 1992). Besides, it has been shown that there are linear correlation between the iodine value and the intensity ratio of two bands at 1658 and 1443 cm^{-1} (I_{1658}/I_{1443}) due to the C=C stretching and CH_2 scissoring modes. Therefore, this ratio (I_{1658}/I_{1443}) may be used as a practical indicator for estimating the degree of unsaturation of a variety of foods containing lipids. It has also been described that changes in lipid are related to modifications in C—H stretching vibrations, which are normally seen within the 2800–300 cm^{-1} region (Li-Chan, 1996; Ozaki et al., 1992).

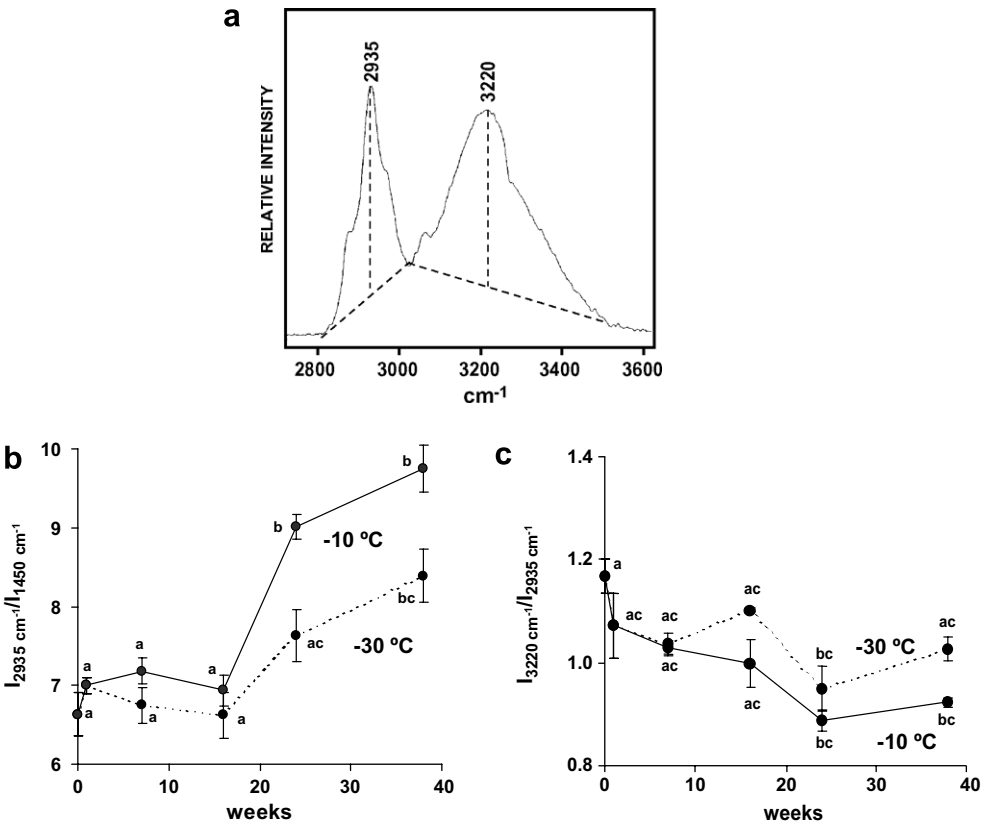

Fig. 2. Raman spectra in the C—H (2800–3000 cm^{-1}) and O—H region (3100–3500 cm^{-1}) of fish muscle (fresh hake muscle) (a). Changes in relative intensity of the C—H stretching band centred at 2935 cm^{-1} (b) and νO—H/νC—H intensity ratio (I_{3220}/I_{2935}) (c) from fish muscle (hake) during frozen storage at −10 and −30 °C (mean ± SEM). Different letters for the same temperature indicate significant differences ($P < 0.05$). Adapted to Careche et al. (1999) and Herrero et al. (2004).

4. Raman spectroscopy and traditional methods to asses muscle food quality

4.1. Raman spectroscopy and sensory analysis methods

A preliminary investigation of the application of Raman spectroscopy to the prediction of the results of sensory analysis has been carried out (Beattie, Bell, Farmer, Moss, & Patterson, 2004; Brøndum, Byrne, Bak, Bertelsen, & Engelsen, 2000; Careche, Herrero, & Carmona, 2002; Mikkelsen et al., 1999). It has been studied the relationships between Raman spectra data and results of warmed-over flavour development during chill storage of pre-cooked of porcine meat using partial least squares regression (PLRS) (Brøndum et al., 2000). Results showed that Raman spectra data in the 0–3000 cm^{-1} region correlated successfully with sensory evaluations of flavour, some of the sensory attributes of flavour such as astringent, metallic, monosodium, sour, can be predicted by Raman spectroscopy with some accuracy (Brøndum et al., 2000).

Other studies to correlate Raman spectra data with sensory attributes such as juiciness and texture using PLSR have been performed in cooked beef samples (Beattie et al., 2004). A strong positive correlation between the aliphatic residues region at 1460–1483 cm^{-1} (CH_2 and CH_3 bending vibrations) of the Raman spectra and juiciness determined by sensory analysis were found. These results could be indicated that the most important factor determining juiciness may be resulted from hydrophobic interactions of aliphatic residues. Besides, the results obtained with Raman spectroscopy data and sensory analysis from the regression coefficients of the PLRS models it was suggested that increased β-sheets (amide I band at 1675 cm^{-1}) and decreased α-helices (C—C stretching vibration at 920–945 cm^{-1}) were correlated with increased juiciness. Therefore, it could be suggested that these changes in secondary structure determined by Raman spectroscopy can be related to juiciness estimated by sensory analysis. Raman data showed also good correlation with others texture sensory attributes such as toughness. It was observed an increase intensity in the amide I (1669 cm^{-1}) and amide III (1235 cm^{-1}) bands, which it was correlated with and increase in β-sheets content in the tough meat. Moreover, the Raman spectra showed that the environment of trypthophan is more hydrophobic (881 cm^{-1}) and tyrosine more involved in hydrogen bonding (855 cm^{-1}) in the tough samples.

On the other hand, Raman spectra data have been employed for identify modifications in some sensory attributes such as general appearance of crustaceans such as shrimp (Careche et al., 2002; Mikkelsen et al., 1999). Some works reported the application of Raman spectroscopy to obtain more information about the formation of white

spots formed during frozen storage in shrimp shell which is a common quality problem which affect mainly the appearance of shrimp shell but not the taste or other eating qualities. Firstly, it has been investigated the dried white spot isolated from the shell of the shrimp and it was observed a Raman band at 1070 cm^{-1} due to the totally symmetric CO stretch which is attributed to ikaite (calcium carbonate hexahydrate) which is initially formed in the shell of the shrimp during frozen storage and subsequently, the ikaite crystals dehydrate upon thawing at ambient temperature and transform into a mixture of the calcium carbonate crystal forms, calcite and vaterite (Mikkelsen et al., 1999). The Raman spectroscopy has been used also to identified *in situ* these white spots on the shrimp shell (Careche et al., 2002). Spectral results showed that ikaite (1071, 719, 278, 221 and 204 cm^{-1} bands) is the predominant calcium carbonate crystal formed on the inner shell side during frozen storage of shrimp, while ikaite crystals are accompanied by significant amounts of calcite (1085 cm^{-1} band) on the outer side (Careche et al., 2002).

4.2. Raman spectroscopy and physico-chemical methods to determine muscle food quality

Raman spectroscopy has been compared to different traditional methodologies (protein solubility, apparent viscosity, water holding capacity, peroxide values, fatty acid composition, instrumental textural methods, etc.) to evaluate quality in fish and meat muscle treated under different conditions of handling, processing and storage through the changes of proteins, water and lipids of muscle food.

4.2.1. Raman spectroscopy and methods to measure modifications in proteins and water of muscle food

The protein changes occurring in isolated muscle proteins (myosin) extracted from cod muscle by effect of the addition of formaldehyde (FA) have been compared to subsequent frozen storage at −18 °C during 8 days by Raman spectroscopy and by the conventional measurements of protein solubility determined by extraction of muscle protein with 0.6 M NaCl at pH 7.0 (Careche & Li-Chan, 1997). The decrease in the protein solubility by more than 90% by effect of FA addition and frozen storage was accompanied by protein structural changes reflected in the Raman spectra (Careche & Li-Chan, 1997). The analysis of the amide I region (centred at 1650 cm^{-1}) and C—C stretching band (940 cm^{-1}) showed a decrease in the intensity of these Raman bands which indicated a decrease in α-helix structure from 95% to 60% by effect of FA addition and frozen storage at −18 °C. Besides, changes in vibrational modes assigned to aliphatic residues (CH stretching neat 2940 cm^{-1} and CH bending near 1450 cm^{-1}) were observed which suggested involvement of hydrophobic interactions after FA addition and frozen storage.

Other studies have related Raman spectroscopy with other methods traditionally used to evaluate quality control of muscle food such as apparent viscosity, Kramer shear-compression cell method and dimethylamine (DMA) content (Careche, Herrero, Rodriguez-Casado, Del Mazo, & Carmona, 1999; Herrero, Carmona, & Careche, 2004; Herrero, Carmona, García, Solas, & Careche, 2005). This studies have been carried out *in situ* in fish muscle (hake) freezing and frozen storage during several months at temperatures known to render very different practical storage lives (−10 and −30 °C). At high frozen storage temperature (−10 °C) a drastic decrease of ∼90% of apparent viscosity was observed, which was determined in a homogenate of thawed muscle obtained with 5% NaCl and pH 7. DMA content was determined with a colorimetric assay and a significant increase about of 80% in DMA content was observed in samples frozen stored at −10 °C. Similarly, the values of hardness, measure by Kramer shear-compression cell method, increased (∼20%) during storage at high frozen temperature (−10 °C). These results were related to Raman spectroscopy data involving secondary and tertiary protein structures. The changes in secondary structure were quantified mainly analysing amide I spectral profile (1600–1680 cm^{-1}) (Fig. 1) and 940 cm^{-1} band and the results showed an increase of β-sheet at the expense of α-helix structure at both frozen temperatures (−10 and −30 °C). Raman spectra also showed a decrease of peak intensity at 759 cm^{-1} at −10 and −30 °C which indicated changes in protein tertiary structure by exposure to buried tryptophan residues. Moreover, changes in tertiary structure were observed due to a decrease in the intensity of the 1450 cm^{-1} band (CH_2 and CH_3 bending vibrations) related to hydrophobic interactions of aliphatic residues at both frozen temperatures (−10 and −30 °C) (Careche et al., 1999; Herrero et al., 2004). It was also observed that changes in tertiary structure were showed by an intensity increase in the 2935 cm^{-1} band (*ν*C—H stretching band) (Fig. 2a and b) which is related to exposure of aliphatic hydrophobic groups to the solvent (Herrero et al., 2004). It is important to notify that all structural changes were more pronounced at high frozen temperature (−10 °C) but occurred at both storage temperatures (−10 and −30 °C), whereas changes in apparent viscosity, DMA content and texture only occurred in samples storage at −10 °C. Raman spectroscopy could be a technique more sensible to determine modifications during frozen storage of fish muscle compared to traditional methods used to determine quality of muscle food, since spectroscopy techniques are more sensible to study structural changes in the muscle. In addition, Raman spectroscopy has the advantage that the sample used can be intact and as no homogenisation or pre-treatment is required prior to analysis of the samples. Moreover, some Raman bands directly related to frozen storage time can be observed. In this way, there was a trend of the intensity ratio of O—H/C—H Raman bands (3100–3500 cm^{-1}/2800–3100 cm^{-1}) to increase during frozen storage at −10 and −30 °C (Fig. 2c) (Careche et al., 1999; Herrero et al., 2004). There also were Raman bands more prone to changes due to frozen storage temperature (2935 cm^{-1}, C—H stretching vibration) and other

bands (940 cm^{-1}, C—C stretching vibration) more insensitive to frozen temperature, but still presenting changes with frozen storage time. This shows the potential for Raman spectroscopy as a promising tool for establishing the time and temperature of frozen storage of fish muscle. In this respect, partial least squares regression (PLSR) models obtained with Raman spectra data correlated with frozen storage time and temperature support the suggestion above (Herrero, 2004; Herrero et al., 2004).

Raman spectroscopy and the traditional study of water holding capacity (WHC) have also been related in a study *in situ* of fish muscle (hake) during frozen storage at −10 and −30 °C (Herrero et al., 2005; Pedersen, Morel, Andersen, & Engelsen, 2003). The decrease in WHC (about 20%) determined by a centrifugation method, with storage time for high (−10 °C) and low (−30 °C) frozen temperature could be related to some Raman spectroscopy data, such as a decrease in the intensity of *v*sOH band (3100–3500 cm^{-1}) during frozen storage at −10 and −30 °C (Fig. 2a). This could be attributed to transfer of water to larger spatial domains during frozen storage. Raman spectra also showed a decrease of the strong Raman band at 160 cm^{-1} during frozen storage at both frozen temperatures (10 and −30 °C) which can be related to conformational transitions of muscle proteins, to changes in the structure of muscle water, and/or to alterations in protein–water interactions. In other studies, Raman spectroscopy results have been correlated with WHC data using chemometric analysis with the aim to use this spectroscopic technique to determine WHC of meat (Pedersen et al., 2003). This purpose is similar to other works which used PLSR models to correlated Raman spectroscopy and sensory analysis (Beattie et al., 2004; Brøndum et al., 2000). Pedersen et al. (2003) obtained different PLSR models based on three Raman spectral regions (500–3200 cm^{-1}, 3071–3128 cm^{-1} and 3128–3071 cm^{-1} + 951–876 cm^{-1}) and WHC values measured as drip loss (Table 1). It was indicated that Raman spectral regions of interest to WHC according to PLSR results are 3128–3071 and 951–876 cm^{-1}. Both spectral regions are related to protein conformation due to 3128–3071 cm^{-1} Raman region containing NH stretching of primary amides in proteins and changes in this spectral region, which could indicate protein denaturation and 951–876 cm^{-1} region (C—C stretching vibration) and provide information about secondary structure of proteins (α-helix structure). Taken into account these results, one could suggest the possibility of using Raman spectroscopy to determine WHC in muscle food with the advantage that this spectroscopic technique could make the measurement *in situ*.

Table 1
Regression results for PLSR models based on Raman spectra of meat samples for prediction of water holding capacity (WHC) (adapted to Pedersen et al., 2003)

Spectra (cm^{-1})	No. of PCs[a]	r[a]	RMSECV (%)[a]	WHC range (%)[a]
3200–500	3	0.98	0.27	0.7–8.0
3128–3071	3	0.95	0.38	0.7–8.0
3128–3071 + 951–876	3	0.98	0.23	0.7–8.0

[a] The number of PLS components (PCs), the correlation coefficients (r), the prediction errors (RMSECV) and the range of WHC. r and RMSECV indicate how well the model applies with the measurement data.

4.2.2. Raman spectroscopy and methods to determine modifications in lipids of muscle foods

Several studies have related Raman spectroscopy to traditional methods to determine modifications in lipids of muscle foods which can help to determine muscle food quality (Beattie, Bell, Borgaard, Fearon, & Moss, 2006; Olsen, Rukke, Flatten, & Isaksson, 2007; Sarkardei & Howell, 2007). In this way, Raman spectroscopy results and oxidation levels, determined by a traditional colorimetric method such as peroxide values, were related in lipids extracted from both mackerel and horse mackerel, by effect of freeze-drying and storage at −22 °C (Sarkardei & Howell, 2007). Peroxide values showed a general trend to increase in lipid oxidation products with frozen storage time in both fish species, although a decrease after 8 weeks was observed. In a parallel way, Raman spectra data showed an increase in the 3011 cm^{-1} band and 2960–2850 cm^{-1} region in the spectra of oil extracted of both fish species which could be attributed to alterations in lipid structure involving CH groups and hydrophobic interactions. Raman spectra results also indicated that lipid structural changes were more obvious in freeze-dried mackerel oil, compared to freeze-dried horse mackerel. These results were confirmed by the formation of primary oxidation products (peroxide value) were significantly higher in mackerel than in horse mackerel (Sarkardei & Howell, 2007). Moreover, fatty acid composition of adipose tissue from beef, lamb, pork and chicken has been investigated by Raman spectroscopy and gas chromatography analysis (Beattie et al., 2006). It is well known that gas chromatography analysis, although it is the most common method for determining the fatty acid profile of adipose tissue, is a time-consuming method and requires destructive sample extraction and methylation. Raman spectroscopy could be an alternative, since this spectroscopic technique is non-invasive and does not require pre-treatment of sample. Partial least squares (PLS) analysis was carried out between the Raman spectra from unextracted adipose tissue from beef, lamb, pork and chicken in the 800–1800 cm^{-1} region and gas chromatography results to confirm the predictive ability of Raman spectroscopy to determine fatty acid composition (Beattie et al., 2006). Results of PLS showed that the level of unsaturation (the number of *cis* unsaturated bonds per chain, i.e., molar unsaturation) was well predicted. It was indicated that due to the low range of relative abundances of *trans* unsaturated bonds in the adipose tissue (0.031, compared with 0.24 bonds per FA for *cis* unsaturated bonds) it was not possible to find a good prediction model for *trans* unsaturation. In addition, Raman spectroscopy successfully predicted

total unsaturation, defined as sum of *cis* and *trans* (Beattie et al., 2006). Raman spectra results also indicated that the prediction for individual fatty acid compounds by Raman spectroscopy using PLS models was very good as compared to gas chromatography analysis. Determinations of fatty acid composition by Raman spectroscopy have been carried out with new accessories such as a ball probe connected to the Raman spectrometer by an optic fibre cable which allows *on-line* measurements directly on the sample surface. Some authors have used these probes and have done partial least square regression (PLRS) between Raman spectroscopy data and gas chromatography analysis to determine the regression models in order to predict the amount of poly-unsaturated fatty acid (PUFA), mono-unsaturated fatty acid (MUFA) and saturated fatty acid (SFA) and the degree of unsaturation (IV) in melted fat and adipose tissue from pork (Olsen et al., 2007). The Raman spectral regions between 775 and 1800 cm^{-1}, and between 2600 and 3100 cm^{-1} was selected for the regressions models due to the fact that it contains bands related to lipids. Results of PLRS showed that Raman measurements of melted fat and adipose tissue provided good prediction compared to gas chromatography analysis (Olsen et al., 2007). Therefore, it has been shown that Raman spectroscopy is a promising tool to determine the fatty acid composition in pork adipose tissue with the advantage that the measurement is non-invasive and can be made *on-line*.

5. Raman spectroscopy and authenticity in muscle foods

Authenticity in muscle foods is an important quality factor for consumers, retailers and food regulatory bodies, as it guarantees that food has not been subjected to adulteration by any lower-grade material either by accident of for economic gain. Besides, the identification of these foods is crucial for the human diet in cases of religious, moral, cultural or dietary health considerations. Therefore, the possibilities of Raman spectroscopy have been investigated for identification of muscle food in substitution of traditional techniques such as detection of DNA or RNA, immunological, electrophoretic and chromatographic techniques which are destructive and time consuming. Ellis, Broadhurst, and Clarke (2005) studied the possibility of Raman spectroscopy to discriminate between important poultry species (turkey and chicken) and to distinguish between muscle groups (breast and leg) within these species with the aim of studying the problem of authenticity in muscle food products. Qualitative interpretation of Raman spectra (0–3000 cm^{-1}) at the species and muscle group levels was possible using cluster analysis which classifies a sample into a small number of exclusive groups based on the similarities among them. Cluster analysis showed that the major discrimination was between legs from the most expensive breast muscle. On closer inspection of data, it is also possible to see discrimination between the both closely related poultry species (chicken and turkey). The results highlight again that the differentiation between leg and breast muscle is more evident than discrimination at species level (Ellis et al., 2005).

6. Conclusion

Raman spectroscopy has been successfully correlated with traditional quality methods commonly used to determine quality of muscle food such as, protein solubility, apparent viscosity, water holding capacity, instrumental texture methods, DMA content, peroxide values, and fatty acid composition among others. In some cases, this correlation has been carried out with chemometric analysis (PLSR, cluster analysis, etc.) to establish if Raman spectroscopy data could predict the results obtained by traditional quality methods used in muscle food and elucidate if this spectroscopic technique could be used as a tool to asses muscle food quality.

In addition, Raman spectroscopy provides great information compared to the traditional methods of muscle food quality evaluation, since it allows identifying structural changes *in situ* about various muscle food components (proteins, lipids and water) which are implicated in the loss of quality of the meat and fish due to handling, processing and storage. Understanding the structural changes involved in the loss of muscle food quality could help to optimize the conditions of application of this process with the purpose of obtaining meat and fish products of greater quality.

Besides, Raman spectroscopy presents several advantages compared to traditional methods used to elucidate quality of muscle food as it is a direct and non-invasive technique which requires little or no pre-treatment of samples, and small portions of samples are necessary in the analysis.

Raman spectroscopy can be also used for identification of specific meat muscle groups and also to assist any future quantitative studies concerning the adulteration of one meat type by another. However, further work with other species of meat and fish and in other processes conditions is required to establish this spectroscopic technique as a tool for identification of muscle food.

The application of Raman spectroscopy for the evaluation of muscle food quality should be implemented in the next years due to the advantages that this technique provides compared to traditional quality methods used in muscle food and thanks to the general trends in the development of instrument advances such as the use of probes allowing *on-line* measurement, the designing of new portable Raman spectrometers and the software integrating data acquisition. All these possibilities and advantages offered by Raman spectroscopy open a wide range of possibilities to apply this spectroscopic technique as an adequate *on-line* tool in the industry of muscle food, meat and fish.

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References

Anklam, E., & Battaglia, R. (2001). Food analysis and consumer protection. *Trends in Food Science and Technology, 12*, 197–202.

Baeten, V., Meurens, M., Morales, M. T., & Aparicio, R. (1996). Detection of virgin olive oil by Fourier transform Raman spectroscopy. *Journal of Agricultural and Food Chemistry, 44*, 2225–2230.

Beattie, J. R., Bell, S. E. J., Borgaard, C., Fearon, A., & Moss, B. W. (2006). Prediction of adipose tissue composition using Raman spectroscopy: Average properties and individual fatty acids. *Lipids, 41*, 287–294.

Beattie, R. J., Bell, S. J., Farmer, L. J., Moss, B. W., & Patterson, D. (2004). Preliminary investigation of the application of Raman spectroscopy to the prediction of the sensory quality of beef silverside. *Meat Science, 66*, 903–913.

Botta, R. (1995). *Evaluation of seafood freshness quality*. New York: VCH Publishers.

Bourne, M. C. (2002). Principles of objective texture measurement. In M. C. Bourne (Ed.), *Food texture and viscosity: Concept and measurement* (pp. 107–188). San Diego, USA.

Bremner, H. A. (2000). Toward practical definitions of quality for food science. *Critical Reviews in Food Science and Nutrition, 40*, 83–90.

Brøndum, J., Byrne, D. V., Bak, L. S., Bertelsen, G., & Engelsen, S. B. (2000). Warmed-over flavour in porcine meat – a combined spectroscopic, sensory and chemometric study. *Meat Science, 54*, 83–95.

Careche, M., Herrero, A. M., & Carmona, P. (2002). Raman analysis of white spots appearing in the shell of argentine red shrimp (*Pleoticus muelleri*) during frozen storage. *Journal of Food Science, 67*, 2892–2895.

Careche, M., Herrero, A. M., Rodriguez-Casado, A., Del Mazo, M. L., & Carmona, P. (1999). Structural changes of hake (*Merluccius merluccius* L.) fillets: Effects of freezing and frozen storage. *Journal of Agricultural and Food Chemistry, 47*, 952–959.

Careche, M., & Li-Chan, E. C. Y. (1997). Structural changes in cod myosin after modification with formaldehyde or frozen storage. *Journal of Food Science, 62*, 717–723.

Colaianni, S. E. M., & Nielsen, O. F. (1995). Low-frequency Raman spectroscopy. *Journal of Molecular Structure, 347*, 267–284.

Council Regulations (EC) No. 2406/96 of 26 November (1996). Laying Down Common Marketing Standards for certain fishery products. In *Official Journal of European Communities, No. L-334/2* (23.12. 1996).

Ellis, D. I., Broadhurst, D., & Clarke, S. J. (2005). Rapid identification of closely related muscle foods by vibrational spectroscopy and machine learning. *Analyst, 130*, 1648–1654.

Grunert, K. G., Bredahl, L., & Bunsø, K. (2004). Consumer perception of meat quality and implications for product development in the meat sector – a review. *Meat Science, 66*, 259–272.

Herrero, A. M. (2004). Cambios estructurales, Físico-Químicos y sensoriales en el músculo de pescado congelado. Evaluación de la calidad. Doctoral Thesis. Facultad de Ciencias Químicas, Universidad Complutense de Madrid, Spain.

Herrero, A. M. (in press). Raman spectroscopy for monitoring protein structure in muscle food systems. *Critical Review in Food Science and Nutrition*.

Herrero, A., Carmona, P., & Careche, M. (2004). Raman spectroscopic study of structural changes in hake (*Merluccius merluccius* L.) muscle proteins during frozen storage. *Journal of Agricultural and Food Chemistry, 52*, 2147–2153.

Herrero, A. M., Carmona, P., García, M. L., Solas, M. T., & Careche, M. (2005). Ultrastructural changes and structure and mobility of myowater in frozen-stored hake (*Merluccius merluccius* L.) muscle: Relationship with functionality and texture. *Journal of Agricultural and Food Chemistry, 53*, 2558–2566.

Herrero, A. M., Huidobro, A., & Careche, M. (2003). Development of a quality index method for frozen hake (*M. capensis* and *M. paradoxus*). *Journal of Food Science, 68*, 1086–1092.

Lee, H. S., & O'Mahony, M. (2005). Sensory evaluation and marketing: Measurement of a consumer concept. *Food Quality and Preference, 16*, 227–235.

Li-Chan, E. C. Y. (1996). The applications of Raman spectroscopy in food science. *Trends in Food Science and Technology, 7*, 361–370.

Li-Chan, E. C. Y., Nakai, S., & Hirotsuka, M. (1994). Raman spectroscopy as a probe of protein structure in food system. In R. Y. Yada, R. L. Jackman, & J. L. Smith (Eds.), *Protein structure. Function relationships in foods* (pp. 163–197). London: Blackie Academic and Professional, Chapman and Hall.

Maeda, Y., & Kitano, H. (1995). The structure of water in polymer systems as revealed by Raman spectroscopy. *Spectrochimica Acta, 51*, 2433–2446.

Martinsdottir, E., Luten, J. B., Schelvis-Smit, A. A. M., & Hyldig, G. (2003). Developments of QIM: Past and future. In J. B. Luten, J. Oehlenschlager, & G. Olafsdottir (Eds.), *Quality of fish from catch to consumer: Labelling, monitoring and traceability* (pp. 265–272). New York: Wageningen Academic Publishers.

McKelvy, M. L., Britt, T. R., Davis, B. L., Gillie, J. K., Lentz, L. A., Leugers, A., et al. (1996). Infrared spectroscopy. *Analytical Chemistry, 68*, 93–160.

Mikkelsen, A., Andersen, A. B., Engelsen, S. B., Hansen, H. C. B., Larsen, O., & Skibsted, L. H. (1999). Presence and dehydration of ikaite, calcium carbonate hexahydrate, in frozen shrimp shell. *Journal of Agricultural and Food Chemistry, 47*, 911–917.

Monin, G. (1998). Recent methods for predicting quality of whole meat. *Meat Science, 49*, S231–S243.

Müller, A., & Steinhart, H. (2007). Recent developments in instrumental analysis for food quality. *Food Chemistry, 102*, 436–444.

Ólafsdóttir, G., Martinsdóttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B., Undeland, I., et al. (1997). Methods to evaluate fish freshness in research and industry. *Trends in Food Science and Technology, 8*, 258–265.

Olsen, E. F., Rukke, E. O., Flatten, A., & Isaksson, T. (2007). Quantitative determination of saturated-, monounsaturated- and polyunsaturated fatty acids in pork adipose tissue with non-destructive Raman spectroscopy. *Meat Science, 76*, 628–634.

Ozaki, Y., Cho, R., Ikegaya, S., Muraishi, S., & Kawauchi, K. (1992). Potential of near-infrared Fourier transform Raman spectroscopy in food analysis. *Applied Spectroscopy, 46*, 1503–1507.

Pedersen, D. K., Morel, S., Andersen, H. J., & Engelsen, S. B. (2003). Early prediction of water-holding capacity in meat by multivariate vibrational spectroscopy. *Meat Science, 65*, 581–592.

Pelton, J. T., & McLean, L. R. (2000). Spectroscopy methods for analysis of protein secondary structure. *Analytical Biochemistry, 277*, 167–176.

Röhr, A., Lüddecke, K., Drusch, S., Müller, M. J., & Alvensleben, R. V. (2005). Food quality and safety – consumer perception and public health concern. *Food Control, 16*, 649–655.

Ruiz-Capillas, C., & Moral, A. (2001). Correlation between biochemical and sensory quality indices in hake stored in ice. *Food Research International, 34*, 441–447.

Sarkardei, S., & Howell, N. K. (2007). The effects of freeze-drying and storage on the FT-Raman spectra of Atlantic mackerel (*Scomber scombrus*) and horse mackerel (*Trachurus trachurus*). *Food Chemistry, 103*, 62–70.

Schweitzer-Stenner, R. (2006). Advances in vibrational spectroscopy as a sensitive probe of peptide and protein structure: A critical review. *Vibrational Spectroscopy, 42*, 98–117.

Sikorski, Z. E., & Kolakowska, A. (1994). Changes in proteins in frozen stored fish. In Z. E. Sikorski, B. Sun Pan, & F. Shahidi (Eds.), *Seafood proteins* (pp. 99–231). New York: Chapman and Hall.

Tuma, R. (2005). Raman spectroscopy of proteins: From peptides to large assemblies. *Journal of Raman Spectroscopy, 36*, 307–319.

Wood, J. D., Holder, J. S., & Main, D. C. J. (1998). Quality assurance schemes. *Meat Science, 49*, S191–S203.