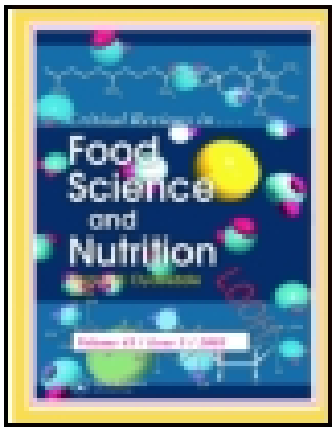

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An Updated Review of Meat authenticity methods and applications

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An Updated Review of Meat authenticity methods and applications

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Abstract

Adulteration of foods is a serious economic problem concerning most foodstuffs, and in particular meat products. Since high-priced meat demand premium prices, producers of meat-based products might be tempted to blend these products with lower cost meat. Moreover, the labelled meat contents may not be met. Both types of adulteration are difficult to detect and lead to deterioration of product quality. For the consumer, it is of outmost important to guarantee both authenticity and compliance with product labelling. The purpose of this article is to review the state of the art of meat authenticity with analytical and immunochemical methods with the focus on the issue of geographic origin and sensory characteristics. This review is also intended to provide an overview of the various currently applied statistical analyses (multivariate analysis such as Principal Component Analysis, Discriminant Analysis, Cluster Analysis etc) and their effectiveness for meat authenticity.

Keywords: authenticity, meat, quality control, multivariate analysis

ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
ANN	Artificial neural network
ANOVA	Analysis of variance
AP-PCR	Arbitrarily primed Polymerase chain reaction
APLSR	ANOVA partial least squares regression
BSE	Bovine spongiform encephalopathy
CAR/PDMS	Carboxen polydimethylsiloxane
CDA	Canonical discriminant analysis
CE	Capillary electrophoresis
CF	Chicken forward
CNS	Central nervous system
CO	Country of origin
CR	Chicken reverse
CSC	Certificate of specific character
CSCB	Same type of bacon
DHS	Dynamic headspace
DVP/CAR/PDMS	Divinylbenzene/ carboxen/ polydimethylsiloxane
EC	Electrochemical detection
ELISA	Enzyme-linked immunosorbent assay
FGC	Fast-growing chicken
FR	Feeding regime
FT-IR	Fourier transform - InfraRed
GA-MLR	Genetic algorithms - multiple linear regression
GCC	Gross chemical composition
GFAP	Glial fibrillary acidic protein
GMO	Genetically modified organisms
HATR	Horizontal attenuated total reflectance
HPLC-EC	High-performance liquid chromatography method with electrochemical detection
HR-MAS	High-resolution magic angle spinning
ICP-MS	Inductively coupled plasma mass spectroscopy
ICP-HRMS	Inductively coupled plasma high resolution mass spectrometry
IEF	Isoelectric focusing
IRMS	Isotope ratio mass spectrometry
LDA	Linear discriminant analysis
LRI	Linear retention indices
MA	Modified atmosphere
MBM	Meat and bone meal

MBP	Myelin basic protein
MGB	Minor groove binding
MLP	Multilayer perceptron
MOFSET	Metal oxide semi-conductor field-effect transistors
MPLS	Multi-way partial least-squares regression
MS	Mass spectroscopy
MVA	Multivariate analysis
NADH	Nicotinamide adenine dinucleotide
NF	Neurofilament
NMR	Nuclear magnetic resonance
NIRR	Near infrared reflectance reflection
NIRS	Near infrared reflectance spectrospopy
NIRT	Near infrared reflectance transmission
NPSD	Nitrogen purge and steam distillation
NSE	Neuron-specific enolase
PCA	Principal component analysis
PCR	Polymerase chain reaction
PDO	Protected designation of origin
PGI	Protected geographical indication
PLOT	Porous layer open tubular
PLS	Partial least-squares regression
QC-PCR	Quantitative competitive polymerase chain reaction
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RT-PCR	Real time – polymerase chain reaction
SDE	Simultaneous distillation extraction
SE	Solvent extraction
SECV	Standard error of cross-validation
SEP	Standard error of validation
SGC	Slow-growing chicken
SIMCA	Soft independent odelling of class analogy
SINE	Short interspersed nuclear element
SNDV	Standard normal variance and detrend
SPME	Solid phase micro extraction
SYBR Green I	N',N'-dimethyl-N-[4-[(E)-(3-methyl-1,3-benzothiazol-2-ylidene)methyl]-1-phenylquinolin-1-ium-2-yl]-N-propylpropane-1,3-diamine
TBARS	2-thiobarbituric acid reactive substances
TBA	2-thiobarbituric acid
TF	Turkey forward
TMA-N	Trimethylamine nitrogen
TR	Turkey reverse
TVB-N	Total volatile basic nitrogen
VIS	Visible infrared spectrospopy

VP	Vacuum package
WBSF	Warner-Braztler shear force
WHC	Water holding capacity
WOF	Warmed-over flavour

INTRODUCTION

Nowadays, people are very much interested in the quality of food they consume. Consumers are aware of the importance of food origin, labelling and ingredients and opt for high food quality. On the other hand, food authenticity is a very important issue for consumers because the latter, often, are not sure of what they eat. Although there are a various national and international laws for labelling and trade of food including meat, unfortunately, these laws are not effective towards preventing adulteration.

New techniques have been developed for the determination of foodstuff authenticity. Food authenticity is also of concern to food processors that do not wish to be exposed to unfair competition from unscrupulous processors who would gain an economic advantage from the misrepresentation of the food they are selling (Reid et al., 2006).

Most of meat authenticity tests are based on lipid or protein analysis of meat and meat products. All of them have advantages and disadvantages. The analytical method that will be used depends on several variables such as type, quantity, part, condition and processing of meat (Rodriguez-Ramirez et al., 2011). In recent years, because of nutritional scandals such as bovine

spongiform encephalopathy (BSE), the most used methods are those which based on DNA analysis. The polymerase chain reaction (PCR) method is preferred by the researchers for authenticity issuses such as the authenticity of meat and meat products (Reid et al., 2006). A review of PCR-based methods with several applications for animal origin foodstuffs was published by Mafra and colleagues (2008). Vallejo-Cordoba and his coworkers (2005) reported that the applications of PCR-based capillary electrophoresis (CE) were enhanced during the last decade. It should be noted that CE was applied both in food analysis and authenticity, but its use in meat authenticity was temperate. Last years, in several studies PCR-based CE is used for the determination of meat authenticity (Prado et al., 2007; La Neve et al., 2008; Fajardo et al., 2009; Pun et al., 2009; Wang et al., 2010). The integrity of DNA-based methods may be severely affected by meat handling such as storage, cooking and processing. It was reported that meat cooked at high temperatures resulted in a low overall DNA yield (Aslan et al., 2009).

The overview of analytical methods is provided in the first part of this review (Luykx and van Ruth, 2008). Many recent studies related to the analytical methods are also presented. Moreover, relevant applications of chemometric techniques have been reviewed. Chemometric analysis of the data obtained with analytical equipment which have the ability to determine more than one ingredient in a sample can be applied to determine the food origin. Chemometrics provides the possibility to identify characteristic patterns and is effectively supportive when the number of ingredients necessary to discriminate samples from different geographical origins increases.

The importance of multivariate analysis (MAV) in the field of authenticity is also stressed in this review. All sensory attributes undergo a statistical treatment. MAV such as discriminant

analysis could give important results with regard to the sensory features of different kind of meat. The geographical origin of meat could be determined by applying several multivariate techniques to differentiate among various kinds of meat from different geographical regions (Arvanitoyannis and van Houwelingen-Koukaliaroglou, 2003).

A Swiss assessment indicated that the food origin is of great importance for the purchase decision of 82% of customers, with the origin of meat being a very important reason for 71% of them (Franke et al., 2005). The reason for this increasing interest by consumers focused on health, media attention, specific gastronomic or sensory qualities related to regional commodities, and environmental friendly production methods and reduced confidence in the quality and safety of foodstuffs produced outside their local region (Gilb and Battershill, 1998; Ilbery and Kneafsey, 1998; Luykx and van Ruth, 2008).

This review aims at presenting a synopsis of the possible and potential methods used for the detection of meat authenticity. The occurrence of meat adulteration is difficult to determine. However, meat authenticity could be detected with different analytical methods such as chromatography, PCR, mass spectrometry, spectroscopy, electronic spin resonance, electrophoresis, immunological assays, enzymatic and near-infrared spectroscopic tests and DNA-based tests.

INSTRUMENTAL DETERMINATION OF SELECTED COMPOUNDS AS MEAT AUTHENTICATION MARKERS

Among DNA-based methods, PCR technology provides the food analyst with a simple, specific, and highly sensitive tool for the authentication of species in food products (Fei et al., 1996, Ballin et al., 2009; Montowska and Pospiech, 2011). PCR has been implicated in a wide variety of foods and food products of great concern not only to food authorities, since the incorrect labelling of foodstuffs could represent a commercial fraud (Mafra et al., 2008). It is a powerful tool able to provide answers to the increasing demands of consumers about the presence of allergens, genetically modified organisms (GMO), bovine meat and adulterations, especially the real-time PCR (RT-PCR) technique.

On the other hand, the PCR - restriction fragment length polymorphism (RFLP) technique was frequently employed for the generation of DNA fingerprints of an extensive variety of animal species (22) by amplifying specific regions within the cytochrome b gene and digesting the amplified product using Hae III and Hinf I (Meyer et al., 1994; Partis et al., 2000). Application of these two restriction enzymes led to satisfactory discrimination except for kangaroo and buffalo; regardless whether the tissue was cooked or not. In the tested mixtures, pig preferentially showed a higher rate of amplification and dominated over all species tested, even at levels of 1%. Besides, quantitative competitive PCR (QC-PCR) has been implicated in the detection and quantification of porcine DNA-through a new porcine specific PCR system based on the growth hormone gene of *Sus scrofa* allowing differentiation between contamination and admixture (Wolf and Lüthy, 2001).

Among mitochondrial genes, the cytochrome b (Matsunaga et al., 1998, 1999; Verkaar et al., 2002; Wolf et al., 1999), the 12S and 16S ribosomal RNA subunits (Bottero et al., 2003; Fajardo et al., 2006; Rodríguez et al., 2003a, 2003b, 2003c, 2004), and the displacement loop region (D-

loop) (Fei et al., 1996; Gao et al., 2004; Montiel-Sosa et al., 2000) are the most widely used markers in the development of DNA methods for species identification. Fajardo et al. (2007a, 2007b). Moreover, the 12S RNA mitochondrial gene has been used for qualitative and quantitative identification of duck species in meat mixtures at low levels (Martin et al., 2007a).

A PCR assay for the identification of beef based on the amplification of bovine 1.709 satellite DNA has been reported by Guoli et al. (1999). Its flexibility to amplify not only raw beef DNA, but also cooked or autoclaved meat DNA was strongly linked to the selected amplified sequence of a 218 bp DNA fragment lying in the 1.709 satellite DNA of bovine. Moreover, a pair of synthetic oligonucleotides flanked in this sequence was employed as primers, while genomic DNA extracted from beef samples served as templates. The resulting amplification products, after a 33 cycle procedure in a Taq DNA polymerase mixture, were rapidly electrophorized in 3% agarose gel and visualized because of the UV illumination of the ethidium bromide staining. Thus, a minimum amount of 33.6 fg of DNA from raw beef samples and 0.32 pg of DNA from cooked or autoclaved beef samples were precisely detected, respectively, and a 100% correct identification of hundred and three (103) tested beef-samples.

The PCR amplification of species-specific amplicons in conjunction with rapid visualisation with vistra green -binding to the backbone of the dsDNA- was reported by Hird et al. (2003) in an attempt to detect turkey and chicken in processed meat products. The DNA amplification in the PCR was accomplished with the species specific primers namely; chicken forward (CF), chicken reverse (CR), turkey forward (TF) and turkey reverse (TR), permitting their (amplicons) detection in less than 5 min after the end of the PCR. Both chicken and turkey assays provided a clear detection of the appropriate DNA when used in the assay neat and at a dilution of 1:10

DNA:water; nevertheless neither assay was able to statistically detect DNA at dilutions of 1:100 or 1:1000, both of which were below 250 for emission at 585 nm.

Ebbehoj and Thomsen (1991a) proposed a quantification assay for pork in heat-treated meat products based on the isolation of DNA from meat samples in conjunction with agarose gel electrophoresis in order to determine the average size of DNA fragments. After its immobilization on nylon membranes the DNA has been hybridized with a 32P-labelled probe made from genomic porcine DNA, whose signal intensity counted with laser densitometry of the autoradiographs. The resultant functional relationships between the aforementioned parameters and the use of appropriate DNA standards resulted in satisfactory outcomes, reaching the 0.1% and approximately 0.5% pork in beef in pure and heat-treated samples, respectively.

The specificity of genomic DNA probes for species differentiation by slot blot hybridization has been used for the study of species differentiation between monkey and human and between cattle, goat and sheep (Ebbehoj and Thomsen, 1991b). The cross hybridization between probe and DNA sequences from closely related species was reduced by addition of unlabelled DNA from the cross hybridizing species, promising a quantitative species differentiation. The detection limits for differentiation between cattle and sheep or goat and the closely related species sheep and goat were found to be less than 0.01% and about 10%, respectively.

Koh et al. (1998) aimed at establishing a library of reference fingerprint patterns for the various meat species employing the random amplified polymorphic DNA (RAPD) method to generate fingerprint patterns for 10 meat species, namely: wild boar, pig, horse, buffalo, beef, venison, dog, cat, rabbit and kangaroo. At the same time, the most suitable primer sets for the characterization of specific meat species were identified. High molecular weight DNA was

successfully extracted from all samples with a satisfactory A260: A280 ratio range from 1.6 to 2.0. Thus, a total of 29 10-nucleotide primers, with GC contents ranging from 50-80% were evaluated for their specificity and efficiency. These included 50% GC (6 different sequences), 60% (8 sequences), 70% (5 sequences) and 80% (10 sequences). The generated fingerprint patterns were found in some cases to be species-specific, i.e. one species could be differentiated from another.

Matsunaga et al. (1999) employed the PCR technique for the identification of six meats (cattle, pig, chicken, sheep, goat and horse) as raw materials for products. The appropriate ratios resulting by mixing seven primers revealed species-specific DNA fragments by only one multiplex PCR. A forward primer was based on a conserved DNA sequence in the mitochondrial cytochrome b gene, whereas additionally reverse primers on species-specific DNA sequences for each species. The PCR primers offered different length fragments from the six meats revealing species-specific DNA fragments of 157, 227, 274, 331, 398 and 439 bp from goat, chicken, cattle, sheep, pork and horse meats, respectively. Meat species prepared at high temperature were detected with multiplex PCRs, whereas the amplification of all heated- at all temperatures- meat DNAs was possible except for horse DNA fragment (at 120ºC) because of its great length (439 bp). The detection limits of the DNA samples were 0.25 ng for all meats.

Similarly, the discrimination between red and sika deer was shown to be successful after the digestion of the PCR products with a restriction enzyme (EcoRI, BamHI, ScaI) and the analysis by 4% agarose gel electrophoresis. The first species fragment was successfully digested with EcoRI to 67/127bp fragments (but not with the other two), while the second-one was digested to

481/46 bp and 49/145 bp fragments with the two other enzymes, thus permitting the differentiation of the two kinds of deer (Matsunaga et al., 1998).

Pascoal et al. (2005) reported a novel set of bovine-specific primers namely; CYTbos1 (forward) and CYTbos2 (reverse). These primers allow the specific amplification of a 115 base pair fragment of the bovine cytochrome b gene (cytb) between nt 844 (mitochondrial site 15,590) and nt 958 (mitochondrial site 15,704), with the significant absence of cross-reaction with DNA from another twelve frequent commercial meat species. The confirmation validity of the PCR technique was extensively increased through the specific cleavage by the endonucleases ScaI and TspE1, while the sensitivity reached 0.025%.

The incidence of incorrect labelling in food products containing one or more meat species has been examined by Pascoal et al. (2004) with PCR coupled to RFLP. The completion of this target employed the universal primers CYT b1/CYT b2, qualified for the amplification of a variable region of the mit cytb of vertebrates, and the endonucleases PalI, MboI, HinfI and AluI. A large number of food products, raw and/or cured, were investigated resulting in 30% of incorrect qualitative label displaying, only 11.1% of the raw/cured products, and 34.2% of the products subjected to some type of heat-processing were not correctly labelled. Significant was the undeclared presence of turkey since it was detected in 14% of the food products.

The PCR-RFLP technique of the mitochondrial 12S rRNA gene was applied to identify the origin of meats such as cattle, buffalo, sheep (mutton) and goat (chevon) (Girish et al., 2005). PCR amplification yielded a 456-bp fragment in each of these species, and after their digestion with AluI, HhaI, ApoI and BspTI restriction enzymes a pattern able to identify and differentiate each of the above species has been resulted. The restriction enzymes HhaI, ApoI and BspTI

corresponded to unique sites in buffalo, sheep and goat sequences, respectively; while AluI gave 97 and 359 bp fragments in cattle and 246 and 210 bp fragments in sheep and goat. Therefore, the results were unambiguously interpreted; the method could be applied with equal efficiency to both fresh and processed meats, however, with little success to meat mixtures.

Klossa-Kilia et al. (2002) developed a similar assay in order to prevent the famous fish roe of Messolongi (Greece), processed seafood originating from the whole ovaries of the fish *Mugil cephalus* captured in the homonymous lagoon. The PCR-RFLP method was based on the amplification of the mitochondrial 16s rRNA gene segment, digestion of PCR products with the restriction enzymes BstNI, TaqI and HinfI, and finally the electrophoresis on agarose gel. The *M.cephalus* could be identified with only one restriction enzyme (BstNI or HinfI), with non-existent difference in RFLP pattern resultant from DNA extracted either fresh fish or fish roe. Thus, the resulted species specific restriction patterns allowed the unequivocal discrimination of the fish roe of Messolongi from the fish roe originating from the other Mugilidae species coexisting in the same area.

The PCR-RFLP technique has also been employed by Fajardo et al. (2006) for the identification of meats from red deer (*Cervus elaphus*), fallow deer (*Dama dama*), roe deer (*Capreolus capreolus*), cattle (*Bos taurus*), sheep (*Ovis aries*), and goat (*Capra hircus*). It was based on the amplification of a conserved fragment from the mitochondrial 12S rRNA gene of ~712 base pairs. Therefore, the selection of MseI endonuclease, in parallel with the combination of MboII, BslI, and ApoI enzymes, proved potential tools for the suitable identification of above mentioned meats species. The MboII endonuclease cleaved the 12S rRNA gene products of both red deer and fallow deer into two DNA fragments of 384/328 and 489/223 bp, respectively. Two

identical MboII restriction sites for roe deer, goat, cattle, and sheep PCR products yielded three conserved DNA fragments of 384, 223, and 105 bp. Similarly, the presence of four restriction sites for BslI endonuclease in both red deer and fallow deer 12S rRNA gene sequences caused five DNA fragments. The three restriction sites for this enzyme occurring in roe deer, cattle, and sheep PCR products yielded four DNA fragments of, while goat amplicons gave five DNA fragments. Finally, digestions performed with ApoI endonuclease resulted in three DNA fragments of 553, 96, and 63 bp in red deer and fallow deer, whereas a single restriction site present in roe deer sequence generated two DNA fragments of 649 and 63 bp. Two restriction sites for this enzyme identified in sheep samples yielded three DNA fragments of 412, 204, and 96 bp, and cattle and goat samples, with one ApoI restriction site, caused two DNA fragments of 616 and 96 bp. The technique has satisfactorily differentiated the game meats from those of bovine, caprine, and ovine species.

Furthermore, Murugaiah et al. (2009) applied the above mentioned assay in the mitochondrial gene towards beef (*Bos taurus*), pork (*Sus scrofa*), buffalo (*Bubalus bubali*), quail (*Coturnix coturnix*), chicken (*Gallus gallus*), goat (*Capra hircus*), rabbit (*Oryctolagus cuniculus*) species identification and Halal authentication. The detection of smaller bands was made possible using CE due to its better resolution than agarose gel. Thus, PCR products of 359-bp were identified from the cyt b gene of these six meats using the AluI, BsaJI, RsaI, MseI, and BstUI enzymes as potential restriction endonucleases. Any genetic difference within this gene was easily detected with PCR-RFLP offering a reliable typing scheme for species corresponding to their genetic differences. The intensity of bands of chicken or beef opposed to pork were

considerably lower when presented at 1%, 3% or 5% in a meat mixture, but still clearly detectable on the agarose gel.

The wide spreading of chicken of various quality marks in European meat market and the secrecy that the breeding companies demonstrate concerning the breeding process spontaneously pose critical questions to the consumer. More often the ambiguity is related to the certified products with specifications of slow-growing chicken strains (SGC-at least 81d) versus the fast-growing ones (FGC-about 42d). Against this question Fumiere et al. (2003) employed the amplified fragment length polymorphism (AFLP) technique to search for molecular markers able to discriminate the mentioned strains and to authenticate certified products. The use of two pairs of restriction enzymes (EcoRI/MseI and EcoRI/TaqI) and 121 selective primer combinations gave significant data resulting from a wide range of individual DNA samples of both breeding categories. Thus, a band of 333 bp has been generated with the EcoRI + AAC/MseI + CAA primer combination is amplified in all SGC-individuals (strain ISA 657), while it is absent in the FGC (strain Cobb 500). In contrast, the EcoRI + AAC/TaqI + ATG primer combination generated a band of 372 bp present in all individuals of the FGC (strain Cobb 500) and in almost all of FGC-specimens from the market and none of the ISA 657 strain specimens. Analysis of commercialized products of various origins led to their successful identification.

A species-specific PCR assay was employed for the detection of low levels of pork, horse and donkey meat in cooked sausages by Kesmen et al. (2007). Two series of binary meat mixtures were prepared from raw minced beef or lamb by adding horse, donkey and porcine meats at five different levels (0.0%, 0.1%, 0.5%, 1.0% and 5.0%). The detection limits of specific PCR assay were determined with PCR amplification of mitochondrial DNA extracted

from each species at levels from 0.01 to 100 ng of DNA in water. The sensitivity of the method was determined as 0.01 ng DNA per species. The implementation of the assay to DNA extracts from the above-mentioned samples gave the possibility each species to be detected when spiked in any other species at the 0.1% level.

Similarly, Ilhak and Arslan (2007) determined the origin of horse, dog, cat, bovine, sheep, porcine, and goat meat with the PCR technique. The studied mixtures were prepared by adding 5%, 2.5%, 1%, 0.5%, and 0.1% levels of pork, horse, cat, or dog meat to beef, sheep, and goat meat. The amplified species-specific fragments of the mtDNA were found to be of 439, 322, 274, 271, 225, 212, and 157 bp for horse, dog, cat, bovine, sheep, porcine, and goat meat, respectively. The barrier of the 1.0% mixture, as earlier reported by Meyer et al. (1994), Hopwood et al. (1999) and Partis et al. (2000), was overcome with the increase of the PCR cycles; thus, the 0.1% level required 35 cycles while for the rest only thirty, with satisfactory accuracy.

Lockley and Brardsley (2002) presented a novel one-step assay to discriminate DNA originating from chicken and turkey. The detection was based on the PCR and primers which exploit intron variability in α-cardiac actin to generate single products of a characteristic size for each species. The lack of cross-reactivity fragments with porcine, ovine or bovine DNA templates and the possible detection of chicken/turkey admixtures up to 1% turkey in 99% chicken and vice versa was noteworthy.

The multiplex PCR technique was employed to feedstuff analysis for the identification of various species in rendering plants, including pork materials (Dalmasso et al., 2004). Primer binding sites (PBS) were chosen to generate specific amplimers of less than 300 bp in length

thereby sustaining the possibility to apply the assay to samples DNA of which is highly degraded by heat following the treatment indicated by the EU Law for thermal processing of animal ingredients (134.4–141.9 0C and 3.03–4.03 bar for 24 min). The designed primers, located in different regions of mitochondrial DNA and characterized by alternate well-conserved regions (12S rRNA-tRNA Val), were of 290bp in length and detection limit 0.002%.

Martin et al. (2007b) have applied a similar assay for qualitative detection of four duck species in meat mixtures, and a second PCR assay for the specific identification of Muscovy duck basing on oligonucleotide primers targeting the 12S rRNA mitochondrial gene. Therefore, the necessity of the conventional PCR and realtime PCR for the detection of Mule duck for separate primers sets, that is 12S and 5S ribosomal RNA, and α-actin genes (Rodríguez et al., 2001, 2003a, 2003b, 2004) overcame. The specificity of both assays comprised a wide range of animal species and was applied to raw and sterilized muscular binary mixtures offering detection limit range from 0.1% to 1.0% (w/w). The strong point of this technique was the successful amplification in samples with highly degraded DNA, and consequently, in inspection programmes (labelling regulation enforcement) due to the short length (less than 100 bp) of the amplified DNA fragments.

The power of PCR amplification of selected α-actin fragments by species-specific oligonucleotides actG, actD, actC and actP, together with the actinv primer has been used for the qualitative/semiquantitative discrimination among goose, mule duck, chicken and pork samples (Rodríguez et al., 2003c). The interpretation of the results could be unambiguously achieved visually- without computer use- and was complementary to those earlier reported for the identification of goose and mule duck foie gras and their mixtures (Rodríguez et al., 2003a).

The PCR method was implemented for the qualitative identification of chicken (*Gallus gallus*), turkey (*Meleagris gallipavo*), duck (*Anas platyrhynchos* x *Cairina muschata*), and goose (*Anser anser*) tissues in feedstuffs (Martin et al., 2007b). The designed oligonucleotide primers- specific for each avian species- has been targeted the 12S rRNA mitochondrial gene and the generated amplicons were of 95, 122, 64, and 98 bp length, respectively. Their specificity was extensively tested using DNA obtained from muscle samples of twenty nine animal species (13 mammals, 12 fish, 4 birds) and 8 plant species without any cross-species amplification. Satisfactory results have also been obtained when heat-treated samples (120°C/50 min, 110°C/120 min, or 133°C/300 kPa/20 min) underwent the amplification patterns and strongly resembled those obtained for raw samples (LOD 0.1%). Similar results were obtained from raw or heat-treated when time was extended and temperature dropped simultaneously.

In a similar approach, species specific primers- based on the nucleotide sequence variation in the 12S rRNA mitochondrial gene- were designed by Martin et al. (2007c) for the qualitative detection and identification of cat, dog and rat or mouse in food and feedstuffs. The detection limit of these tissues in meat/oats mixtures was as low as 0.1%, with no effect of prolonged heat-treatment (up to 133°C for 20 min at 300kPa); thus proving very useful with regard the origin verification of raw materials in food and feedstuffs submitted to denaturing technologies.

The direct and highly species-specific method for the detection of pork meat and fat in meat products which has been developed by Montiel et al. (2000) was based on highly species-specific primers of pork D-loop mtDNA. It was one step forward from the nuclear 18S ribosomal RNA and growth hormone genes or Y chromosome PCR amplification by Meyer and colleagues (1994) and Meer and Eddinger (1996), as well as the porcine mtDNA previously reported by

Ghivizzani et al. (1993). Thus, the designation of these primers in conjunction with restrictive PCR amplification conditions allowed the identification of pork meat and fat, even heated, by the presence of an amplified band of 531 bp. Furthermore, the absence of this band in PCR-amplified products from bovine, ovine, chicken, and human samples; and the facile distinction of wild boar and pork amplified DNA by digestion with AvaII restriction enzyme (production of two bands of 286 and 245 bp vs none, respectively) qualify it gradually.

Fajardo et al. (2007a) developed a PCR assay for the identification of meats from three caprinae species based on the selective amplification of mt D-loop sequences from chamois, pyrenean ibex, and mouflon by using oligonucleotides targeting to mitochondrial D-loop sequences. The use of mitochondrial DNA (mtDNA) sequences for species identification with PCR is preferable over other genetic markers, i.e. the cell nucleus DNA, whereas its detection might be of low yield. On the other hand, the utilization of mtDNA enhances the PCR amplification sensitivity because of the enhanced number of mtDNA copies per cell. Moreover, the numerous forms of mtDNA offer reliable identification and differentiation of precise species in food mixtures (Girish et al., 2004). The D-loop region (~700–1000 bp) was primarily amplified and sequenced from various game and domestic meat DNAs, and was used as matrix for the following designation of three primer sets. Thus, the PCR amplification of the targeted D-loop fragments from chamois (88 bp), pyrenean ibex (178 bp), and mouflon (155 bp) meats, successfully resulted in adequate specificity and reproducibility against a number of game and domestic meats. Equally satisfactory results were reported for the amplification accomplished in the analysis of experimentally pasteurized (72ºC for 30 min) and sterilized (121ºC for 20 min) meats, with a detection limit of ~0.1% for each of the targeted species.

Mane and co-workers (2009) expanded PCR abilities towards the detection of chicken meat using designed primer pair based on mitochondrial D-loop gene for amplification of 442 bp DNA fragments from a variety of meat and meat products. The high specificity of the used restriction enzymes HaeIII and Sau3AI was successfully cross tested with nine red meat species-except from quail and turkey-with detection limits being less than 1% in admixed meat and meat products.

Since the increasing trend towards low fat content in the diet was a fact, the pronounced interest in consuming meats from game species was the anticipated consequence. For example, venison-very popular in European markets- consisted of red deer (*Cervus elaphus*), fallow deer (*Dama dama*), and roe deer (*Capreolus capreolus*). The specific identification of meats from red deer, fallow deer, and roe deer, with a selective PCR amplification of DNA fragments on the mitochondrial 12S rRNA gene was studied by Fajardo et al. (2007b). The use of a common reverse primer, together with forward specific primers for the mentioned species allowed the selective amplification of the desired cervid sequences. Amplification and sequencing of a conserved 12S rRNA gene fragment (~720 bp) from red deer, fallow deer, roe deer, chamois, mouflon, pyrenean ibex, cattle, sheep, goat, and swine meats was accomplished with the use of the primers, namely: 12S-FW and 12S-REV oligonucleotides (Fajardo et al., 2006). After the alignment of 12S rRNA gene sequences specific primers were then designed based upon detailed analysis and comparison of the aligned 12S rRNA sequences. These comprised a reverse primer (12SCERV-REV) common to all targeted species, and three forward species-specific primers: 12SCE-FW for red deer, 12SDD-FW for fallow deer and 12SCC-FW for roe deer. The combination of each forward primer, along with the common reverse oligonucleotide, was

expected to yield DNA fragments of ~170-175 bp in the 12S rRNA gene from each deer species. Thus, PCR of species-specific primers directed to short DNA fragments seems a versatile tool towards meat authentication, through the target-species identification from a pool of different DNAs, without the need for further sequencing or digestion of the PCR products with restriction enzymes.

Dooley et al. (2004) developed assays around small (amplicons <150 base pairs) regions of the mitochondrial cytochrome b (cytb) gene, the speciation of which was based on species-specific primers. Thus, species-specific real-time PCR (TaqMan) assays were introduced for the detection of beef, pork, lamb, chicken and turkey, in parallel with two TaqMan probes. The first was specific to the mammalian species (beef, lamb and pork), and the second to the poultry species (chicken and turkey). PCR was limited to 30 cycles under normal end-point TaqMan PCR conditions to DNA extracts from raw meat admixtures, giving the possibility to detect each species when spiked in any other species at a 0.5% level. The experimentally determined limits for beef, lamb and turkey were below 0.1%.

A Duplex PCR assay has been upgraded by Di Pinto et al. (2005) to determine the adulteration of horse fresh sausages with pork meat. The approach involved the extraction of DNA with a procedure in which total DNA was bound on a silica membrane followed by amplification of a fragment of the cytochrome b gene of mitochondrial DNA (mt DNA). This revealed the presence of two-439 and 398 bp- specific amplicons. It has been thereby confirmed that the primer specificity and detection limit were in good agreement with those reported by Matsunaga et al. (1999), while none of the bovine DNA samples gave positive results. The

developed assay revealed the presence of pork meat in 6/30 and the total absence of horse meat in 1/30 of the analyzed horse sausage samples.

In the same direction, the efforts of Colombo et al. (2002) were driven about the typical product of the Lomellina zone (Italy)- "Mortara" goose salami. The PCR assay was set up using Italian goose breeds meat and "Mortara". The identification of the species *Anser anser* in salami was successful with the designation of two primer pairs: one zone of the cytochrome b of mitochondrial DNA of goose and a specific primer. Primers were indicated as "cytb1" and "cytb2" and were named "Goose1" (5'-ccatctgcttagccacacaaatccta-3') and "Goose2" (3'-tatgtttctttggactttgtgtcctc-5'). The control species included pig and duck because they possess the two principal fraud components for salami content, or envelope concerning the second. Chicken was excluded because it is rarely substituted with goose and its homology with goose is much lower than ducks.

In an attempt to restore consumers distrust to beef or beef containing products because of the recently increasing cases of BSE and variant Creutzfeld Jacob Disease (vCJD) Brodman and Moor (2003ms) applied TaqManTM real-time PCR analysis targeting to even smaller amounts of highly degraded DNA detection. The two methods used for the detection of mammal DNA and beef DNA included apart from TaqMan Universal PCR Master Mix: 0.3 μM of the primers BtaGH-1and BtaGH-2, 0.1μM of the probe and BtaGH-S and 0.45 μM of the primers GH-1 and GH-4, 0.1 μM of the probe GH-S, oligo storage buffer, respectively. Amplification fragments as short as 66 and 76 bp, respectively, allowed their determination even in meat and bone meal (MBM) products with a detection limit of 0.02 ng which corresponds to about 5 or 6 genome copies.

Minute amounts of beef and pork in processed foods were successfully detected by Laube et al. (2003) using two TaqManTM-PCR systems based on the amplification of the phosphodiesterase gene (104bp) (with cattle-specific primers) and the ryanodin gene (108bp) (with swine-specific primers). The overall assay was further enforced with a third system which reliably excluded false-negative results by detecting meat from a variety of sixteen different animals-mammals or poultry- in the material to be tested. However, the results concerning the discrimination between cattle and deer were not satisfactory.

Bovine, porcine, lamb, chicken, turkey, and ostrich DNA in complex samples have been quantitatively detected with six TaqMan real-time PCR systems using minor groove binding (MGB) probes (López-Andreo et al., 2005). This low cost assay was accomplished by combining only two fluorogenic probes and 10 oligonucleotide primers targeting mitochondrial sequences showing limits of detection from 0.03 to 0.80 pg of template DNA. It was shown that the proposed method could effectively detect more than 1% of pork, chicken or turkey and more than 5% of cattle or lamb in blends containing between 2 and 4 species.

Calvo et al. (2001) developed a rapid, more specific and more sensitive PCR method for pork detection of pork in heated and unheated meat, sausages, canned food, cured products and pâtés. Therefore, a new DNA-specific porcine repetitive element was isolated by non-specific PCR, whose analysis led to the synthesis of a pair of primers. Its effectiveness and specificity through the fifty five pig blood DNA samples (from different breeds) test gave positive results and totally negative in two hundred samples from various species. The number of the PCR amplification cycles was determinative regarding the detection limit; 0.005% pork in beef and 1% pork in duck pâté after thirty and twenty cycles, respectively. Similarly, the detected amount of porcine DNA

in cattle DNA yielded 1.25 and 250 pg following the same procedure, respectively. Conclusively, pork has been identified in all the above mentioned meat products in a rapid, inexpensive, simple and reliable manner.

A PCR assay was developed to quantitatively establish pork adulteration in ground beef and pâté-accidental or intentional- in heated and non-heated meat and pâtés by densitometry using a specific and sensitive repetitive DNA element (Calvo et al., 2002). That is known as the Short Interspersed Nuclear Element (SINE) repetitive element, well known for its specificity and sensitivity for pork detection. Therefore, detection limits were established up to 0.005%, 0.1%, and 1% pork in beef (raw and heated) and pork in duck pâté, after 30, 25, and 20 PCR cycles, respectively. The best standard curve and correlation between pork content and band intensity was opted after the completion of twenty-five PCR cycles. Thus, fraud was successfully determined in commercial pâtés, even in known samples containing 0% to 100% pork in beef after heating, autoclaving at 50, 80, and 120°C for 30 min. The analysis of raw samples was similarly performed, as well as pork and duck pâté mixtures varying between 0% and 100% pork.

The great significance of cytochrome b gene also proved in the detection of low levels of horse or donkey meat in commercial products. Chisholm et al. (2005) have developed real-time PCR assays specific for these animals using primers, based on the mitochondrial cyt b gene, and were 3’ position mismatched to closely related and other commercial species. It thus became possible to overcome the disadvantages of using probes common to many mammalian species and specific primers or multiprimer assays with sense and species-specific antisense primers (Brodmann and Moor, 2003; Dooley et al., 2004; Walker et al., 2003). Amplification of non-

target species DNA was prevented by truncation of primers at the 5' position, thereby conferring complete specificity. Independent primer and probe sets for horse and donkey maximised design flexibility for specificity and allowed amplicons to be less than 150 base pairs. Both assays revealed high sensitivity and the limits of detection were 1 pg and 25 pg of donkey and horse template DNA diluted in water, correspondingly. The applicability of the assays to model food samples, spiked with horse or donkey muscle and commercial products containing horse, was successfully tested.

Sawyer et al. (2003) introduced a technique greatly contributing to the development of quantitative PCR assays, with the use of universal and species specific PCR primer pairs. A comparison of the cycle number at which universal and species specific PCR products were first detected, in comparison with reference standards of known species content, have been used as the basis for the determination of wide range of mixed samples (0.1-100%) of beef and lamb admixtures.

Yman et al. (1987) investigated by means of starch gel electrophoresis and appropriate staining the residual blood in the carcass which contains both serum albumin and esterase thereby facilitating the examination of genetic variants of these proteins in meat extract from horse, donkey and their hybrids, mule/hinny. Because of the absence of any of the donkey albumin variants C and D in horses and none of the horse variants A, B and I were detected in donkeys, it was possible to differentiate the two species from each other and from their hybrids, mules and hinnies. On the other hand, the serum enzyme carboxylesterase, as earlier shown, is able to segregate fresh meat from horses and donkeys and analysed with the mentioned techniques. In view of similarities in electrophoresis zones of serum albumin of the most

interesting species, it has been suggested that for the identification of an unknown sample suspected of being horse, donkey or mule/hinny meat one must start with immuno-diffusion or protein staining of IEF gels. Then, identification of these animals as a group different from other species, e.g. beef, swine, etc., must take place and finally determination the genetic variants of serum albumin by stage and further confirmation by staining the other slice of the starch gel for serum carboxylesterase.

Polyacrylamide gel electrophoresis gives characteristic protein patterns for the most consumable kind of meats, e.g. beef, pork, mutton, venison, and reindeer, while disc electrophoretograms could differentiate the meats of various animal species and provide a quantitative determination of beef and pork. The muscle extracts from beef, pork, venison, reindeer, mutton, and a mixture of these meats were examined electrophoretically by Skrökki and Hormi (1994). The amounts of beef and pork in commercial minced-meat mixtures were determined quantitatively by reference to standard electrophoretograms thus providing a valuable tool against meat adulteration. As a result, a set of 49 minced-beef and 49 minced-beef-and-pork samples electrophoretograms were compared optically with standard ones obtained in the same manner (potential 280 V, run time 1 h 30 min, amount of sample 30μL). The identification of pork, venison, and reindeer from the same meat mixture proved easy, while the main bands for beef, reindeer, and mutton showed high similarity. This difficulty could be possibly overcome if the run time is prolonged.

Saez et al. (2004) applied a single step DNA-based test for the simultaneous identification of multiple meat species which was based on the generation of species-specific fingerprintings by two different arbitrary DNA amplification approaches (RAPD- and arbitrarily primed PCR, AP-

PCR) using the primers OPL4 and OPL-5. The large variety of representative samples reflected the applicability of the techniques. Thus, application of RAPD-PCR fingerprintings facilitated the discrimination amongst pork, beef, lamb, chicken and turkey in all cases, achieving a satisfactory species cluster at similarity levels ≥75%. The application of AP-PCR using the primer M13 also allowed the detection of five species-specific fingerprintings. AP-PCR also permitted the identification of the five tested species in every sample although more complex patterns were generated, including some low intensity bands. These difficulties were overcome in both cases by introducing a ramp time between annealing and extension temperatures, thereby making this technique suitable for meat authentication in routine analysis.

The interesting point in a real-time PCR assay is focused on the direct monitoring ability of amplification products during each amplification cycle. Thus, the quantification is possible at an early stage in the PCR process, which is inherently more accurate than at the end point analysis. Furthermore, the use of a DNA binding dye like SYBR green, which adheres to the minor groove of the double-stranded DNA in a sequence-independent way, enhances the method flexibility without the need for individual probe design and optimization steps. This technique has been developed by Fajardo et al. (2008) for quantification of red deer, fallow deer, and roe deer DNAs in meat mixtures through the combination of cervid-specific primers-amplifying a 134, 169, and 120 bp of the 12S rRNA gene fragment, respectively-and universal primers-amplifying a 140 bp fragment on the nuclear 18S rRNA gene- from eukaryotic DNA. The Ct (threshold cycle) values resulting from the latter primers were used to normalize those obtained from each of the former, working as endogenous control for the total content of PCR-amplifiable DNA in the sample.

Therefore, the obtained detection and quantification of the target cervid DNAs ranged from 0.1to 0.8%, depending on the species and the preceding treatment.

The real-time quantitative PCR, employed by Rodriguez et al. (2005), for the quantitation of pork (*Sus scrofa*) in binary pork/beef muscle mixtures was based on the amplification of a fragment of the mitochondrial 12S ribosomal RNA gene (rRNA). The pork-specific primers amplified a 411 bp fragment from pork DNA while mammalian-specific primers a 425-428 bp fragment from mammalian species DNA, which were used as endogenous control. The internal fluorogenic probe (TaqMan) was used for monitoring the amplification of the target gene. Thus, a comparison of the cycle number (Ct) at which mammalian and pork-specific PCR products were first detected, in conjunction with the use of reference standards of known pork content, allowed the determination of the percentage of pork in a mixed sample. The following analysis demonstrated the specificity and sensitivity of the assay for detection and quantification of pork in the range 0.5–5%. The detection limit for pork-specific PCR was 0.01 ng DNA, which corresponds to 0.1% pork DNA.

The identification and quantification of bovine, porcine, horse, and wallaroo DNA in food products was examined by López-Andreo et al. (2006) with real-time uniplex and duplex PCR assays with a SYBR Green I post-PCR melting curve analysis. The threshold-cycle (Ct) data obtained from serial dilutions of purified DNA enable the quantification of these species, resulting in limits of detection in uniplex reactions: 0.04 pg for porcine and wallaroo DNA and 0.4 pg for cattle and horse DNA. The species specificity test of the PCR products was based on the identification of peaks in DNA melting curves, measured as the decrease of SYBR Green I fluorescence at the dissociation temperature (74.3, 73.2, 78.7, and 76.0ºC, respectively). The

duplex assay, by use of either single-species DNA or DNA admixtures containing different shares of two species, allowed the following minimum proportions of each DNA species though the resolution of Tm peaks: 5% (cattle or wallaroo) in cattle/wallaroo mixtures, 5% porcine and 1% horse in porcine/horse mixtures, 60% porcine and 1% wallaroo in porcine/wallaroo mixtures, and 1% cattle and 5% horse in cattle/horse mixtures. The results obtained from SYBR Green I uniplex and duplex reactions with single-species DNA were very much comparable to those obtained previously with species-specific TaqMan probes.

In 2007, Rastogi and his colleagues suggested that mitochondrial markers were more effective than nuclear markers for the purpose of species identification and authentication. Therefore, they were focused on the mitochondrial markers viz. 16S rDNA and nicotinamide adenine dinucleotide (NADH) dehydrogenase subunit 4 (ND4) and a nuclear marker viz. the actin gene to identify the specimens of animal origin for several reasons (forensic identification, food regulatory control, illegal trading prevention, poaching and conservation of endangered species).Among the five tested primers (Operon I.D. C4, D5, F4, F7 and F5) only one of them (F4) was selected for RAPD-PCR fingerprinting on the basis of number, intensity and distribution of bands to clearly discriminate various species, such as buffalo, cow goat, pig and chicken tissues. Species identification based on intron sequences present between exon 6 and 7 of actin gene was not satisfactory because only chicken and pig samples were identified perfectly up to species level using this nuclear marker.

The difficulty of infrared spectroscopy to discriminate between muscle foods was of limited success, in particular regarding the closely related poultry species, chicken and turkey, lead to the implementation of more powerful spectroscopic techniques. These include, among others, the

first application of HATR (horizontal attenuated total reflectance) Fourier transform – InfraRed (FT-IR) and Raman spectroscopy to the study of muscle foods and the collection of novel data able to discriminate between both the species and the distinct muscle groups within these species (Ellis et al., 2005). Application of discriminant function analysis and genetic algorithms provide a qualitative and in (bio) chemical terms interpretation of FT-IR and Raman spectra-though specific wavenumbers-of the just mentioned two categories. Thus, a total of only eight FT-IR wavenumbers were used by genetic algorithms - multiple linear regression (GA-MLR) to discriminate between general muscle type, species and each of four separate muscle groups from the closely related muscle foods. The discrimination of muscle type included three wavenumbers; namely 1413 cm^{-1}, 1444 cm^{-1} and 1729 cm^{-1}, which were assigned to C–N stretch from amides, N–H bend from amides and C=O stretch from saturated aliphatic aldehyde, respectively. Moreover, the wavenumbers 1729 cm^{-1} and the pair 1575 cm^{-1} and 1606 cm^{-1} satisfactorily discriminated samples coming from muscle type and species level, correspondingly; the last two wavenumbers being ascribed to a CNH combination vibration from amide II and NH_2 deformation from amines, respectively.

A vast number of different meat muscle species, such as beef (n=100), lamb (n= 140), pork (n=44) and chicken (n=48), have been identified and authenticated with visible infrared spectrospopy (VIS) and near infrared reflectance spectroscopy (NIRS) (Cozzolino and Murray, 2004). The homogenised samples were scanned in the region of 400 to 2500 nm, giving an excellent differentiation of muscle species in conjunction with both Principal Component Analysis (PCA) and dummy partial least-squares regression (PLS) models. Thus, a more than 85% correct classification of the studied samples has been obtained. The most satisfactory

classification for PLS resulted when three wavelength segments (400-700, 1100-2500, and 400-2500 nm) have been used, while for both PCR and PLS methods with either the NIR or the VIS+NIR region. In contrast, the visible region alone proved of low importance.

The feasibility of applying quality controls to typical Spanish sausages by performing a proximate analysis (fat, moisture and protein) on the finished product (intact and homogenized) was made feasible with NIRS technology (diode array instrument) (Gaitán-Jurado et al., 2008). The studied quality controls reflected the various stages once the finished product was obtained, whereas finished product, storage, distribution and marketing. The selected models were subjected to calibration and evaluation by cross and external validation. Thus, for end-products, coefficients of determination for calibration (R2) for fat, moisture and protein were 0.98, 0.93 and 0.97, respectively, whereas the corresponding values for homogenised products were 0.99, 0.98 and 0.97, respectively. In homogenised products, these values were lower: 0.71%, 0.41% and 0.95%, respectively.

NIRS was also employed by Ortiz-Somovilla et al. (2005) for the detection of meat mixture ingredients in Iberian sausages. Each of the five homogenized meat mixture treatments of Iberian (I) and/or Standard (S) pork was analyzed by NIRS as a fresh product (N = 75) and as dry-cured sausage (N = 75). The most representative absorption peaks and bands were selected for both homogenized dry-cured and fresh sausages and hereafter a discriminant analysis procedure revealed the mixture prediction equations. The best results were obtained for fresh products versus the dry-cured sausages, with 98.3% and 91.7% (calibration) and 60% and 80% (validation) correct classification, correspondingly. Afterwards, two instrumental modes of analysis were compared; that is “Down-view” and “Up-view”. The first was based on a spinning

circular capsule which allowed the collection of spectral information at different points of the sample, whereas the second required the inversion of the instrument and the direct place of the sample over the quartz window Ortiz-Somovilla et al. (2007). The resulting data from proximate analysis (fat, moisture and protein) were put into a calibration model with a diode array NIR spectrometer- spectral range 515-1650 nm – through multi-way partial least-squares regression (MPLS) and PCR.

The predictions of Kang et al. (2001) for fat, moisture and protein in ground pork sausages with NIRS of greater scanning range (400–2500 nm) gave values very similar to those reported by Ortiz-Somovilla et al. (2007) -0.98, 0.98 and 0.93 (R^2) and 1.38%, 1%, 0.83% (standard error of validation, SEP), respectively. In contrary, the predictions Chan et al. (2002) for the same parameters about pork quality characteristics, with the Up-view mode, were not any after cross validation (r^2) 0.76, 0.8 and 0.69, and standard error of cross-validation (SECV), 0.62%, 0.58% and 0.43%, respectively.

Different types of bovine meat were differentiated with NIRS by Alomar et al. (2003) and predicted main chemical fractions on samples from two breeds, three muscles and six grading (Chilean system) categories-namely V, A, C, U, N, O. The samples were scanned (400–2500 nm) and then analyzed for dry matter, crude protein, ether extract, total ash and collagen content, after freeze drying. The best calibrations for protein and fat were obtained with a second order derivative (2-20-20 and 2-10-10, respectively) and without any scatter correction of the spectral data. Thus, the discriminant analysis in conjunction with PLS technique and cross validation, have been correctly identified breed and muscle type for most samples. The results outline the NIRS as a helpful and effective tool for identifying breed and muscle type in beef meat, on an

objective, composition-related basis and to abtain rapid predictions of meat composition with reasonable accuracy, particularly for protein and fat content.

NIRS has also been employed by Ding and Xu (2000) for the detection of beef hamburgers adulterated with 5-25% mutton, pork, skim milk powder, or wheat flour (accur. ~92.7%, increasing with the adulteration level). In the positive cases the adulteration level was further predicted using calibration equations especially for mutton, pork, skim milk powder, and wheat flour with standard errors of cross-validation of 3.33, 2.99, 0.92, and 0.57% and coefficients of variance of 0.87, 0.89, 0.99, and 1.00, respectively. Discrimination has also successful for normal and abnormal chicken carcasses (Chen and Massie, 1993), broiler and local chicken carcasses (Ding et al., 1999), and fresh and frozen-then-thawed beef (Thyholt and Isaksson, 1997). An intrinsic review on the last case products-primarily whole meat from *Bos taurus* (cow), *Sus scrofus* (pig) and *Gallus gallus* (chicken)- concluded that the best analytical choice for this discrimination is bound to be a combination of analytical methods (Ballin and Lametsch, 2008).

Moreover, beef and kangaroo meat (Ding and Xu, 1999) could also be differentiated by comparing the NIR spectra due to their physical and chemical differences. Thus, the resulting differences in chromatic values ($P<0.001$) were attributed to the lower fat content and higher percentages of polyunsaturated fatty acids in kangaroo meat than in beef (Sinclair et al., 1982). Therefore, selected wavelengths in the near-infrared region (2384, 2278, 2236, 2210, 2096, 1742, 1680, 1308, 1246, 1204 and 926 nm) were strongly related to C-H bonds and explained the noticed differences. Furthermore, spectral bands at 2236, 2210 and 1680 nm were related to

unsaturated =C-H groups therby enhancing the differentiation capability of the polyunsaturated fatty acids towards the classification.

The physical parameters of adult steers (oxen) and young cattle meat samples were investigated with NIRS to evaluate their quality mark (Prieto et al., 2008). Hence samples of *Longissimus thoracis* muscle coming from young animals extensively reared (in extensive conditions) were analyzed for pH, colour (L*, a*, b*), water holding capacity (WHC) and Warner–Braztler shear force (WBSF). The meat colour parameters obtained were of great importance since they represent a parameter closely related to factors such as freshness, ripeness, desirability and food safety and often affect greatly the consumers' decision. The satisfactory prediction of these parameters in young cattle meat samples was strongly correlated with the intramuscular fat content and was more accurate (L* -redness; R2 = 0.869; SECV = 1.56 and b*-yellowness; R2 = 0.901; SECV = 1.08) than in the case of oxen.

Liu et al. (2004) used generalized two-dimensional (2D) correlation analysis of VIS-NIRS for the characterization of the spectral intensity variations of chicken muscles induced by either storage time/temperature regime or shear force values. Since an asynchronous 2D correlation spectrum consists exclusively of off-diagonal cross-peaks, it appears if the basic trends of dynamic variations observed at two different wavelengths of the cross-peak spectral coordinate are dissimilar. Thus, the sign of an asynchronous cross-peak could possibly determine a specific sequence of events occurring at different temperatures/perturbation variables. A negative cross-peak indicates that the spectral intensity change observed at $\lambda 1$ occurs after that at $\lambda 2$, whereas positive peaks indicate the opposite. The positive cross-peaks located between the 445 and 560 nm bands, whereas negative cross-peaks observed between the 475 nm band and the 445 and 560

nm bands. This observation is indicative of an increase in spectral intensities at 445 and 560 nm while at 475 nm it decreases as the storage temperature drops. The 445, 475, and 560 nm bands have been assigned to deoxymyoglobin, metmyoglobin, and oxymyoglobin species, respectively. Furthermore, shear value-induced NIR spectral intensity variations detected significant differences in spectral features between tender and tough muscles.

The authentication of beef and ox kidney and liver with mid-infrared (MIR) spectroscopy has been effectively carried out by Al-Jowder et al. (1999). At any spectra recorded (800 to 4000 cm^{-1}), sixty four interferograms have been co-added and a triangular apodization was employed prior to Fourier transformation. All absorbance spectra were truncated to 470 data points in the range of 900-1800 cm^{-1} (the "fingerprint" region), not rarely the most useful part of the MIR spectrum. To the major constituents (water, protein, and fat) of the samples: beef (brisket, silverside and neck), kidney and liver were included; in the last one must be added an appreciable amount of carbohydrate, present in the form of glycogen. Significant variation between these sets of spectra was recorded for the group of features in the region 1000-1200 cm^{-1} present in the spectra of liver and attributable to the glycogen content of these specimens. The fat content indicated by the slight shoulder at 1744 cm^{-1} appears to be highest in the brisket and silverside specimens, lower for the cuts of neck, and lower still or absent for the offal.

The discrimination of raw pork, turkey and chicken meat (n = 74) based on the recorded spectra in the visible, NIR and MIR regions, was carried out by Rannou and Downey (1997). Thus, the development of discriminant models led to satisfactory results in all ranges as follows; the best results from NIR spectra received using the 400–1100 nm range, with seven principal components working optimally for all of the calibration samples and a success rate of 91.9%.

The optimal factorial discriminant model developed from MIR spectra employed five principal components for all of the calibration samples and 86.5% correct identification in the validation step. Finally, the combination of NIR and MIR spectra gave the best results using the data in the wavelength ranges 400–750 plus 5000–12500 nm. The use of six principal components was adequate to totally and correctly classify the calibration sample set in comparison with the 94.6% of samples in the validation set. The individual spectral regions were correctly separated concerning the pork samples from the poultry while some overlap of chicken and turkey samples was apparent.

Xia et al. (2007) have developed a fiber optical probe to count spatially resolved diffuse reflectance from beef samples in VIS-NIR bandwidth of 450-950 nm. It is well known that beef absorption coefficients depend heavily on the chemical composition of the samples, such as the concentration of myoglobin and its derivatives; while scattering coefficients are rather based on meat structural properties such as sarcomere length and collagen concentration. Since the above-mentioned factors also affect the determination of beef tenderness, the hypothetical existence of a correlation between optical scattering and cooked WBSF was rightful. Thus, the higher the scattering coefficient the higher the cooked WBSF association, as easily revealed from the linear regression analysis ($p < 0.0001$ vs R2 of 0.59, respectively).

The correlation of BSE with bovine MBM increased consumers' preference in meat originated from birds fed exclusively vegetable diets. Carrijo et al. (2006) examined the presence of MBM in broiler diets through the analysis of samples of major breast muscle (*Pectoralis major*) using mass spectrometry of stable isotopes (carbon and nitrogen). A total of 150 day-old chicks were reared in five randomized treatments with increasing MBM dietary inclusion levels

(0, 1, 2, 4 and 8%). On day 42, breast muscle samples were prepared for the determination of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotope ratios. The determined isotope values for the above mentioned MBM levels were: -18.74±0.11, -18.51±0.19, -18.24±0.10, -17.79±0.12, and 17.15±0.15 for the carbon and 1.65±0.14, 1.65±0.28, 1.72±0.08, 1.95±0.16, and 2.52±0.09 for the nitrogen, respectively. The significant differences-enrichment- in these isotopic pairs allows the tracing of MBM in bird diets and/or indicates exclusively vegetable diets.

Application of an up-to-date technique for purging and trapping gaseous compounds-from both high and low volatility samples-at low temperature on short alumina-coated porous layer open tubular (PLOT) columns was carried out by Lovestead and Bruno (2010). The target marker compounds could be indicative for poultry spoilage either fresh or spoiled. After a two-week purposely severe spoilage the headspace was collected with cryoadsorption and the volatiles were subjected to separation, identification, and quantification with GC-MS. Six dominant substances were thereby noticed, namely dimethyl disulphide; dimethyl trisulphide; phenyl sulphide; methyl thiolacetate; allyl methyl sulphide; and 2,4,6-trimethylpyridine. The presence of isophorone in both fresh and spoiled chicken-in spite of its unknown origin-was attributed to the packaging materials. It is noteworthy that the determined differences between the samples analysed according to the classical approach and the new approach were minor and rather negligible (only one out of six compounds diferred).

Sultan et al. (2004) introduced a novel assay based on Western blotting for the detection of brain tissue in processed (heated) meat products through the development of central nervous system (CNS)-specific antigens. The bands of antigen-bound primary antibodies were visualised using secondary anti-antibodies labelled with peroxidase, which produced the necessary

chemiluminescence for the photographic film documentation. Ponceau-S staining has been used before antibody incubation providing an intermittent control for the efficacy of the extraction and blotting procedures by judging the intensity of the protein profile on the blotted paper. The molecular mass information on detected antigens after immunoreactions provided additional information towards the correct identification of brain tissue in the meat products. Thus, the B50/growth-associated protein (B50), glial fibrillary acidic protein (GFAP), myelin basic protein (MBP), neurofilament (NF), neuron-specific enolase (NSE) and synaptophysin proteins were detected in raw luncheon meat and a liver product enriched with brain tissue at a level of 5% (m/m). However, only MBP and NSE were considered suitable biomarkers for detection of 1% (m/m) brain tissue in meat products pasteurised at 70ºC or sterilised at 115ºC. On the other hand, the use of an anti-monkey MBP instead of anti-human MBP enabled speciation of the CNS material whether from bovine and ovine brains or from porcine brain tissue.

One the most frequently used adulterant in meat products, the addition of non-declared and lower quality protein, has been studied by Flores-Munguia et al. (2000) implementing an immunodiffusion assay in agar gel. The added portions of protein, from bovine, porcine, equine and avian species, in two slightly processed meat products, uncooked commercial hamburger and Mexican sausage (chorizo) were successfully identified. Thus, in a total of forty (40) samples from local food stores, undeclared equine species was detected in 9 of the 23 of the hamburger meat samples, while Mexican sausage undeclared equine and porcine species were also found in 5 of the I7 samples.

The identification of animal species in ground meat mixtures was succeeded by Scarpeid et al. (1998) based on intensity profiles from isoelectric focusing of water-soluble proteins in

conjunction with a multivariate calibration model. Therefore, the isoelectric focusing images in immobilized pH gradients from samples containing various amounts of beef, pork and turkey meat were analyzed and transferred to a digital format. The latter was also reduced background and optimized signal strength, thereby giving profiles which allowed the determination of sample composition with prediction errors close to 10%.

The presence of limited accessible data on the detection of irradiated quails meat was endorsed by Erel et al. (2009) using DNA comet assay EN13,784 (CEN, 2001); a screening test for the identification of the irradiated food but not the applied dose. The obtained DNA comets were examined by both photomicrographic and image analysis after exposure of samples to six radiation doses (0.52, 1.05, 1.45, 2.00, 2.92 and 4.00 kGy in gamma cell). The isolated muscle cells (chest, thorax) were subjected to the assay analysis following certain days post radiation after cells lysing, electrophoresis, staining and evaluation of the slides through a fluorescent microscope. Therefore, fragmented DNA moved towards the anode and damaged cells appeared as a comet, with the density of the DNA in the tails to be proportional to the radiation dose. The differences on the values of tail DNA%, tail length and tail moment were totally significant, except for the dose exposure between 0.9 and 4.0 kGy and also among storage times on day 1, 4 and 8.

The high importance of a clear and trustworthy identity of the species of the meat samples due to possible economic, medical and religious reasons and the need for more rapid, sensitive and specific methods than the genetic ones for routine analysis led to the implementation of enzyme-linked immunosorbent assay (ELISA) in this field (Asensio et al., 2008). The results of the quantitative evaluation of pork adulteration in raw ground beef with ELISA by Martin et al.

(1998) were successful (Chen and Hsieh, 2000; Liu et al., 2006) porcine thermal-stable muscle protein and quantified the pork meat amount in raw and heat-processed meat and feed products identified successfully and presented. The detection limit reached the level of 0.5 to 0.05% (w/w) pork in meat mixtures.

The substitution of meats with others of low cost and/or quality is a common practice of adulteration of inexpensive and fast foods, such as hamburgers, where meat of different animal species can be used. As regards this product, Macedo-Silva et al. (2000) employed the dot-ELISA method and by using anti-sera to bovine, chicken, swine and horse albumin could detect the homologous species at concentrations as low as 0.6%. The produced anti-albumin anti-sera could detect the just mentioned meat species with satisfactory specificity and sensitivity both in isolation and in hamburger as additives. Conclusively, no adulteration has been confirmed in the examined commercial samples.

The hemoglobins from cow, lamb or pork meat samples (extracted with Milli-Q water and filtered on a cellulose acetate filter) were qualitatively identified with cation exchange chromatographic separation and diode array detection from the different peak patterns at 416 nm (Wissiack et al., 2003). The common point in the chromatograms of the investigated meat species was an intense peak at its beginning: 1.6 min for the pork meat, and 1.7 min and 1.9 min for cow meat and lamb meat partially separated, which might correspond to myoglobin or denaturated hemoglobin, both hardly being retained on the analytical column under the applied conditions. The characteristic hemoglobin peaks for the identification of pork, cow and lamb were eluted at 27.5 min and at 30.5 min, at 8.7 min and 13.2 min, and at 8.6 min, 11.3 min and 13.3 min, respectively. Therefore, approximately 10% of one meat species in the other could

easily be detected, while the differentiation between lamb and cow meat was more difficult because of the co-elution of characteristic peaks.

The high-performance liquid chromatography method with electrochemical detection (HPLC-EC) was employed by Chou et al. (2007) to differentiate fresh or cooked meat products from fifteen food animal species, such as cattle, pigs, goats, deer, horses, chickens etc., following the routine approach. Satisfactory coefficients of variation (<6%) were obtained for species-specific markers which displayed reproducible peak retention times irregardless different runs, body regions and subjects. For example, incubation of fresh beef, pork or chicken at room temperature for 24 h or repeated freezing and thawing affected only the intensity and by no means the pattern of species-specific peaks.

A comprehensive summary of methods used for determination of meat authenticity is given in Table 1.

GEOGRAPHIC ORIGIN DETERMINATION

One of the consumers' strongest demand concerning food and food products over the last two centuries, at least, is reliable information for guaranteeing quality, safety and animal welfare (Verbeke and Viaene, 1999). However, this is almost practically impossible without trustworthy scientific data originated from independent technologies able to protect regional designations and ensure fair competition (Ilbery et al., 2000). Similar pressure of the respective magnitude becomes intensively determinative when the above mentioned awareness is accompanied with health concerns and, non-rarely, animal diseases such as BSE.

At the same time globalization and facilitation of goods' transportation between countries and continents are a constant sourve of apprehension. On the other hand, there are numerous foodstuffs directly associated with specific geographic area using prescribed techniques that may be unique to that region, such as the European adapted name schemes PDO (protected designation of origin), PGI (protected geographical indication) and CSC (certificate of specific character) (Regulation (EEC) No 2081/92). In any case, it is about a complicated subject since geographic origin is clearly affected by factors such as water, feeding, feed supplements, breed-strain, geology, pollution and housing system (Franke et al., 2005).

In this direction several regulations and techniques have been introduced or implemented (Regulation (ECC) No 2081/1992; Regulation (EC) No 2772/1999; Renou et al., 2004; Schmidt et al., 2005; Hintze et al., 2001; Kelly et al., 2005; Piasentier et al., 2003) and a recent review by Luykx and van Ruth (2008). Isotope Ratio Mass Spectrometry (IRMS) analysis holds one of the top positions among the applicable methods towards the determination of the geographical origin of foods, because isotopic compositions of them reflect many factors in natural environment. This is a result of the high-precision analysis of the abundance of naturally present stable isotope pairs of light elements in biological materials such as honeys, juices, spirits, wines, oils and several other plant-derived foods (Kelly, 2003; Renou et al., 2004; Boner and Förstel, 2004; Rossmann, 2001). Lately, the IRMS technique has been applied for the authentication of organically farmed Atlantic salmon by Molkentin et al. (2007). The H and O isotope analysis has been especially applied for the authentication of regional origins correlated to regional climatic conditions (Boner and Förstel, 2004; Renou et al., 2004). On the other hand, C and N isotopes

were extensively used to detect dietary components, such as maize or concentrates (Boner and Förstel, 2004; Gebbing et al., 2004; Quilter, 2002), whereas S was of limited use.

Schmidt et al. (2005) successfully combined the above presented types of the IRMS technique to analyse the natural stable isotope compositions of carbon, nitrogen and sulphur for the verification of the geographical origin and feeding history of beef cattle. Thus, beef reared in the USA (23 samples) and Brazil (10 samples) proved to be isotopically different from that bred in northern Europe (35 samples), mainly because of contrasting proportion of plants with C_3 and C_4 photosynthetic pathways in the cattle diets. The natural abundance stable-isotope ratios of carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$) and sulphur ($^{34}S/^{32}S$) were measured on the de-fatted muscle residue by continuous flow IRMS (Scrimgeour & Robinson, 2004). The significant difference from the mean values of terrestrial C_3 and C_4 plants of $\delta^{13}C$ values (-27‰ and -13‰, respectively; Kelly, 2000) and the determined $\delta^{13}C$ values of the conventional Irish (-24.5 ‰± 0.7‰) and other European (-21.6‰ ± 1.0‰) samples and those from US (-12. 3‰ ± 0.1‰) and Brazilian (-10.0‰ ± 0.6‰) beef reflect the almost exclusive type of foodstuffs.

The isotopic ratios of D/H, $^{18}O/^{16}O$, $^{15}N/^{14}N$, $^{34}S/^{32}S$ and $^{13}C/^{12}C$ of two hundred and twenty three (223) beef samples from Germany and Chile have been safely determined for their authenticity and possible fraud as reported by Boner and Förstel (2004). The isotopic composition effectively identified the geographical origin, the specific location of the breeding and the kind of fodder supplied. Data from the same seasonal trend and D/H-$^{18}O/^{16}O$ have earlier revealed correlation between samples from northern and southern Germany (D=4.5×18O-18.6 {‰}, r=0.88; and D=5.5×18O-22.2 {‰}, r=0.89, respectively), with the first parameter to be the most determinative. Thus, the seasonal variation stands out the geographical pattern through the

isotope ratios decrease from north to south for D/H from about -45 to -85‰ and for $^{18}O/^{16}O$ ratios from -7 to -12‰ (IAEA, 1983). The geographic origin within Germany was plotted and revealed mean and median values of the D/H ratios in north German samples of -36‰; south German samples slightly lower between mean -56‰ and median -55‰, being in good agreement with the expected north-south difference caused by the isotopic fractionation called the "continental effect". The $^{18}O/^{16}O$ ratios were almost the same with the mean and the median of northern samples differ slightly (northern -3.7‰ and -4.1‰; southern –5.9‰ and -6.3‰). Distinction between conventional and organic farming could be obtained through the $^{13}C/^{12}C$ ratios of samples (21) from conventional farming compared to samples (223) from so-called Bio-meat.

The data combination of the $^{15}N/^{14}N$ and (between 5–6‰ and 5–7‰, respectively) isotopes from northern German farms allowed a direct differentiation between three farms out of eleven by their low $^{34}S/^{32}S$ and high $^{15}N/^{14}N$ values. In spite of the small overlapping, $^{34}S/^{32}S$ ratios above 8.5‰ were satisfactorily correlated with Argentinean origin beef-samples, while $^{34}S/^{32}S$ ratios below 4.8‰ were certainly of German origin. Additional differentiation is provided from the $^{15}N/^{14}N$ ratios data (Argentinean and Chilean sample-values ranging above 5.9‰ and 15.2‰, respectively, and 75% of the German ones below 6.1‰) and the $^{18}O/^{16}O$ data. Therefore, a multi-elemental analysis seems to be obligatory to improve reliability of authentication (Hegerding et al., 2002).

Nakashita et al. (2008) examined the isotopic signatures (C, N and O) of beef samples from Australia, Japan, and USA in order to declare the geographical origin of that commercially distributed in Japan. Thus, the carbon isotopic composition of beef defatted dry matter followed

the order; USA, Japan and Australia according the following ranges: −13.6‰ to −11.1‰, −19.6‰ to −17.0‰ and −23.6‰ to −18.7‰, respectively. Controversially, the oxygen isotopic composition found: +15.0‰ to +19.4‰, +7.3‰ to +13.6‰ and +9.5‰ to +11.7‰ for Australia, Japan and USA, respectively. Finally, the nitrogen isotopic composition values from Japan found slightly higher (+7.2‰ to +8.1‰) than that from USA (+5.1‰ to +7.8‰) and Australia (+5.7‰ to +9.3‰); not permitting an instant use except the similarity reflectance in the diet δ15N values among these countries basically as a result of cattle diet (deNiro & Epstein, 1978, 1981). Thus, the first two parameters appear more powerful towards, not only, a provenance but also in a region discrimination possibility.

In the same year Heaton et al. (2008) analyzed a vast number (>200) of beef samples of worldwide origin (Europe, USA, South America, Australia and New Zealand) using both the IRMS and ICP-MS techniques. The C and N and H and O isotopic composition of the specimens were determined in defatted dry mass and the corresponding lipid fractions. Additionally, the type of pasture feeding was reflected on the ^{13}C content offering a prior discrimination between Brazil and the USA and British cattles (C_4 and C_3 pasturage and fodder, respectively). The mean δ^2H‰ and $\delta^{18}O$‰ values of beef lipid was satisfactory correlated with the latitude of production regions and went with the Meteoric Water Line supporting the hypothesis that the systematic global variations in the ^2H and ^{18}O content of precipitation are transferred through drinking water and feed into beef lipid. Canonical discriminant analysis was selected for the best result on the basis of the broad geographical areas (Europe, South America and Australasia) through six key variables ($\delta^{13}C$‰ (defatted dry mass) and Sr (function 1) and Fe, δ^2H‰ (lipid), Rb and Se (function 2)).

Bong et al. (2010) attempted the geographic discrimination of American, Mexican, Australian, New Zealand and Korean beefs which that period circulated in the Korean markets based on their carbon, nitrogen and oxygen isotopic compositions of defatted dry samples. Thus, statistically distinct isotopic compositions have been recorded, particularly in $\delta^{18}O‰$ and $\delta^{13}C‰$ values due to the different isotopic percentages in their water and feed. The $\delta^{13}C$ values of Korean beef ranged from -19.10 to -14.21%, while those of New Zealand beef recorded the lowest and most distinctive values being overlapped only with those of Australian beefs-which covered the entire range of the analysis. The samples from the USA and Mexico revealed the highest $\delta^{13}C$ values. Finally, the ^{18}O isotopes were specifically high in Mexican and Australian beef in antithesis to those originated from Korean, USA and New Zealand.

Franke et al. (2007) measured the concentrations of a total of seventy two different elements with inductively coupled plasma high resolution mass spectrometry (ICP-HRMS) in order to determine the geographic origin of dried beef. The samples were prepared from *M. biceps femoris* and *M. semitendinosus* produced in Switzerland, Austria, Australia, United States, and Canada out of raw meat originating either from these or from other countries. Sixty-six out of 72 elements/isotopes (all except ^{9}Be, ^{149}Sm, ^{165}Ho, ^{172}Yb, ^{202}Hg, and $^{20}9Bi$) were found in the samples, while only fifteen were statistically significant in differing between-country raw meat and sixteen according to site of processing. The group included the following eighteen elements: B, Ca, Cd, Cu, Dy, Eu, Ga, Li, Ni, Pd, Rb, Sr, Te, Tl, Tm, V, Yb, and Zn. The highest concentrations of the elements among the countries were attributed to: Australia in Ca, Cd, Ga, Pd and Tl; Switzerland in Ca, Cu and Ni; USA in Ca, Cd, Ga and Pd; Austria in Cu and Sr;

Canada and Brazil in Ni and Rb. The lower concentrations were those of Zinc (Zn) in Australian and US samples comparing to those of other origins.

The analysis of stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) with IRMS was also employed as an evaluation method for feeding and geographical origin authentication in fractions of lamb meat (Piasentier, Valusso, Caminand Versini, 2003). The study included meat from twelve lamb types, produced in couples in six European countries (country of origin) and divided in three groups according to the feeding regime during their finishing period: suckled milk only, pasture without any solid supplementation and supplementation containing maize grain (feeding regime). The representative muscle samples were analysed in duplicate and displayed significant differences in $\delta^{13}C$ values with higher being in protein than in fat (average difference 5.0‰). However, the pairs $\delta^{13}C$ values of crude fat and protein were highly correlated (r=0.976) and mainly depended on the lamb type thus reflecting animals' feeding regime. The $\delta^{15}N$ values of meat protein fraction revealed significant differences between lamb types but were not correlated to the feeding regime. In fact, lambs fed on similar diets, but in different countries, gave meat with different ^{15}N relative abundances and provided the discrimination of lamb types within the same feeding regime.

The geographic origin of poultry breast meat was studied based on the concentration results of a total of 72 different elements in parallel with gross chemical composition (GCC) as they obtained with ICP-HRMS (Franke et al., 2007). The twenty-five poultry breast filets samples originated from Switzerland, France, Germany, Hungary, Brazil, and Thailand. Only forty-six of the elements and isotopes were detected in poultry, while statistically significant were only the most abundant isotopes per element. Thus, for poultry meat the differentiation of the origin was

possible using those elements, which were significantly different across countries (As, Na, Rb, and Tl); revealing the inadequacy of GCC to authenticate the origin of this meat type. A multi-element (signature, fingerprinting) approach proved to be more promising, since single characteristic elements triggered doubts about the discriminative power of their concentration for an entire country since the correspondingly enriched soils are present in a relatively small production region.

The addition of the oxygen isotope ratio to above mentioned seventy two trace elements contents of a great number of poultry breast (78) and dried beef (74) samples was applied toward the improvement of the accuracy of the prediction of the geographic origin in conjunction with combining promising methods (Franke et al., 2008). Any element content either varying with time or being below detection limit was excluded from the followed statistical analysis. A good classification rate of 83% (using the ^{82}Se, ^{85}Rb, ^{88}Sr, ^{155}Gd, ^{205}Tl, ^{23}Na, ^{51}V, and δ^{18}O amounts) resulted from a cross-validation matrix based on ANOVA (Analysis of variance) and LDA (linear discriminant analysis). The validation of the method, as examined through the selected ^{75}As, ^{82}Se, ^{85}Rb, ^{88}Sr, ^{95}Mo, ^{142}Nd, ^{205}Tl, ^{23}Na, ^{44}Ca, ^{51}V and δ^{18}O contents, found 50%. The respective values of dried beef samples found were 73% and 43%, thereby underlining the need of more work.

The remarkable benefits of nuclear magnetic resonance (NMR) as a non-destructive technique, little sample preparation time and rapidity were successfully utilized by Shintu et al. (2007) through the ^{1}D ^{1}H HR-MAS NMR spectroscopy for the selection of potential molecular markers of one specific quality, geographic origin. This target was earlier confirmed for beef through characterization of the diet by analyzing fatty acid contents (Renou et al., 2004). Thus,

dried beef samples of certified origin were tested giving interesting results on a limited sample set confirmed the good aptitude of this method for rapid food analysis. Fat content as well as specific metabolites, probably linked to feeding system, proved to be good candidates for markers of origin. The content of fatty acids was found to be higher in American and Australian samples, while the concentration of several small metabolites, such as proline, phenylalanine, glutamic acid and/or glutamine, were relatively more concentrated in the Canadian and Brazilian samples. The Swiss sample displayed a discriminatorily high content of phenylalanine, and a relatively low amount of alanine and/or methionine. A lower content of carnitine and a higher content of succinate and/or an unknown compound (both resonating at 2.40–2.45 ppm) discriminated between North American samples and Australian and Brazilian ones.

Sakazaki et al. (2007) developed six DNA markers for the discrimination between Japanese and Australian beef. Two Bos indicus-specific markers SRY (803bp) and ND5 (527bp); and MC1R (219/218bp) marker were used as possible candidate markers, while even more markers were developed afterwards with AFLP (BIMA100-465bp; BIMA118-153/148bp; and BIMA119-78/76bp). The 1564 primer combinations provided three markers converted into single nucleotide polymorphisms markers for high-throughput genotyping. In these markers, the allele frequencies in cattle from both countries were investigated for discrimination ability using PCR-RFLP. The probability of identifying Australian beef was 0.933 and probability of misjudgement was 0.017 using the above mentioned selected markers. The discriminating power of these markers could be extended against beef samples of other countries.

Sacco et al. (2005) examined twenty-five lamb meat samples from three areas located in Apulia (Southern Italy) through the analysis of moisture, ash, fat and protein content, stable

isotope ratios ($^{15}N/^{14}N$ and $^{13}C/^{12}C$), major elements (Ca, Mg, Na, K) and trace metals (Zn, Cu, Fe, Cr). Besides ^{1}H high resolution magic angle spinning (HR-MAS) NMR spectra has been employed in total offering its significant advantages; the ease of sample preparation and the analysis of different classes of compounds (amino acids, fatty acids, sugars, etc.) in a single experiment. The same signals were present but with different intensities in all the meat spectra. Thus, 100% classification capacity and 96% prediction ability resulted from stable isotope ratios and NMR data for lamb types according to their origin gave.

Camin et al. (2007) have implemented multi-element (H, C, N, S) stable isotope ratio analysis towards the geographical assignment of lamb meat from several European regions. The defatted dry matter (crude protein fraction) from lamb meat was found to be a suitable probe for "light" element stable isotope ratio analysis because of the significant differences between the corresponding ratios from those regions. The mean hydrogen isotopic ratios of the defatted dry matter from lamb were found to be significantly correlated with the mean hydrogen isotopic ratios of precipitation and groundwater in the production regions. Thus, the highest deuterium content for lamb was obtained for Greek lamb (Chalkidiki), with a mean value of −80‰, which is in good agreement with the high deuterium content measured for eastern Mediterranean precipitation and ground waters. Similar values were reported for the other Greek region (Lakonia). The mean values ratios from UK-Sicily, Central Europe and Alpine Mountains were found as follows; -80 – (-90)‰, -90 – (-100)‰ and -100 – (-110)‰, correspondingly. Carbon and nitrogen isotopic ratios were affected by feeding practices and climate. Thus, the nitrogen isotopic values of the lamb defatted dry matter exhibit a relatively small amount of variation between different European regions (range is from +3.8 to +9.2‰). The north-western European

regions of the UK and Ireland have relatively high δ^{15}N‰ values from +7.4 to +9.2‰. The other regions have given relatively homogeneous results for δ^{15}N ‰ (from +5 to +6‰), with the results for the mountain area of Trentino being significantly (p=0.001) lower than for the other regions (+3.8‰), and the data for Greece (Chalkidiki) being slightly higher (+6.5‰). Sulfur isotopic ratios were substantially affected by geographical location and surface geology of the production region along Europe, with δ^{34}S‰ values ranging from +1.6 up to +12.8‰.

Selenium (Se) is an essential nutrient with providing numerous human health benefits and the single most important dietary source of Se is beef. Thus, Se content of beef varies and is potentially a unique supplemental source of dietary Se (Hintze et al., 2002). The Se content of one hundred and thirty-eight cull cows-determined from 21 ranches- in five distinct geographic regions of North Dakota has been studied on the basis of soil parent material, reports of Se deficiency, and previous soil and forage Se surveys (Hintze et al., 2001). Selenium concentrations of all samples were determined with hydride generation atomic absorption spectroscopy. The Se content of all samples was clearly affected ($p < 0.05$) by the geographic origin, whereas any extra Se intake in mineral supplements poorly affected the concentrations of Se in sampled tissues ($p > 0.05$). Therefore, the geographic origin of the animals was a more important determinant for the Se concentration of beef than the presence or absence of supplemental Se. Geographical area resulted in a range of Se concentrations from 0.27 to 0.67 μg of Se/g, whereas inclusion of supplemental Se enhanced the mean Se concentrations only from 0.41 μg/g (no supplemental Se) to 0.46 μg of Se/g ($p > 0.07$).

The geographical origin determination of beef and lamb products with isotopic ratio methods is shown in Table 2 and in Figure 1, the geographical origin determination of meat in UK and Ireland, Central Europe and Central America is presented.

MULTIVARIATE ANALYSIS

The naturally high complexity of the majority of food products is basically due to the vast number of chemical substances they consist of in complex physical structures. Thus, a spherical opinion about the quality of a food product, as perceived by humans, is obviously a result of the most detectable of these properties. The need to analyse, understand and improve a food product is closely linked to powerful instrument action capable of determining a variety of properties and data analysis techniques that can handle the large and complex data matrices produced (Næs et al., 1996).

Piasentier et al. (2003) used canonical discriminant analysis to evaluate whether lamb meat from different CO (country of origin) or FR (feeding regime) or COxFR interaction could be mathematically distinguished by its stable isotope ratios. On the basis of CO, the corrected empirical allocation of 79.2% of the initial observations and the corrected cross-validation of two thirds of the individual meat samples was obtained. FR gave better results: 91.7% of the individual meat samples ware both correctly allocated and cross-validated, indicating the high potential of stable isotope ratio analysis as a tool for lamb diet characterisation. The most satisfactory classification was reached by using K-means clustering technique and canonical discriminant analysis, enabling a good resolution of six COxFR groups of lamb types: Icelandic

fed on pasture; British and French grazing; Italian; suckled and Karagouniko concentrates finished; French Lacaune; Ternasco de Aragon. In other words, a multi-element stable isotope analysis may be considered promising for a trustworthy authentication of lamb meat as, in the same way, was proved for wine, fruit juice, honey and dairy products.

The application of MV analysis to two data sets containing tissue composition results together with the metals contents and ^{1}H HR-MAS NMR spectral data together with isotope ratios, respectively, provided in both cases a satisfactory origin differentiation of lamb meat samples Sacco et al. (2005).

The use of stable isotope data on the hydrogen, carbon, nitrogen, and sulphur coming from lamb meat from different European regions in parallel with PCA and linear discriminant analysis (LDA) has been proved a powerful tool towards their geographic origin (Camin et al., 2007). Thus, PCA applied to the European lamb protein isotope data (δ^{2}H‰, δ^{13}C‰, δ^{15}N‰ and δ^{34}S‰) led to interesting differentiations among the regions. The relative plot of the first two principal components for samples of the year 2005 revealed the differentiation of the north-western regions of Orkney, Ireland and Cornwall from Greece, from the Mediterranean areas of Sicily, Toscana, Carpentras, and from the Alpine sites of Trentino and Allgäu with Mühlviertel. Moreover, LDA based on the same data revealed its ability in separating the lamb products from Bavaria (Germany), France, Greece, Ireland, and the UK. Following double checking, 78.7% of the original grouped cases, 77.6% of the cross-validated grouped cases, and 100% of the Carpentras, Cornish and Muhlviertel lamb samples were correctly classified. Significant classification errors (Limousin vs Carpentras, 50% France; Orkney vs Cornish, 35% UK) were attributed to barn and feeding practices. The obtained poor results were produced in the 40% of

Franconian samples (Bavaria) they characterized as being from Limousin (10%), Allgäu (5%), Trentino (10%) and Sicily (15%). The remaining regions were all classified with success rates of 80% or more, except for Ireland where the success percentage amounted to 69.2%.

Al-Jowder et al. (2002) applied chemometric methods (PCA, PLS and linear discriminant analysis) to MID-IR spectra. The possibility of reporting discrimination between the pure and adulterated sample types, hardly depended on the cooking regime. The data have been resulted on pure beef and beef containing 20% w/w of a range of potential adulterants (heart, tripe, kidney, and liver). The acquired spectra of the two different cooking regimes (8 min at 240 W or 400 W-'level 1' and 'level 2', respectively) gave a cross-validated classification success rate of ~97%. The application of PCA revealed the need of at least two dimensions to reveal a distinct group corresponding to a particular sample type. The spectra obtained from the cooked samples were visually quite different from those obtained from raw meats, reflecting the compositional changes that occur upon cooking. Thus, since the most notable effect of cooking is dehydration, the loss of the water peak was clear at 1650 cm^{-1}, and relatively more prominent fat (1725 cm^{-1}) and protein (1650 and 1550 cm^{-1}) peaks. However, a significant reduction in spectra quality relative to the cooking level was reported.

Investigations into the classification of meat samples as either fresh or frozen-then-thawed have been performed using NIRS and two chemometric techniques: factorial discriminant analysis and SIMCA. Work was performed on meat pieces using a fibre optic probe for spectral acquisition. A sample of meat (*M. longissimus dorsi*) from each of 32 animals of a single species (female Hereford-cross) was collected from a commercial abattoir. Using a standard freeze-thaw regime, results reported show a significant rate of discrimination between fresh and frozen-then-

thawed beef for the factorial discriminant procedure; SIMCA was less successful (Downey and Beauchêne, 1997).

A great number of minced beef (32), lamb (33) and lamb-in-beef mixtures (5%, 10% and 20% (w/w), 33 each) samples were examined for their reflectance in the visible, near and MIR spectral regions. The percentage of lamb content was predicted by means of PLS models using each spectral region alone and combinations of all three. The most promising results derived from those models applied to MIR (800-2000 cm^{-1}) and near IR (1100-2498 nm) spectral data following 2nd derivatization (McElhinney et al., 1999).

The spectra data obtained from beef and ox kidney and liver with MIR spectroscopy have also been used for authentication purposes by Al-Jowder et al. (1999). The successful distinction of specimens was based on development of a combined technique of PLS/CVA, predictive models. Although beef and offal types could be readily distinguished, the distinction among the three cuts of beef proved much less clear. The neck specimens were included in a distinct group, but the silverside and brisket groups were partly separated because of an inherent overlap between these two specimen types. No effect has been attributed to the specimen condition (fresh or frozen-thawed), as earlier reported by Downey et al. (1997) using FDA (factorial discriminant analysis) on Vis/NIR date of beef samples. The used SIMCA class modelling gave a single class model for the pure beef specimens with a type I error rate of ~8%. When this mode was challenged by beef specimens adulterated with quantities in the range of 10-100% w/w of kidney or liver, all of these specimens were rejected by the model suggesting a detection limit for these adulterants of <10% w/w. The use of PLS regression towards the quantification of the amount of added kidney and liver, obtained SEPs commensurate with those suggested with SIMCA

analysis. PLS in conjunction with canonical variate analysis, predictive models have, in parallel, led to satisfactory spectra identification of un-adulterated beef as such, with an acceptable error rate, while rejecting spectra of specimens containing 10-100% w/w kidney or liver. Comparatively good results were revealed when PLS regressions performed towards the quantification of added offal.

Ding and Xu (1999) managed to differentiate of beef from kangaroo meat through the development of a canonical discriminant analysis (CDA) - PCA performance- and stepwise multiple linear regression (MLR) based on visible/near-infrared (NIR) spectroscopic data. The spectral data were first pretreated with standard normal variance and detrend (SNVD) and/or second derivative operation, with gap and smooth both set at 8 nm. For minced meat proved necessary the scatter correction and the derivative treatment of reflectance spectra for a better classification. On the contrary, cut meat could easy be classified from the original reflectance spectra. Thus, the overall classification accuracy vaaried from 83% to 100%, while none of the kangaroo samples was misclassified.

Meat originating from broilers and Chinese local chickens could be differentiated with a near-infrared spectroscopic method in conjunction with a dummy regression technique (Ding, et al., 1999). Thereby satisfactory classification accuracies were achieved for minced thigh meat, minced breast meat, breast cut without skin and breast cut with skin (100%, 92%, 96% and 92%, respectively). Whereas the application of the regression models of MLR, PCR, PLS and MPLS did not show any obvious differences in classification accuracy, the scatter correction and derivative treatment of the spectral data before discriminant analysis improved it for minced meat, while for meat cuts, spectra without pretreatment produced better classification. This target

was also reached with physical and chemical properties of meat samples, which revealed significant differences in collagen and fat contents and pH and chromatic values between the two groups of chickens.

Sante et al. (1996) investigated the relevant objective measurements which for visual assessment of turkey meat colour and the possibility to use them for the early prediction of the colour development of turkey breast meat. These parameters included meat temperature, dielectric loss factor, pH, pigment concentration, L* (lightness), a* (red-ness) and b* (yellowness) colour coordinates determined at different times *post mortem*. The last two obtained the relevant variables when measured at 1 and 4 hr post mortem and were used in prediction models. Thus, application of linear analysis (canonical discriminant analysis) disclosed that the efficiency of prediction was 15%, while a non-linear analysis (neural network) led to better prediction; the colour of the meat being correctly predicted amounted to 70% of the muscles.

A machine learning strategy in a neural network multilayer perceptron (MLP) manner was used for the correlation of FT-IR spectral data with beef spoilage over aerobic storage at chill and maltreatment temperatures (Argyri et al., 2010). Therefore, fresh beef fillets packaged under aerobic conditions and spoiled at various temperatures-from 0 to 20ºC- for almost two weeks were subjected to FT-IR scattering directly to the surface of meat samples in spite of the total viable counts (TVC) of bacteria. The samples firstly were sensorially categorized into three quality classes; fresh, semi-fresh, and spoiled. All three were part of the scale where beef samples had to be ranked in after FTIR spectra data processing of the biochemical profile, and in parallel to predict the microbial load (as TVC) on meat surface. The results revealed high accuracy and correct classification 91.7% of fresh samples, 94.1% of spoiled samples, and 81.2%

semi-fresh samples; demonstrating good correlation of microbial load on beef surface with spectral data and the potential of the proposed method toward the rapid assessment of meat spoilage.

Arvanitoyannis et al. (2000) conducted physico-chemical and sensory analyses on 48 samples of Cavourmas, a Greek traditional cooked meat product, bought directly from 16 known producers. The following physico-chemical attributes were determined: moisture, crude protein, ether-extractable fat, ash, NaCl, $NaNO_2$, $NaNO_3$, pH, 2-thiobarbituric acid (TBA) value, lightness (L*), redness (a*), and yellowness (b*). Principal component analysis based on three main axes (PC1, PC2 and PC3) accounting for 56.4% of overall variation showed that high consumer acceptability of Cavourmas is related to accepted taste and odour and to the presence of meat in pieces with red colour and white fat. Fatty appearance, excessive molten fat and high rancidity were not appealing to the consumer, as confirmed by instrumentally determined high TBA values, high fat content, high percentage moisture and lightness, and should be kept low in order to enhance product acceptability. Arvanitoyannis and van Houwelingen-Koukaliaroglou (2003) performed MVA as a very powerful and effective tool in classifying and grouping individual products. PCA, anonical analysis, cluster and PLS were found to be indispensable for classifying food products according to variety and/or geographical origin. Meat and meat products were correctly classified for authentication purposes to various groups following instrumental and/or sensory analyses.

Papadima and her colleagues (1999) conducted chemical, physical, microbiological and sensory analyses on 31 samples of Greek traditional sausages. The following points were recorded: fat 15.49–56.86%, moisture 21.92–65.40%, protein 14.73–26.74%, sodium chloride

2.36–4.13%, nitrites 0.0–3.26 ppm, mean nitrates 38.19 ppm, TBA value 0.42–5.33 mg malonaldehyde/kg, pH 4.74–6.74, water activity (a_w) 0.88–0.97, firmness 0–64 Zwick units, lightness (L*) 25.03–35.37, redness (a*) 2.55–11.42, yellowness 4.42–12.96, aerobic plate count 5.48–9.32 cfu/g, lactic acid bacteria (LAB) 5.26–9.08 cfu/g, micrococci/staphylococci 4.11–6.91 cfu/g and Gram (-) bacteria 1.78–6.15 cfu/g. Two statistical analysis programmes (Praxitele and SPSS) were used for characterising and assessing the properties of sausages. The first two principal components (PC1-2) derived by SPSS (50.5% variance) describe more satisfactorily the variance than the corresponding PC1-2, PC1-3 obtained by Praxitele (40.4% variance).

Pappa et al. (2000) used response surface methodology to determine the optimum salt level (1.3–2.1%) and pectin level (0.25–1.0%) when olive oil replaced pork backfat (0–100%) for the production of highly acceptable low-fat frankfurters (9% fat, 13% protein). The test ingredients significantly affected (P<0.05) jelly separation of the batter, skin strength, hardness, saltiness, odour and taste and the overall acceptability of the low-fat frankfurters. Low-fat frankfurters with high salt levels tended to have very hard skins and increased (P<0.05) saltiness while those with a high pectin level were very soft, tasted like cream and had the lowest (P<0.05) score for odour and taste. The low-fat frankfurters with 1.8–2.1% salt, 0–35% olive oil and 0.25–0.45% pectin had the highest overall acceptability. However, low-fat frankfurters produced with 1.3% salt, 0.25–0.30% pectin and 80–100% olive oil were also acceptable. Such low-fat frankfurters, compared to commercial products, have 48% lower salt content (from 2.5 to 1.3%) and 66.6% lower fat content (from 30 to 10%), in which 80–100% of the added fat is olive oil. However, further research is needed to improve the acceptability of these frankfurters.

The main points of MA applications in meat and meat products are summarized in Table 3.

SENSORY ANALYSIS

One of the important, if not the most, sensorial characteristics of food and food products is its external view and more specific color which, concerning meat, is mainly dependent on myoglobin chemistry and pigment redox stability. Moreover, the contribution of animal genetics, *ante*- and *post-mortem* conditions, fundamental muscle chemistry, and many factors related to meat processing, packaging, distribution, storage, display, and final preparation for consumption (Mancini and Hunt, 2005). Rousset-Akrim et al. (1997) thoroughly studied the effect of sheep age and diet on several odours and flavours based on a variety of feed and growth. Moreover, meat flavour is strongly correlated with the aroma volatiles produced over cooking, because uncooked meat has little or no aroma and only a blood-like taste. These could be divided into two groups; water-soluble components and lipids, and related with Maillard reaction between amino acids and reducing sugars, and the thermal degradation of lipid (Mottram, 1998). A vast number of volatile compounds (~1000) were identified in meat and may be even more in the most extensively investigated beef meat. Several methods have been applied for separation, isolation and identification of the volatiles such as NPSD (nitrogen purge and steam distillation), SPME (solid phase micro-extraction), SE (solvent extraction) and SDE (simultaneous distillation extraction), DHS (dynamic headspace) in conjunction with GC/MS (Ai-Nong and Bao-Guo, 2005; Ai-Nong, Bao-Guo, Da-Tingand Wan-Yun, 2008; Sun et al., 2009; Jerković, Mastelicand Targaglia, 2007; Jerković et al., 2010; Bianchi et al., 2007). Furthermore, several techniques, such as VIS-NIR spectroscopy, electronic noses, image processing and RT-PCR among others

(Eklöv et al., 1998; Ortiz-Somovilla et al., 2006; Balamatsia et al., 2007; Otero et al., 2003; Bernard et al., 2007; Gerrard et al., 1996; Berzaghi and Riovanto, 2009) and sensory panels (Bernard et al., 2007; Kriese et al., 2007; O'Sullivan et al., 2003; Balamatsia et al., 2007) have been employed in the sensory analysis of meat and meat products.

Ahn et al. (2000) investigated the production of volatiles of irradiated and non-irradiated pig samples (*Longissimus dorsi* muscle strips) packed either aerobically or under vacuum in bags and stored at 4ºC for five days. Fluorescence TBARS (2-thiobarbituric acid reactive substances) method was employed for the analysis of lipid oxidation, while a purge-and-trap/GC/MS method applied for qualitative and quantitative determination of volatiles components. Although, irradiation slightly affected the production of volatiles related to lipid oxidation, it affected significantly the production of few sulfur-containing compounds absent in non-irradiated samples. The major contributor of off-odors in irradiated meat was not lipid oxidation, but radiolytic breakdown of sulfur-containing amino acids which gave thio-bismethane, 3-methoxy-1-propene, thioacetic acid methyl ester, 2,3-dimethyl trisulfide, toluene, and 2,3-dimethyl disulfide; with the latter accounting for approximately 75% of the total of new volatiles. There was also a considerable decrease if many of the irradiation-dependent volatiles to 50-25% at the end of the fifth day in aerobically storage conditions. Finally, ~70% of sensory panels characterized irradiation odor as barbecued-corn-like odor and the acceptance being unsusceptible from meat irradiation.

The implicit role of volatile amines as indicator(s) of poultry meat (fresh chicken breast fillet) spoilage has been investigated by Balamatsia et al. (2007) through four different package atmospheres: air (A), vacuum (VP) and two modified atmospheres (MAs), namely M1,

30%/65%/5% ($CO_2/N_2/O_2$) and M_2, 65%/30%/5% ($CO_2/N_2/O_2$). The last three proved the most effective against the development of aerobic spoilage microbial flora. The decrease of *Pseudomonas* spp. in the samples stored under M_2 gas mixture and VP was substantial comparing to the rest samples after 15 days of storage. Seven experienced panellists in poultry evaluation had sensorially evaluated the acceptability as a composite of odour, taste and appearance based on a scale ranging from 9 (excellent) to 6 (acceptable). Moreover, with regard to chicken spoilage and freshness, trimethylamine nitrogen (TMA-N) and total volatile basic nitrogen (TVB-N) limit values of acceptability, namely 10.0 mg N/100 g and 40 mg N/100 g for chicken samples stored in air, contributed to the definition of the upper limit 6. All vacuum- and MA-packaged chicken samples were given higher sensory scores ($p < 0.05$) than air-packaged samples; the values of the former were further enhanced after day 3 over the entire period of refrigerated storage.

Bernard et al. (2007) reported new molecular markers related to beef sensory quality produced from the transcriptomes of *Longissimus thoracis* muscle from Charolais bull calves after the analysis with microarrays and comparison between high and low meat quality groups. A total of 215 genes were thereby differentially expressed according to tenderness, juiciness, and/or flavor. A subset of twenty three were correlated (or up-regulated) to the tenderest, juiciest, and tastiest meats, and eighteen were highly correlated with both flavor and juiciness (e.g. PRKAG1), interpretting up to 60% of their variability. Nine were down-regulated in the same samples, but only DNAJA1, which encodes a heat shock protein, revealed a strong negative correlation with tenderness that alone explained 63% of its variability, thus standing for a new marker of beef sensory quality.

The critical and common way of fermentation in sausages manufacturing was examined by Eklöv et al. (1998) who applied a sensor array and pattern recognition routines (electronic nose) to record the variation of the emitted volatile compounds during this process. It consisted of ten metal oxide semi-conductor field-effect transistors (MOFSET) of different gate metal (Pd, Pt, Ir), metal structure and operating temperature and in parallel with principal component regression and an artificial neural network (ANN) gave satisfactory results. Therefore, the detection of a large number of compounds was accomplished and these results were compared with those from a seventeen-member sensory panel analysis, carried out both at the early stage of the process and on the final product (sausages). The sensitivity and the discrimination capability between the two techniques were of the same grade, allowing any difference to be noticed between the batches just after 4 h and by the end of the whole process. This is equal to prediction of the final sensory quality of fermented sausage at the very early of the process.

The alternative method for the taste and flavour analysis of meat products after trained sensory panels, that is the GC-MS, has been applied in the case of aged Iberian hams (Garcia et al., 1991). The distilled volatiles were isolated under vacuum in cool traps and collected with dichloromethane. The GC-MS analysis revealed seventy-seven compounds in the volatile fraction which mainly included alkanes (12), branched alkanes (14), aldehydes (13), and aliphatic alcohols (9). Moreover, small quantities of lactones (5), esters (9) and ketones (7) and other miscellaneous compounds were detected as well. The large amount of olfactory volatiles found in Iberian hams is due to an intense proteolytic and lipolytic breakdown during maturation, which is expected due to the higher temperatures it is aged at and the longer times of procession.

Gerrard et al. (1996) examined steaks of various degrees of marbling and color employing a ten-member sensory panel and image processing simultaneously. These parameters were assigned to each steak with USDA marbling score cards and a lean color guide. Color has been evaluated by trained panel in an eight-point scale from 1 (bleached red) to 8 (very dark red), whereas marbling in a nine-point scale from 1 (devoid) to 9 (moderately abundant). All tests were conducted under the same conditions where image processing successfully predicted the lean color ($R_2 = 0.86$) and marbling scores ($R_2 = 0.84$), therefore confirming its effectiveness as a potential tool towards the determination of USDA quality attributes of fresh meat.

The role of irradiation in refrigerated and frozen chicken relative to sensory properties was extensively examined on skinless boneless breasts (white) and leg quarters (dark) by Hashim et al. (1995). The appearance of moistness and glossiness of raw chicken (white or dark) was not affected by irradiation, as well as the higher "fresh chickeny", bloody, and sweet aromatic aroma intensities compared to non-irradiated samples. Moreover, irradiation had no effect on frozen or re-refrigerated or frozen sensory characteristics of cooked white meat. Irradiation did affect only two sensory characteristics of the dark meat investigated in the cooked state, namely frozen and refrigerated states which displayed more chickeny flavour and tenderness ($p < 0.05$) than corresponding controls.

Besides the applications of NIR technique towards the quantitative assessment of the major beef components (i.e. protein, fat and water) and the heat treatment (Ellekjær & Isaksson, 1992), Hildrum et al. (1994) investigated the possible changes of beef muscles in NIR spectra during conditioning and ageing and correlation to sensory parameters. Using the reflection (NIRR) and transmission (NIRT) modes of analysis in the period of two weeks of aging (at 20ºC) significant

conclusions were derived about the sensory hardness, tenderness and juiciness of *M. longissimus dorsi* muscles. Thus, NIRR in conjunction with principal component regression satisfactorily predicted only the first two sensory variables thereby resulting in coefficients in the range 0.80-0.90. Furthermore, good predictions resulted from NIRR measurements of frozen and thawed samples. On the contrary, all variables were not sufficiently predicted with the NIRT mode.

One of most important sensory characteristics of meat is tenderness, which is mainly affected by *rigor mortis* phenomenon and proteolytic reactions, both of which take place in the refrigeration phase of the carcass. Kriese et al. (2007) proposed that broiler breast fillet tenderness could be further improved and it is matter of whether or not meat was excised from the carcass. Evaluation of tenderness was conducted with myofibrillar fragmentation index determination, shear force analysis and sensorial testing on post-harvest samples (from 0 to 72 h after slaughtering at 2 ± 2ºC). The most acceptable and tender intact samples were those of the 24 h (30.6% and 41.7% more excised and control samples, respectively), as it was also confirmed from the sensory test, demonstrating that a minimum refrigeration time (24h at 2±2 ºC) was necessary step to obtain very tender meat.

The use of VIS/NIRS in 400–1080 nm region has been employed by Liu et al. (2003b) for the prediction of color, instrumental texture, and sensory attributes of beef steaks in a certain day post-mortem. Dimensionally random measurements all over the samples of the Hunter L (lightness), α (redness), b (yellowness) and the indirect fraction E* (redness relative to those of yellow-ness and lightness) resulting from the modified equation of Liu et al. (2003b ms): E*= α/b + α/L, yielded the prediction coefficients of determination (R_2) in calibration between 0.78 and 0.90 (Hunter α, b, and E*), and 0.49 and 0.55 (tenderness, Hunter L*, sensory chewiness and

juiciness). Divergence in the prediction R2 for tenderness have been noticed when an ageing partition of the samples applied. The implication of the PLS and soft independent modeling of class analogy of PCA (SIMCA/PCA) models on tenderness successfully classified the samples into tender and tough (83% and 96%, respectively). Decrease was reported in Bratzler shear force, sensory chewiness and E* (possible loss of meat redness) with time (Hildrum et al., 1995; Liu et al. 2003a).

Reflectance and transmittance spectroscopy has also been used for quantification of the cooking process on intact chicken patties in terms of both time-temperature integrated indices (C and F) and endpoint temperature (T_{max}) (Chen and Marks, 2006). Calibrations for the above-mentioned indices resulted from reflectance overcame those of transmittance in the employed wavelength ranges (400 to 700 nm, 1100 to 2500 nm, and 400 to 2500 nm), with visible/near-infrared being the most accurate.

The differences in colour, texture and yield between vacuum-packaged cooked sausages -treated at 500 MPa for 5 or 15 min at 65ºC-and those prepared with conventional heat pasteurisation (80–85ºC for 40 min) were examined by Mor-Mur and Yuste (2003). Therefore, pressurized sausages were shown to be more cohesive and less firm, and resulted in higher yield than the heated ones. The work of the sensory panel was not always easy since, on several occasions, they could not detect the differences between both types of sausages. Moreover, even if there were differences, they were rather due to appearance (lesser gelatin on the surface), taste (stronger and more pleasant, especially in 15min pressurised sausages) and, especially, texture (juicier, less grainy and more uniformly consistent).

Ai-Nong et al. (2005) studied the volatiles from Chinese traditional smoke-cured bacon (CSCB) trapred by condensing and dissolving in organic solvent (ether and n-pentane) with the NPSD technique. Afterwards, this extract was both qualitatively and quantitatively characterized by means of GC-MS and gas chromatography (GC-FID) in conjunction with a flame ionization detector, respectively. Thus, twenty seven new components-among them and four phenols (o-tertbutylphenol, butyl hydroxy toluene, 2,3,5-trimethoxytoluene and 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl]-phenol)-were identified, thereby confirming the excellence of NPSD method in carrying the aroma ingredients and preventing the oxidation of the cellular components. The most important result was the phenolic-derivative affinity of the volatiles, which by far differentiated from fresh pork and Jinhua ham were strongly related to smoking during manufacturing.

In a more recent report (Ai-Nong et al., 2008) on the same type of bacon (CSCB) the volatile compounds were examined by means of SPMS-GC/MS. Thus, a total of forty eight volatile components were identified and quantified, which belonged to several chemical classes: 1 alkane, 16 aldehydes, 5 ketones, 9 alcohols, 4 thioethers and thiols, 3 furans and 10 phenols compounds. The majority of volatiles-apart from alkane-contributed to the CSCB aroma and originated from smoking, oxidation and Maillard reaction, etc. The increase in number of the volatile substances was mainly due to the isolation methodology, which now employed fibers coating such as CAR/PDMS (carboxen/polydimethylsiloxane; with the highest area counts for most volatile compounds) and DVB/CAR/PDMS (divinylbenzene/ carboxen/ polydimethylsiloxane) giving better extraction to those compounds with low and high linear retention indices (LRI), respectively.

Three ways of volatile isolation, whereas SE, SDE and NPSD, in conjunction with GC and GC–MS firstly have been used by Jerković et al. (2007) towards the autochthonous Dalmatian prosciutto. Thus, forty six and eighty one compounds were obtained by SDE and SE (including fatty acids, aldehydes, phenols, esters, ketones and others) and NPSD (headspace volatiles including phenols, aldehydes, hydrocarbons, ketones, alcohols, esters and heterocyclic compounds), respectively. Significant was the increase in the amounts of aldehydes and esters during the ripening time mainly due to the dry curing. Fried and raw samples were quantitatively differentiated with respect to aldehydes (SDE and SE), while NPSD method provided more information about fried ham volatiles since the identification of both pyrazines and most of the lower aldehydes was only thus possible.

The authentication of two typical Italian dry-sausages, namely “Salame Mantovano” (7) and “Salame Cremonese” (5), in terms of their volatile composition was investigated by Bianchi et al. (2007). The dynamic headspace extraction technique (DHS) coupled with gas chromatography–mass spectrometry (GC–MS) gave mainly among the one hundred and four volatiles identified, terpenes, aldehydes, ketones and alcohols. Peak area data for all the substances from this group in conjunction with, on the one hand, the PCA (visualization of data trends and detection of possible clusters within samples) and, on the other hand, the linear discriminant analysis (LDA) (detection of the volatile compounds able to differentiate the two kinds of sausages) worked satisfactorily. The most important contributors to the differentiation of the two kinds of salami proved were volatile compounds, i.e. 3-methylbutanal, 6-camphenol, dimethyl disulfide, 1-propene-3,3’-thiobis, ethyl propanoate, 1,4-p-menthadiene and 2,6-

dimethyl-1,3,5,7-octatetraene, and prediction ability at 100% by the "leave-one-out" cross-validation.

Jerković et al. (2010) isolated and identified 119 organic compounds from famous Slavonian salami "kulen" with NPSD in conjunction with gas chromatography and mass spectrometry (GC and GC–MS). The majority of the volatiles originating from lipid oxidation, amino acid degradation, smoke treatment and added spices showed different distribution among NPSD traps with little or no interference from abundant lipid constituents in the samples. They mainly included methylphenols, methoxyphenols, organosulphur compounds (diallyl sulphide, diallyl disulphide, methylallyl disulphide, diallyl trisulphide and methional) and several derivatives of 2-cyclopenten-1-one such as ethyl cyclotene. Conclusively: (1) the lower amount of terpenes (only α-pinene, δ-3-carene, limonene and β-ionone) detected in "kulen" volatiles was in contrast with those identified in different European salamis; (2) the high percentages of methoxyphenols and methyl-phenols as well as 2-cyclopenten-1-one derivatives (such as ethyl cyclotene) could be attributed to smoking, in view of its rare use in many European salamis; (3) the diallyl disulphide compounds possessed higher percentages than other organosulphur ones; and (4) the NPSD isolation may be responsible for the minute presence of low-molecular lipid oxidation carbonyl compounds and alcohols (lower than C7).

The warmed-over flavour (WOF) of pork samples (*M. longissimus dorsi* and *M. psoas*) following four different diets was sensorially evaluated after refrigeration at 4ºC for up to 5 days in parallel with gas chromatography mass spectrometry (GC/MS) and Electronic nose analysis (O'Sullivan et al., 2003). The results clearly confirmed that iron supplementation enhanced the susceptibility to WOF development for both *M. longissimus dorsi* and *M. psoas* major compared

with the other dietary treatments (O'Sullivan, et al., 2002). The *M. psoas* major muscle produced greater WOF compared with *M. longissimus dorsi* and iron supplementation of animal diets resulted in greater WOF than any of the other dietary treatment. Thus, this variability in WOF among samples provided a good model for subsequent GC/MS and electronic nose analysis. The majority of the compounds correlated to lipids oxidation was responsible for the oxidative sensory to the panel and specially those with the greater levels of WOF development, i.e. pentanal, 2-pentylfuran, octanal, nonanal, 1-octen-3-ol and hexanal. The electronic nose device in conjunction with the MAV (APLSR) offered an unambiguous separation on the basis of muscle type, applied treatment and degree of WOF development.

A semiconductor multisensorial system constructed with tin oxide layers-pure or enriched with amounts of Pt, Pd and TiO_2 has been implicated in the quality evaluation of dry-cured Iberian hams with good responses obtained from the 12 elemetns forming the multi-sensor at different operating temperatures (Otero et al., 2003). The electrical resistance of each sensor was measured in air (R_a) and in the sample (R_s) to evaluate the sample sensitivity (S) of each sensor defined as: $R(\%) = \frac{(R_a - R_S)}{R_a} \times 100$.

Discrimination between the two types of ham was satisfactorily carried out through PCA, while the sensitivity to ham aroma gave best results at elevated temperature, with 250ºC being the temperature of maximum response. Moreover, the thinnest sensor revealed maximal response at 150 and 200ºC, with totally minor differences and best sensitivities and discriminations when functioning at 250ºC. The dopant incorporation rarely improved the sensitivity to ham aroma.

The second most wide consumed dry-cured sausage in Spain after chorizo is salchichón, a product manufactured from a mixture of chopped meat (pork, beef/pork or beef), lard, salt, additives (nitrate, nitrite, antioxidants), starter cultures (optional) and spices. A highly trained and descriptive sensory panel worked on this subject for recording and editing its main sensory characteristics with descriptive and discriminative power (Ruiz Pérez-Cacho et al., 2005). The initial 108-term vocabulary was reduced down to a 15-attribute total about appearance, odour, texture and flavour. Employment of Kruskal-Wallis test revealed the significant difference of all attributes among samples ($p < 0.001$) leading to in three groups of salchichónes; group I (with strong other spices smell and aroma and high juiciness), group II (with high mould smell and aroma), and group III (with acid and salty taste).

A summary of sensory analysis surveys is presented in Table 4.

GENETICALLY MODIFIED ORGANISMS (GMO)

Over the last 30 years, there has been an impressive and stable increase of GM crops. The main GM crops are corn, soybean and oilseed rape (canola). Over the last years, soybean proteins are added in the processed meat-based products (Belloque et al., 2002; Cardarelli et al., 2005).

Processed meat-based foodstuffs have high fat content but their content in meat proteins is low. Meat proteins as food emulsifiers do not allow the coalescence of fat during heating process. In case the meat protein content is not high then another kind of protein such as soybean protein is added to prevent the coalescence of fat (Belloque et al., 2002). Soybean proteins were

determined with two different methods; direct and indirect methods. Direct methods focused on the detection of soybean protein whereas indirect methods on the determination of substances or compounds related to soybean proteins (Olsman, 1979).

Rencova and Tremlova (2009) employed indirect competitive ELISA method to detect soybean protein in meat products. The detection limit of ELISA was 0.5% of the added soybean protein weight. A total of 131 meat products were collected and analysed for the presence of soybean protein. The results confirmed that a percentage of 84% of samples was positive in the presence of soybean protein.

DNA-based techniques are used for the determination of small amounts of DNA in foods. PCR technique was tested in a variety of foodstuffs, such as meat and blended products (Meyer et al., 1996). PCR assay was used to detect trace amounts of soybean in meat sausage by Soares et al. (2010). The PCR protocol aimed at detecting the soybean lectin gene when a percentage of 0.1% and 0.5% of hydrated textured protein were added in the meat sausage. These percentages meant that 0.01% and 0.06% (w/w) of soybean protein, respectively, were added to raw and heat-processed pork meat. In this survey, 18 samples were detected and resulted that nine of them contained soybean protein and four of them contained soybean protein without labelled in formation for soybean in the package.

Treml and Maisonnave-Arisi (2008) detected 47 samples of processed meat products for determination of soybean proteins using DNA-based technique. Soy-specific primers LEC1/LEC2 intensified a 164 bp fragment of the lectin gene. The positive samples were further tested by nested PCR using primers GMO5/GMO9 and GMO7/GMO8 intensified a 447 bp and 169 bp fragments, respectively. The lectin fragment was not found in 5 of 47 samples. The 42

samples were positive for presence of soybean protein. PCR revealed that 6 meat samples contained roundup ready soybean.

Immunochemical methods are used for the detection of soybean proteins in processed meat-based foodstuffs and present high specificity and sensitivity. The AOAC International granted an ELISA as the AOAC official method for determination of soybean proteins in raw and heat processed meat products (AOAC, 1998).

Based on the above mentioned method, Castro-Rubio and her colleagues (2005) developed a simpler and cheaper method for the determination of soybean proteins in the meat products. According to the method, the samples of meat are defatted with acetone and the soybean proteins are solubilized in a 30mM Tris-HCl buffer containing 0.5% (v/v) 2-mercaptoethanol. In the chromatograph, two peaks from soybean proteins were determined. This method seemed to be specific, precise, exact, forceful and sensitive, thereby making possible the detection and the quantitation of additions of 0.07% (w/w) and 0.25% (w/w), respectively of soybean proteins in meat products (related to 1g of initial product).

Criado et al. (2005) applied perfusion liquid chromatography to identify the soybean proteins in cured meat products. As the previous study, the samples were collected and defatted with acetone and soybean proteins were solubilized with a buffer solution at basic pH. The use of water-aceto-nitrile-trifluoroacetic acid and water-tetrahydrofuran-trifluoroacetic acid linear binary gradients at a flow rate of 3 mL/min at a temperature of 50ºC and use of UV at 280 nm allowed the chromatographic analysis of soybean proteins in cured meat products in less than 3 min.

CONCLUSIONS

Over the last years, legislation related to labelling to avoid the adulteration of foodstuffs was entry into force. Although numerous surveys were published in regards to foodstuffs authenticity and great efforts were made toward improving quality and authenticity control including the evaluation of the final foodstuff, since each analytical method has its limitations which restrict its applicability.

However, there has been an increasing research into the development of new applications for existing analytical and chemometric techniques for food authentication. Moreover, it must be stated that the majority of new applications occurred within the areas of novel technologies such as DNA-based techniques, SNIF-NMR and IRMS. The DNA-based techniques are very promising and have potential to be applied in determining the authenticity of animal origin samples, such as species determination in meat products. Nowadays, DNA-based techniques display drawbacks related to food authentication. Reid et al. (2006) gave an example based on the exclusion by means of DNA digestion techniques of pork protein prior to addition of pork into poultry products.

The SNIF-NMR and IRMS technologies cannot be possibly applied widely in the food industry because of instrument high cost. However, these technologies have the benefit of being impossible to be outwitted due to their specificity to analyse atoms occurring in molecules within the food sample.

IR spectroscopy has been repeatedly shown as a sensitive and rapid technique in the detection of adulteration of a wide variety of foodstuffs. It also has the ability to be applied easily in combination with chemometric analysis of foodstuffs. Application of GC technology led to a

considerable decrease in the analysis time with chromatographic runs holding only a few minutes. This technique has potential for industrial use.

Another technique such as electronic nose technology has the advantage that it is of comparatively low cost, rapid and facile to operate. However, the main problems of this technique are related to sensors used in the electronic nose instrumentation.

The target of established techniques remains the continuing development of simple, rapid and accurate applications focused on the detection and determination of foodstuff adulteration.

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Table 1. Meat authenticity: Type of meat product and meat(s) to be detected, QC method, %correct classification and advantages and disadvantages of the applied method

A/A	No of samples	Type of meat/ product(s)	Treatment/ Package	Type of meat(s) to be detected/ excluded	QC Method	Specific remarks	LOD/ % correct classification	Advantages and disadvantages	References
1	10	Red meat animal species	No	wb, ca, dg, h, v, r, k, and p	RAPD-PCR	29 10-noucleotide primers	-	Rapid Qualitative Reproducible Discriminative	Koh et al., 1998
2	-	Sausages	Cooked	sw h and dk	ss-PCR	ss-primers of (bp): 227 153 145	1% (w/w)	Rapid Cost effective	Kesmen et al., 2007
3	103	Beef	Raw Cooked Autoclaved	e, sw, c, o, g, d, cm, dk, m, ms, f, ch, gs, bf, and y	PCR	bovine 1.709 satellite DNA	33.6fg 0.324pg 0.322pg	Sensitive Specific Rapid Convenient	Guoli et al., 1999
4	7	Processed	Yes	t, ch	PCR	mt *cyt b* gene Vista green visualization	1:10 DNA:water	Rapid High sensitive	Hird et al., 2003
5	18	Various products	Raw Cooked at 100^0C and 120^{0C}	ct, p, ch, sh, g and h	PCR	mt *cyt b* gene 6 primers	0.25ng	Rapid Sensitive Qualitative	Matsunaga et al., 1999
6	13	Foodstuffs & SRMs	Severe heat-processing under overpressure	ct, sh, g, p, wb, ch, t, dc, q, rd, o, r, and h	PCR	CYTbos1(fw) CYTbos2(rv) 115bp specific amplif. of mt *cytb*	2.5pg bovine DNA	Direct Specific	Pascoal et al., 2005
7	60	Several (very commercial)	Wide variety of raw and cooked methods	b, p, wb, ch, t, dc, q, d, rd, os, r, sh, g, and h	PCR-RFLP	CYTb1/CYTb2 universal primers	-	Rapid Easy-to-perform Qualitative	Pascoal et al., 2004
8	16	Commercial	Raw	ct, b, sh, and g	PCR-RFLP	mt 12S rRNA	-	Qualitative Good efficiency	Girish et al., 2005
9	150	Dry salt-cured	Raw muscle	fd, ct, sh, and g	PCR-RFLP	mt 12S rRNA 12S-FW & 12S-REV primers	-	Simple Rapid	Fajardo et al., 2006

		Heated		rdr rd				Cost effective	
10		Muscle of: cm pi mo	Raw	rdd, fd, rd, ct, sh, g, and sw	PCR	mt D-loop region	0.1%	Rapid Straight-forward	Fajardo et al., 2007a
11	161	Chicken	Raw	Growing: Slow vs Fast	PCR-AFLP	2 primer combinations *Eco*RI + AAC/*Mse*I + CAA *Eco*RI + AAC/*Taq*I + ATG	- 68-100% - /88-100%	Tedious Reliable Promising	Fumiere et al., 2003
12	27	Beef Sheep Goat	Raw	h, dg, ca, and p	PCR	ss-mtDNA primers 35 PCR cycles	0.1%/ -	Rapid Easy Reliable	Ilhak & Arslam, 2007
13	35	Commercial	Raw Autoclaved	Fishes Ruminats Poultry Pork	Multiplex-PCR	Primers based on: 12S Rrna tRNA Val 16S rRNA	0.004%/ - 0.002%/ -	Advanced Wide use	Dalmasso et al., 2004
14	16	Chicken Turkey	Raw "chilled foods"	t and ch	PCR	α-cardiac actin ss-products of: 372bp 159bp 35-40 PCR cycles	1%/ -	One-step method Susceptible of improvement	Lockley & Bardsley, 2002
15	-	Duck Moscovy duck	Raw Heat-treated meat mix.	gs and numerous other species	PCR	mt12S rRNA gene	Binary dc/gs: 0.1% 1.0%	Quick Sensitive Cost effective Routine anal.	Martin et al., 2007
16	26	Goose Duck (*foie gras*)	Raw Pasteurized	ch and pk	PCR	α-actin gene	0.1%/ -	Qualitative Specific Sensitive	Rodríguez et al., 2003c
17	11	Meat mixtures	Heated Dry-cured	pk	PCR-RFLP	a 531bp of the D-loop mt DNA region	5%/ -	High ss primers Reliable Rapid	Montiel-Sosa et al., 2000
18	22	Meat Meat products	Fresh Processed Autoclaved	ch	PCR	a 442bp of the D-loop mt DNA region	<1%/ -	Rapid Specific (except for t and q)	Mane et al., 2009
19	300	Venison (mixt of rd, rdd, and fd)	Raw Pastreurized Dry-cured	mo, pi, ct, sh, g, sw, h, r, dc, t, ch, and gs	PCR	~170-175bp fragments mt12S rRNA gene	-/ -	Reliable High specific Rapid , Simple	Fajardo et al., 2007b
20	25	Meat mixtures	Minced Lean	pk, ch , bf, la and t	ssRT-PCR	a <150bp fragment of mt cyt b gene	0.5%/ - 0.1%/ -	Reliable Accurate	Dooley et al., 2004

21	30	Horse sausages	Fresh	Pk	Duplex-PCR	two 398 and 439bp fragments of mt cyt b gene		-	Qualitative Rapid Accurate	Di Pinto et al., 2005
22	8	Goose salami	Boiled Spiced	dc, ch and t	PCR	a 100bp fragment of mt cyt b gene		-	Easy to applied Reproducible	Colombo et al., 2002
23	21	Complex meat products/samples	Freeze-dried	pk, ch, t, os, ct, and la	RT-PCR	TaqMan^{R} MGB probe		>1% >5% / 0.03-0.80pg	Cost effective Quantitative Good accuracy	López-Andreo et al., 2005
24	69 10	Numerous commercial products Beef	Heated Canned Sausages Pâtés Raw	pk	PCR	SINE motif 161bp ampl. fragment	Cycles 30 20 20	0.005%[a]/ - 1%[b]/ - 1%[a]/ -	Rapid Simple Specific Sensitive Cost effective	Calvo et al., 2001 &2002
25	-	Commercial Products	Raw meats Sausages Salami, etc.	h and dk	RT-PCR	mt *cyt b* gene	119bp 69bp	- /25pg - /1pg	Specific High sensitive Wide applicability	Chisholm et al., 2005
26	8[c]	Beef and lamp admixtures	Raw	Bf	RT-PCR	Primers: Species specific / COW1 COW2	104bp	2%/ -	Quantitative Ease handle of unknown samples	Sawyer et al., 2003
						Universal / UNIV P UNIV Q	237bp			
27	96	Beef- meat mixtures	Minced	pk, la, v, mu and rnd	PAGE	280-300V 1½ - 2¼ h		-	Rapid Cheap Large N^{o}samples/run	Skrökk et al., 1994
28	18	Meat and meat products	Raw	pk, ch, bf, la and t	RAPD-PCR AP-PCR	primers: OPL-04 and OPL-05 M-13		differentiation ss-detection	Reproducible Rapid Simple	Saez et al., 2004
29	15	Meat mixtures in swine	Raw	rdd, fd and rd	RT-PCR	Primers:134bp 169bp 120bp	of 12SrRNA	0.1-0.8%/ -	Accurate Simple Relat. Rapid	Fajardo et al., 2008
30	80	Beef mixtures	Raw	pk	RT-PCR	Primer: 411bp	of mt 12SrRNA	0.5-5.0%	Quantitative Rapid High-sensitive	Rodriguez et al., 2005
31	72	Food products	Raw	wl, pk,	RT-PCR uniplex	205 &100bp	SYBR Green I	- /0.04pg	Simple/cost	López-Andreo et al.,

		and mixtures		h,and ct	duplex	250 & 92bp	TaqMan probe	-/ 0.4pg	effective	2006
32	8	Meat and meat mixtures	Raw	p, ch, b g, cw, fg, sn and fs	PCR-: RAPD actin barcoding	ss "fingerprinting"	mt 16SrDNA & ND4 actin gene	-	Discriminative Accurate & efficient/ -	Rastogi et al.,2007
33	7	Meat and meat mixtures	Raw	ch, pk, t, la and bf	HATR FT-IR Raman spect/py	942, 988, 1382, 1413, 1444, 1575, 1606 and 1729nm	GA-D-MLR PC-DFA	-	Rapid Cost effective	Ellis et al., 2005
34	332	Muscles	Raw and Homogenized	bf, la, ch and pk	Vis-NIR	400-2500nm	PLS PCA DA	>85% correct classification	Reliable Rapid Objective	Cozzolino & Murray, 2004
35	100	Spanish sausages	Dry-cured (sliced)	Homogenized	DAD-NIRS 400-1700nm	Fat Moisture Protein	PCA	-	Rapid Cost effective Non-distractive	Gaitán-Jurado et al., 2007
36	150	Iberian pork	Homogenized: Dry-cured Fresh	standard pk and admixtures	NIRS 400-1700nm	critical wavelengths: 975nm ~1210nm ~1455nm	DA PCA	>80% correct classification	Rapid Cost effective Non-distractive	Ortiz-Somovilla et al., 2005
37	127	Several type of bovine meat	Minced Frozen Thawed	Handle Type	NIRS 400-2500nm		PLS ANOVA	Good accuracy	Rapid Effective	Alomar et al., 2003
38	194	Beef hamburgers	Raw Cooked minced	5-25% of mu, pk, skim milk powder, wheat flour	NIRS 400-2500nm	966, 1212, 1396, 1732, 1748, 1870, 1900, 2310, and 330 nm.	CDA KNN PCA	up to 92.7% accuracy	Rapid	Ding & Xu, 2000
39	41	Beef	Frozen then: Minced or Cut	k	VIS/NIRS 400-2500nm	926, 1204, 1246, 1308, 1680, 1742, 2096, 2210, 2236, 2278 and 2384nm	CDA MLR	up to 100% accuracy	Rapid Effective	Ding & Xu, 1999
40	120	ct	Muscle (raw)	ox	NIRS Physical properties	1100–2500 nm pH, L*, a*, b* colour, WHC, WBSF	Colour parameters	Successful	-	Prieto et al., 2008
41	525	Chicken (42d)	Tender Muscles Tough	Storage time/ temperature regim Shear force values	VIS/NIRS	445 and 560 nm 1200, 1330, 1465, 1670, 1960, and 2380 nm	2D-CA	Good discrimination	Rapid More informative Easy applied	Liu et al., 2004
42	185	Beef	Minced	fresh	MIRS	900-1800nm	PLS/CVA,	<10%	-	

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		Ox kidney Ox liver		frozen-thawed mixtures			SIMCA, and PLS regression			Al-Jowder et al., 1999
43	74	Pork Turkey Chicken	Raw	t, ch pk, ch pk, t	MIRS VIS/NIRS MID-NIRS	5000-12500nm 400-2500nm 400-750nm 5000-12500nm	DA PCA	86.5% 91.5% 94.6%	Qualitative Rapid Non-distructive	Rannou & Downey, 1997
44	32	Beef muscles	Raw	Tenderness	VIS-NIRS	450-950nm	LGA	-	Non-distructive Practical Fundamental Sensitive	Xia et al., 2007
45	150	Poultry	Raw	Bovine meat & bone meal	MS-IRA	$^{13}C/^{12}C$ $^{15}N/^{14}N$	-18.74 - -17.15‰ 1.65- 2.55‰	DA ANOVA	0-8%	Reliable	Carrijo et al., 2006
46	12	Poultry	Fresh	Spoiled	Headspace GC-MS	Alumina-coated PLOTcolumns	-	-	Low temp.collection Rapid Facile	Lovestead & Bruno, 2010
47	2	Products of: Meat Liver	Heated	Brain tissue	Western blotting	Biomarkers: MBP and NSE SDS-PAGE	1% (m/m)	70 ^{0}C pasteurization 115 ^{0}C sterilization	Advanced Sensitive High throughput	Sultan et al., 2004
48	40	Commercial hamburger Mexican sausage	Slightly processed	poultry, bv, e, and pk	Immunodiffusion	Agarose gel assay	3% 1% 1% 10%	-	Rapid High specific Reproducible	Flores-Maguia et al., 2000

Notes

e: equine, sw: swine, c: canine, o: ovine, g: goat, d: deer, cm: camel, dk: donkey, m: mule, ms: mouse, f: fish, ch: chicken, gs: goose, bf: buffalo, y: yak, t: turkey, wb: wild boar, ca: cat, dg: dog, h: horse, v: venison, r: rabbit, k: kangaroo, p: pig, ct: cattle, sh: sheep, dc: duck, q: quail, rd: roe deer, rdd: red deer, fd: fallow deer, os: ostrich, b: beef, pk: pork, mo: mouflon, pi: pyrenean ibex, mu: mutton, bv: bovine, wl: wallaroo (kangaroo).

SRMs: specific risk materials, MGB: Minor Groove Binding, a: pork in beef, b: pork in duck, c: 0.1%, 1%, 2%, 5%, 10%, 25%, 50% and 100% beef in lamb, SINE: Short Interspersed Nuclear Element, PAGE: Polyacrylamide Gel Electrophoresis, RAPD-PCR; Random Amplified Polymorphic DNA Polymerase Chain Reaction, AP-PCR: Arbitrarily Primed Polymerase Chain Reaction, ND4: NADH dehydrogenase subunit 4, ss: species specific, HATR: Horizontal Attenuated Total Reflectance, GA-D-MLR: Genetic Algorithms Discriminant Multiple Linear Regression, PLS: Partial Least Squares, DA: Discriminant Analysis, PCA: Principal Component Analysis, PC-DFA: Principal Components Discriminant Function Analysis, CDA : Canonical Discriminant Analysis, KNN: *K*-nearest-neighbour method, WBSF: Warner–Bratzler Shear Force, 2D-CA: 2D Correlation Analysis, CVA: Canonical Variate Analysis Modeling, SIMCA: Soft Independent Modelling of Class Analogy, PLOT: Porous Layer Open Tubular

Table 2. Geographical origin determination of beef and lamb products with isotopic ratio methods

A/A	Country	No of samples	Measured parameter(s)	QC method	Special remarks on samples	Statistical method(s)	δ (‰) Means/Ranges					References
							^{13}C or $^{13}C/^{12}C$ (*)	^{18}O or $^{18}O/^{16}O$ (*)	^{14}N or $^{15}N/^{14}N$ (*)	^{2}H or $D/^{2}H$ (*)	^{32}S or $^{34}S/^{32}S$ (*)	
1	EU vs Ireland USA Brazil	68	C, N, & S	SIRA[a]	Conventional & organic farming	MANOVA	-21.6 -24.5 -12.3 -10.0	–	7.1	–	7.6	Schmidt et al., 2005
2	Germany vs Argentina Chile	244	H, O, C, N, & S	SIRA[a]	Freeze-dried Extracted water Defatted Raw protein Organic farming	PCA DA	(*)	North: -7.9 South: -11.2 - -	<6.1 >5.9 5.0	-36 -55	<4.8 >8.5 15.2	Boner & Förstel, 2004
3	Japan vs Australia USA	22	C, N & O	EA/IRMS[b]	Defatted dry	-	-19.6 – -17.0 -23.6 – -18.7 -13.6 – -11.1	7.3 – 13.6 15.0 – 19.4 9.5 – 11.7	7.8 – 8.1 5.7 – 9.3 5.1 – 7.8	–	–	Nakashita et al., 2008
4	Eu GB, Irl, Sctl USA Australia S. America Brazil N. Zealand S. Africa	>200	C, N O, H	IRMS ICP-MS	Defatted dry mass Lipid fractions	CDA	-22.6 – -21.2 -25.8 – -24.3 -11.1 -16.9 -20.6 -11.4 -26.32 -15.3 – -12.5	17.7 – 20.3 17.9– 18.3 17.9 23.4 22.3 – 22.8 21.4 19.4 26.1 – 32.3	4.7 – 6.6 6.6 – 7.1 6.0 6.1 6.6 – 6.8 6.7 5.8 7.3	-220.0– -176.4 -208.0– -189.8 -214.2 -157.7 -180.8 -183.0 -214.6 -151.0– -140.5	–	Heaton et al., 2008
5	USA Mexico Australia N. Zealand Korea	85	C, N & O	EA/IRMS[b]	Freeze-dried	ANOVA	~ -12.0 ~ -12.0 ~ -17.0 ~-23.0 ~ -16.0	~ 9.0 ~ 15.0 ~ 14.0 ~ 10.0 ~ 10.0	6.3 7.0 ~ 7.0 5.6 6.15	-	-	Bong et al., 2010
6	Switzerland Australia U.S.A. Brazil and Canada	72	$^{18}O/O$ $^{87}Sr/^{86}Sr$	ICP-MS IRMS	Dried sample	One-way ANOVA	-	-1.84 1.84 -3.35 -1.03 -5.59	-	-	-	Franke et al., 2008

7	Australia Brazil Canada Switzerland USA	23	1D ^{1}H	HR-MAS NMR	Dried	One-way ANOVA PCA Stepwise DA	**Compounds used (ppm)** [e]carns/tyr: 3.20–3.25 [e]carns: 2.70–2.75 [e]glut/pro: 2.35–2.40 carnt/suc/[e]UB: 2.40–2.45 [e]carnt: 2.45–2.50 [e]ala: 3.80–3.85 [e]phe: 3.30–3.35 [e]UA; 3.65–3.70 [e] pro/carnt: 3.40–3.45	Shintu et al., 2007

8	Switzerland Austria Australia U.S.A. Brazil and Canada	23	72 elements	ICP-HRMS	Dried sample	ANOVA LDA PCA

Ca	Cd	Cu	Ga	Ni	Pd	Rb	Sr	Tl	Zn
		√		√					√
√[c]		√					√		
	√				√			√	
	√		√		√				
				√		√			√
				√		√			

Franke et al., 2007

9	Japan Australia	782	DNA markers	PCR-AFLP	Tissue and blood	-

Marker	SRY	ND5	MC1R	BIMA100	BIMA118	BIMA119
Product size (bp)	803	527	219/218	465	153/148	78/76

Sasazaki et al., 2007

10	USA (N Dakota)	138	Se	HG-AAS	Several tissues	

Region	NW	SCentral	SW	Central	SE
Muscle (mg/kg)	0.67	0.47	0.40	0.38	0.27

Hintze at al., 2001

11	Switzerland France Germany Hungary Brazil and Thailand	25 chicken	46 elements	ICP-HRMS	Breast	ANOVA LDA PCA

As	Na	Rb	Tl
√[c]	√ √ √ √ √	√	√

Franke et al., 2007

12	Switzerland France Germany Hungary and Brazil	78 chicken	^{18}O/O ^{87}Sr/ ^{86}Sr	ICP-MS IRMS	Dried	One-way ANOVA

-	-5.69 -1.79 -4.05 -5.39 -2.56	-	-	-

Franke et al., 2008

13	GB Spain France	360 lamb	^{13}C/^{12}C ^{15}N/^{14}N	IRMS	Meat fat and protein fractions	ANOVA CDA

-26.75 d -26.25 -29.30 -24.80	-	6.75 d 6.15 4.55 5.75	-	-

Piasentier et al., 2003

	Greece Iceland Italy						-31.50 -28.45		2.50 5.7			
14	Greece UK (+Sicily) Centr. Europe Alpine Mnts		203 lamb	H, C, N, S	SIRA	Dried	ANOVA PCA LDA	-21.2 - -23.0 -26.2- -28.5 -24.6- -26.1	-	+5.0 - +6.0 +5.0 - +6.0 +7.4 - +9.2 +5.0 - +6.0	-80 -80 - -90 -90 - -100 -100 - -115	+1.6 - +12.8	Camin et al., 2007
15	Italy (Apulia)	N C S	25 Lamb	$^{13}C/^{12}C$ $^{15}N/^{14}N$ Li, Ca, Mg, Na, K Zn, Cu, Fe, Cr, Mn ^{1}H (500MHz)	IRMS HPIC ICP-AES HR-MAS NMR	Dried	PCA DA One-way ANOVA	-25.65 -25.89 -26.02	-	6.9 5.53 7.05	-	-	Sacco et al., 2005

Notes

[a] SIRA: Stable Isotope Ratio Analysis, [b] EA/IRMS: Elemental Analyzer/isotope ratio mass spectrometry, [c] Measured higher levels, [d] mean values of different lamb types and feeding regimes based on protein , [e] carns: carnosine; tyr: tyrosine; glut: glutamine; pro: proline; ala: alanine; phe:phenylalanine, HR-MAS NMR: High Resolution-Magic Angle Spinning Nuclear Magnetic Resonance, HPIC: High Performance Ion Chromatography, ICP-AES: Inductively Coupled Plasma Atomic Emission Spectrometer, HG-AAS: Hydride Generation Atomic Absorption Spectroscopy

Table 3. Application of Multivariate analysis to meat and meat products; adulterant, QC method, (pre)treatment, % correct classification and % detection limit

A/A	No of samples	Type of meat	Adulterant(s)/ mixed with	QC Method	(Pre)Treatment	MV method	Mean Correct Classification (%)	Detection limit (%)	References
1	12	Beef	Heart, tripe, kidney, liver	MIR	Two levels microwave	PCA PLS LDA	~97%	≥20	Al-Jowder et al., 2002
2	185	Beef	Ox kidney & liver	FTIR	Fresh Frozen-thawed	PLS-CVA SIMCA	>95%	>10 <10	Al-Jowder et al., 1999
3	41	Beef	Kangaroo	VIS-NIR	Freezed	CDA stepwise MLR	80-100	-	Ding & Xu, 1999
4	71	Chicken	Broilers	VIS-NIR	None	DA stepwise MLR PCR MLS	92-100	-	Ding et al., 1999
5	32	Beef	Frozen-then-thawed	NIR	None	FDA SIMCA	89.1-95.3	-	Downey & Beauchêne, 1997
6	154	Beef	Lamb	VIS-NIR/ MIR	None	PLSR	~100	<20	McElhinney et al., 1999
7	108	Turkey (m)	Discoloration	pH L^*, α^*, b^*	Homogenized in $C_2H_3IO_2$ None	CDA NNS	70	-	Santé et al., 1996
8	74	Beef	Microbial load	FTIR/ATR	Incubation at 0, 5, 10, 15 and 20^0C for 350h	MLP NN	80-90	-	Argyri et al., 2010
9	203	Lamb	Origin	IRMS (H, C, O, N)	Dried	ANOVA PCA LDA	~78	-	Camin et al., 2007
10	360	Lamb	Origin	IRMS (C, N)	Vacuum-packed Freezed-dried	ANOVA CDA	~66.7	-	Piasentier et al., 2003
11	194	Beef (hamburger)	Mutton Pork Skim milk powder Wheat flour	NIRS	None	CDA KNN PCA Mpls	92.7	-	Ding et al., 2000

Table 4. Sensory analysis of meat and meat products; type of meat, no of panellists (trained or untrained or semi-trained) and attributes investigated.

A/A	No of samples	Meat/Product		QC method	No of panelists	Trained	Attributes	Range	Results	Remarks	References
		Type	Part/ Treatment								
1	25	Beef	Muscle	RT-PCR	10-12	Y	Tenderness Juiciness Flavour	1-10	No of specific expressed genes/markers: 29 21 15 DNAJA1 new!	Expensive Time consuming	Bernard et al., 2007
2	72	Sausage	Fermented	Electronic nose of 14 sensors (2x5 MOSFET[a])	17	Y	19	1-9	Good agreement	Rapid Simple Inexpensive	Eklöv et al., 1998
3	10	Ham	Dry cured	CG-LC GC-MS	-	-	Alkanes Alkanes branced Aldehydes Alcohols aliphatic	-	12 (6.82%) 14 (12.31%) 13 (30.69%) 9 (22.27%)	Intense proteolytic & lipolytic breakdown Questions on the volatiles real origin	Garcia et al., 1991
4	60	Beef	Steak	Image processing	10	Y	Color Marbling	1-8 1-9	R^2 =0.86 R^2 =0.84	Inexpensive Rapid Difficulty on visual evaluation	Gerrard et al., 1996
5	84	Chicken	Irradiated Breast & Leg/ refrigerated or frozen	Descriptive Sensory Analysis	14	Y	Raw[b] Cooked[c]	0-150	White / Dark No effect More chickeny & tender	-	Hashim et al., 1995
6	30	Beef	Fresh Raw Raw frozen-then-thawed	NIR-Reflection NIR-Transmission	9	Y	Hardness Tenderness Juiciness	1-9	2.9-7.1 (4.7) 2.9-7.4 (5.6) 4.6-6.1 (5.4)	Great effect of the age, wheight, fat content & shear-press Rapid Satisfactory results	Hildrum et al., 1994
7	36	Chicken	Breast / Intact	Sensory Analysis	28	N	MFI Shear force	A_{540nm} x 200	The Higher Lower	The most preferred	Kriese et al., 2007

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				Excised				Sensory analysis	Nts 1-9	Higher values		
8	177	Beef	steaks	Frozen	VIS-NIR Sensory Analysis	7	Y	Color values[d] Shear force Chewiness Juiciness	1-5	5.46 3.19 2.37	>90% correct classification Good accuracy [d]	Liu et al., 2003
9	28	Pork	Muscle	Cooked Refrige-rated	GC-MS Electroninc nose Sensory analysis	8	N	odour, flavour, taste and after taste sensory	0-150	Samples with high WOF [E] values: - 5d refrigerated - From iron enriched feeding - *M. psoas major muscle vs M. longissimus dorsi*	Indestructible sensors High reproducibility	O'Sullivan et al., 2003
10		Ham	Dry-cured	20^0C 30^0C	Electronic nose	-	-	-	-	Flavour: Sweet Intense and pleasant	12 TiO_2 sensors Good responses Best results at 250^0C working temperature Non-destructive Non-contact	Otero et al., 2003
11	4	Pork	Muscle	Ir NIr	GC-MSD Sensory analysis	13	Y	Lipid oxidation Volatiles Sensory analysis	0-15	Irradiation had no (negative) effect on the acceptance of the meat	TBARS are: -responsible for the off-odor in Ir samples - non-dose dependent at <10kGy - related to radiolytic degradation of AAs.	Ahn et al., 2000
12	60	Chicken	Package at: Breast	A [g] VP [g] M1 [g] M2 [g]	Sensory analysis	7	Y	Taste Odour Appearance	0-9	6 Most effective against microbial spoilage	The limit of sensory acceptibility Volatile amines as chemical indicators of possible microbial spoilage	Balamatsia et al., 2007
13	117	Ham	Dry-cured		VIS/NIR Sensory analysis	2	Y	pastiness, colour, crusting, marbling and	1-10	*Y* (%)[h] : 80.0 91.1 42.8 70.1	Use of remote refle-ctance fiber optic probe On-line	Ortiz-Somovilla et al., 2006

							ring colour		69.9	classification possibility	
Notes: [a] MOSFET: Metal Oxide Semiconductor Field-Eeffect Transistors, [b] Fresh chickeny, Bloody, Oxidized, Sweet aromatic, Color, Moistness & Glossiness, [c] Color, Juiciness, Sweet, Salt, Sour, Bitter, Chickeny, Warmed-over, Brothy, Sweet-aromatic, Cardboard, Tenderness & Graininess. ReflectionTransmittance, [d] Hunter L (lightness), a (redness), and b (yellowness), [E] WOF: Warm-Over Flavour, [f] I: Irradiated, NI: Non irradiated, [g] A: Air; VP: Vacuum Package; M1: Modified Atmosphere 1, 30%/65%/5% ($CO_2/N_2/O_2$); and M2: Modified Atmosphere, 65%/30%/5% ($CO_2/N_2/O_2$), [h] is the percent variance of the binary response explained by PLS, GC-MSD: Gas Chrmatography-Mass Selective Detector, MFI: Myofibrillar Fragmentation Index										

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Figure 1. Geographical origin determination of meat with isotopic ratio methods for UK, Ireland, Central Europe and Central America