Universidade de Trás-os-Montes e Alto Douro

ADULTERATION OF FOODS FROM ANIMAL ORIGIN

Consumer perception about food labelling and

Spectroscopic methods for detection of fresh food adulteration

Ph.D. Thesis in Veterinary Sciences

Branch - Food Quality and Safety

Maria João Pinho Moreira

Advisor: Professor Cristina Maria Teixeira Saraiva

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VILA REAL, 2019

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of the author’s responsibility

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ABSTRACT

Consumers have become more and more demanding in their meat and fish consumption, in terms of quality, safety, and the origin of the products they consume.

Today, consumers attribute great value to the information on food labels, this is, the indication of the ingredients and about the production processes applied to the final product. The identification and authentication of food have an important role in the healthy diet of consumers.

There is a need to develop fast and efficient techniques to detect food adulteration and re-establish consumer confidence in food manufacturers. There is an increasing interest in methods based on spectroscopic techniques, because they offer several advantages, this is, and they are powerful tools for conducting adulteration tests.

One of the objectives of this study was not only to identify important labelling aspects that consumers would examine at the time of purchasing, but also to determine opinions and the knowledge about adulteration of food products. To understand the usefulness of the information provided for consumers, a survey was carried out to assess the efficacy of the information presented in food labelling.

The Kruskal-Wallis and Spearman test and descriptive analytical tools were used to analyse data. Principal Component Analysis (PCA) was performed to obtain a smaller number of uncorrelated factors regarding the usefulness of food labelling, regarding the confidence of information displayed in food label and perception of food fraud.

Results showed consumers usually do not read food labels due to lack of time and excessive information. Additionally, food labelling was observed to be more useful for specific consumer groups, such as, athletes, consumers with health conditions or consumers concerned with a healthy lifestyle.

The respondents consider that information displayed in food label is useful, but the way the information is presented may decrease the consumer interest. The level of education, a healthy

lifestyle, and practising sport were factors which influenced the opinion of consumers regarding food labelling.

Regarding respondents´ confidence on foodstuffs, over half of them stated that information provided in food label is reliable. However, a lack of confidence on food composition is observed in those processed foodstuffs such as meat products. Food fraud is recognized by over half of respondents with a higher perception of those practices that implies a risk to public health than those related to economic motivation. Age and consumers' education revealed the most important socio-demographic factors regarding food label perception, confidence on its information and also knowledge about food fraud.

The results of the present study highlight the need of information campaigns by public health authorities to show the importance and advantages of reading food labels as well as ensuring food labels with essential information which are not only quickly and clearly seen but also understood by consumers. Thus, implementation of education programs to increase consumer knowledge about food labelling and fraud is essential. Since scarce research is available about consumer perceptions about food label information and food fraud, the respondents´ perceptions observed in the current work could be used as guidelines by food industry to improve food label design to enhance the consumer understand and usefulness.

The potential of the Fourier transform infrared spectroscopy (FTIR) in tandem with chemometric methods such as PCA, Partial Least Squares Regression (PLS-R) and Partial Least Squares Discriminant Analysis (PLS-DA) in detecting the presence/absence of adulteration of fallow deer meat (*Dama dama*) (D) with goat meat (*Capra aegagrus hircus*) (G) and *Atlantic salmon* (SS) with *Onconrhynchus mykiss* (OM) was studied not only for fresh samples but also for samples stored for different storage periods. The PCA model was able to describe the studied adulteration by using four principal components with a variance of 95%. PCA showed that the absorbance in the spectral region from 1138 to 1180, 1314 to 1477,1535 to 1556 and from 1728 to 1759 cm^{-1} may have been attributed to biochemical fingerprints related to adulteration used in the differentiation of fallow deer and goat meat. Furthermore, the absorbance in the spectral region of 721, 1097, 1370, 1464, 1655, 2805 to 2935, 3009 cm^{-1} may have been attributed to biochemical fingerprints related to adulteration and used in the differentiation of the SS and

OM. The PLS-DA and PLS-R model predicted the presence/absence of adulteration in meat and fish samples of an external set with high accuracy.

Keywords: Fresh Food, Fraud, Consumer opinion, Adulteration, Confidence, Labelling

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RESUMO

Os consumidores tornaram-se cada vez mais exigentes com o consumo de carne e peixe, em termos de qualidade, segurança e origem dos produtos.

Atualmente, os consumidores atribuem elevado valor à informação nos rótulos dos alimentos, nomeadamente indicação dos ingredientes e processos de produção. A identificação e autenticação de alimentos têm um papel importante na dieta saudável dos consumidores.

Existe a necessidade de desenvolver técnicas rápidas e eficientes para detetar a adulteração de alimentos e restabelecer a confiança do consumidor na indústria agroalimentar. Há um interesse crescente em métodos baseados em técnicas espectroscópicas, pois oferecem várias vantagens, ou seja, são ferramentas poderosas para a realização de testes de adulteração.

Um dos objetivos deste estudo não foi apenas identificar aspetos importantes de rotulagem que os consumidores examinariam no momento da compra, mas também determinar opiniões e o conhecimento sobre a adulteração de produtos alimentícios.

Para entender a utilidade das informações fornecidas aos consumidores, foi realizada um inquérito para avaliar a eficácia das informações apresentadas na rotulagem dos alimentos.

Os testes *Kruskal-Wallis, Spearman,* análise de componentes principais (PCA) e ferramentas analíticas descritivas foram utilizados para analisar os dados. O nível de escolaridade, o estilo de vida saudável e a prática desportiva foram fatores que influenciaram a opinião dos consumidores em relação à rotulagem dos alimentos.

Os resultados mostraram que os consumidores geralmente não leem os rótulos dos alimentos devido à falta de tempo e excesso de informações. Além disso, observou-se que a rotulagem de alimentos é mais útil para grupos de consumidores específicos, como atletas, consumidores com condições de saúde ou consumidores preocupados com um estilo de vida saudável.

Os inquiridos consideram que a informação exibida no rótulo dos alimentos é útil, mas a forma como a informação é apresentada pode diminuir o interesse do consumidor. O nível de escolaridade, o estilo de vida saudável e a prática esportiva foram fatores que influenciaram a opinião dos consumidores em relação à rotulagem dos alimentos.

Quanto à confiança dos inquiridos, mais da metade afirmou que as informações fornecidas no rótulo dos alimentos são confiáveis. No entanto, a falta de confiança na composição dos alimentos é observada nos alimentos processados, como nos produtos à base de carne. A fraude alimentar é reconhecida por mais da metade dos inquiridos com maior perceção daquelas práticas que implicam risco à saúde pública do que aquelas relacionadas com a motivação económica. A idade e a educação dos consumidores revelaram os fatores sociodemográficos mais importantes em relação à perceção do rótulo de alimentos, confiança em suas informações e também conhecimento sobre fraude alimentar.

Os resultados do presente estudo destacam a necessidade de campanhas de informação das autoridades de saúde pública para mostrar a importância e as vantagens da leitura dos rótulos dos alimentos, bem como garantir rótulos de alimentos com informações essenciais que não só são vistas rápida e claramente, mas também compreendidas pelos consumidores.

Assim, a implementação de programas de educação para aumentar o conhecimento do consumidor sobre rotulagem de alimentos e fraude é essencial. Existem poucas pesquisas sobre as perceções dos consumidores sobre informações de rótulos de alimentos e fraude alimentar. As perceções dos entrevistados que foram observadas no trabalho atual poderiam ser usadas como diretrizes pela indústria de alimentos para melhorar o *design* de rótulos para melhorar a compreensão e utilidade do consumidor.

O potencial da espectroscopia de infravermelho por transformada de Fourier em conjunto com métodos quimiométricos como o PCA, Regressão Parciais de Mínimos Quadrados (PLS-R) e Análise Discriminante de Mínimos Quadrados Parciais (PLS-DA) na deteção da presença ausência de adulteração de carne de gamo (*Dama dama*) (D) com carne de caprino (*Capra aegagrus hircus*) (G) e salmão do Atlântico (SS) com *Onconrhynchus mykiss* (OM) foi estudado não apenas para amostras frescas, mas também para amostras armazenadas para diferentes períodos de armazenamento. O modelo PCA foi capaz de descrever a adulteração estudada usando quatro componentes principais com uma variância de 95%. A PCA mostrou que a absorbância na região espectral de 1138 a 1180, 1314 a 1477, 1535 a 1556 e de 1728 a 1759 cm^{-1} pode ter sido atribuída a impressões bioquímicas relacionadas à adulteração utilizada na diferenciação de gamos e carne de caprino. Além disso, a absorbância na região espectral de 721, 1097, 1370, 1464, 1655, 2805 a 2935, 3009 cm^{-1} pode ter sido atribuída a impressões

bioquímicas relacionadas à adulteração e utilizadas na diferenciação da SS e MO. O modelo PLS-DA e PLS-R previu a presença / ausência de adulteração em amostras de carne e peixe de um conjunto externo com alta precisão.

Palavras-chave: Géneros alimentícios frescos, Fraude, Adulteração, Opinião do consumidor, Confiança na rotulagem.

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ACRONYMS

2-DE - Two-dimensional gel electrophoresis

AFLP - Amplified fragment length polymorphism

D - Fallow deer meat (Dama dama)

EC - European commission

EFSA - European Food Safety Authority

ELISA - Enzyme-linked immunosorbent assay

FAO - Food and Agriculture Organization of the United Nations

FS - Fluorescence spectroscopy

FTIR - Fourier transform technique

G - Goat meat (Capra aegagrus hircus)

GC - Gas chromatography

HIS - Hyperspectral

HPLC – High-performance liquid chromatography

IEF - Isoelectric focusing

KNN- k-nearest neighbor

LC - Liquid chromatographic

MIR - Mid infrared spectroscopy

MSI - Multispectral

NIR - Near infrared spectroscopy

NMR - magnetic resonance imaging

OGM - Genetically modified organism

OM - Onconrhynchus mykiss

PCA - Principal component analysis

PDO - Protected designation of origin

PFGE - Pulsed-field gel electrophoresis

PGI - Protect geographical indications

PLS-DA - Partial least squares discriminant analysis

PLS-R - Partial least squares regression

PNN - Probabilistic neural networks

RAPD - Random amplified polymorphic DNA - Deoxyribonucleic acid

RASFF - Rapid alert system for food and feed

RFLP- Restriction fragment length polymorphism

RS - Raman spectroscopy

SDS–PAGE - Sodium dodecyl sulphate–polyacrylamide gel electrophoresis

SIMCA - Naive-Bayes, soft independent modelling of class analogy

SS - *Salmon salar*

TSG - Traditional Speciality Guaranteed

UHPLC - Ultra-high-performance liquid chromatography

VIS - Visible spectroscopy

WHO - World Health Organization

CHAPTER I. GENERAL FRAMEWORK

In order to fulfil the objectives of this work, the thesis is structured in such a way as to allow a logical sequence in an approach which is intended to be comprehensive, with elements related to consumer perception and confidence in the labelling of food products and the knowledge and application of spectroscopic methods for the detection of different types of adulteration or food fraud.

In Chapter I, an introduction is presented with a review of the in-depth literature on food labelling and adulteration, more specifically on products of animal origin, and a description of methods of authentication in animal products.

Then, in Chapter II, it is approached in depth the application of spectroscopic methods in the detection of adulteration of products of animal origin, trying to establish connection points with the problematic in analysis, having originated a book chapter.

In Chapter III, the objectives of the work are addressed, being explained in order to establish the hypothesis which were the basis of the experimental work, either in the context of consumer surveys or in the laboratory component.

In Chapter IV are presented the methodological bases of the experimental component, namely those related the realization of a consumer survey and the detection of adulteration in fresh food of animal origin. It begins with an exposition of the research methodology that was the basis of the survey, with the description of the study, the characterization of the participants, the description of the data collection instruments and the data processing process. In the second part of the chapter explained the laboratory procedures applied for the determination of adulteration of wild fallow deer (*Dama dama*) (D) meat with domestic goat (G) (*Capra aegagrus hircus*) meat and Atlantic salmon (*Salmo salar*) (SS) with Salmon trout (*Onconrhynchus mykiss*) and data processing.

In Chapter V, the data collected throughout the study of consumer survey and the analysis carried out in relation to the research questions and the proposed objectives. Begins by presentation and analysis of the mode and frequency data, as the participants in the survey read the label, the factors that affect the purchase decision and the usefulness of the label of a food product.

The results are presented in relation to the opinion and confidence in the content of the label on food products, confidence in the constitution of the different products and the knowledge of the different types of adulteration.

In Chapter VI, are presented the results of the analyses carried out by the Fourier transform infrared spectroscopy in two studies of species of animal origin, the first study on meat products and the second study on fish products, with percentages of different mixtures, of adulteration of D with G meat and SS with OM.

The results were also complemented by microbiological, physicochemical and analysed by chemometric methods, namely methods such as Principal Component Analysis (PCA), Partial Least Squares Regression (PLS-R) and Partial Least Squares Discriminant Analysis (PLS-DA) for a more complete approach.

Throughout Chapter VII were analysed, the data collected in the four studies presented previously, establishing a logical sequence of discussion of results and using, where appropriate, to the interrelation of the sources of information under study, in the sense of greater credibility of results and to the presentation of other comparative studies.

SCIENTIFIC PUBLICATIONS IN THE SCOPE OF THE THESIS

BOOK CHAPTER PUBLISHED

Moreira, M. J., Saraiva, C., Almeida, J. M. M. M., 2017. Spectroscopic Methods for Fresh Food Authentication: An Overview. In Trends in Food Safety and Protection, ed. V Ravishankar Rai, Jamuna A Bai, 131 - 166. ISBN: 9781138070912. India: CRC Press, Taylor & Francis Group.

ARTICLES PUBLISHED IN JOURNALS INDEXED IN SCOPUS

Moreira, M. J., Silva, A. C., Saraiva, C., Almeida, J. M. M. M., 2018. Prediction of adulteration of game meat using FTIR and chemometrics, *Nutrition & Food Science,* 48, 2: 1-9. https://doi.org/10.1108/NFS-08-2017-0164

Sousa, N., **Moreira, M. J.**, Saraiva, C., Almeida, J. M.M.M., 2018. Applying Fourier transform mid infrared spectroscopy to detect the adulteration of *Salmo salar* with *Oncorhynchus mykiss*", *Foods*, 7(4), 55; https://doi.org/10.3390/foods7040055

Moreira, M. J., García-Díez, J., Almeida, J. M. M. M, Saraiva, C., 2019. Evaluation of food labelling usefulness for consumers", *International Journal of Consumer Studies,* 00:1–8. https://doi.org/10.1111/ijcs.12511

ARTICLES SUBMITTED TO JOURNALS INDEXED IN SCIENCE CITATION INDEX (SCI)

Moreira, M. J., García-Díez, J., Almeida, J. M. M. M, Saraiva, C., 2019. "Consumer Knowledge about Food Labelling and Fraud" submitted to Food Control.

COMMUNICATIONS IN NATIONAL AND INTERNATIONAL CONFERENCES

ORAL COMMUNICATION

Moreira, M. J., Silva, A. C., Saraiva, C., De Almeida, J.M. M. M., 2017. Advances in the detection of fraudulent fresh game meat using mid infrared spectroscopy In BioMicroWorld2017 – VII International Conference on Environmental, Industrial and Applied Microbiology, 18-20th October, Madrid Spain.

Almeida, J. M. M. M., **Moreira, M. J.,** Saraiva, C., 2016. Spectroscopic methodologies for fresh food authentication In 1st Meeting of the Veterinary Sciences Doctoral Degree Program, ICBAS, UP, 15th April 2016, Porto.

POSTERS PRESENTATION

Moreira, M. J., García-Díez, J., de Almeida, J. M. M. M, Saraiva, C., 2018. "Evaluation of food labelling usefulness for consumers", 8º Encontro de formação da Ordem dos Médicos Veterinários, 14-15th April 2018, in the Lisbon Congress Center.

Sousa, N., **Moreira, M. J.,** Saraiva, C., de Almeida, J. M.M. M., 2018. A aplicação de espetroscopia FTIR para deteção de adulteração de *Salmo salar*, VII Jornadas de Inspeção

Sanitária, 7th April 2018, in the Forest Building of the University of Trás-os-Montes and Alto Douro, Vila Real.

Silva, A. C, Saraiva, C., **Moreira, M. J.,** Nascimento, P., Almeida, J. M. M. M., 2014. Estudo da adulteração de carnes frescas por espectroscopia de infravermelhos e análise quimiométrica, XX Encontro Luso-Galego de Química, 26-28th November 2014, in the FFUP / ICBAS Complex, Porto

In Chapter VIII, "Conclusions", there is a reflection on the opinion and consumer confidence in the labelling of foodstuffs and the application of spectroscopic methods in the detection of meat and fish adulteration, followed by the critical analysis of the study taking into account the research questions and goals.

Some perspectives are also mentioned for future work.

CHAPTER II - INTRODUCTION

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1. FOOD LABELLING

1.1. Outline and Basic Definitions

Labelling enables important communication between consumers and the food system concerning a product or production process. Consumer personal characteristics, confidence, food related lifestyles and environmental and ethical principles can segment consumers in homogeneous groups (Gracia and de-Magistris, 2016). The message provided by labelling can promote the trust in the food manufacturer. However, there may occur a decline in the perceived trustworthiness of the manufacturer if there is inconsistencies in the information provided on labels (Tonkin et al., 2016). Food information can be available to the final consumer through a label, modern technological tools or verbal communication. Labelling consists of indications, mentions, images and/or symbols concerning food products which accompany packaging so as to promote the sale (Ababio et al., 2012). Therefore, the main ingredient is any constituent, including flavouring, additives and food enzymes used in the preparation of food. The ingredients list is a key point pertaining to the list of mandatory factors of labelling. The ingredients list provides the constitution of the final product in a descending order of proportion for example, the ingredients at the end of the list are present in smaller quantities (Miller and Cassady, 2015).

1.2. Role of Food Labelling Information

Currently, the choice of food in modern society is particularly affected by three major trends, health concerns, sustainability and convenience. Important drivers like nutritional information, safety and quality food, taste and price affect the choice of food (Asioli et al., 2017, Bandara et al., 2016). The intrinsic, extrinsic and socio-cultural factors affect consumer preferences for labelled foods. Food labelling enables people to choose foods for a healthy diet. This can help one assess products because it provides several specific qualities namely, certification, organic production or general quality labels. Nutritional information can play an important role as it conveys information concerning the product characteristics that would otherwise be unknown to consumers. Such information represents an aid to consumers in making food choices, as it facilitates comparisons between alternative products, thus contributing to informed purchasing decisions (Cavaliere et al., 2017). In other words, diet affects the appearance of certain human diseases. Other factors such as age, genetic tendency, physical activity, smoking and drug use,

environmental exposure and stress can encourage the beginning of human diseases. Some diseases such as diabetes and allergic reactions like gluten and lactose intolerance have been diagnosed due to the consumption of ingredients such as eggs, vegetables, and/or milk proteins, which explain the need of legal regulations for proper labelling. The use of labels enables one to understand the relationship between certain nutrients and diseases, compares the nutrient content of similar foods and verifies if food is suitable to the overall diet of consumers (Mnerie et al., 2015). Hence, labelling requirements should be applied with regards to claims concerning the reduction of the risk of disease, for example, food labelling with the description 'low fat' or 'low calories' may stimulate the consumer to buying more and larger food portions.

1.3. Awareness of Food Labelling Information

There are several factors which consumers observe on food labels, this is, if the food product is safe or genetically modified, traceability, animal welfare, environmental protection, fair trade, organic food, sustainable agriculture and brand reputation. The food product must comply with regulations, ingredient composition, production protocols, specific practices and technologies as well as genetic identity (Huck et al., 2016). Regulation (EU) nº 1169/2011 has several mentions, for example, a list of mandatory details, additional mandatory specifications for specific food types and/ or categories. It also refers to the availability and location of the mandatory food information and the detailed provisions on mandatory particulars. Labels provide information on the ingredients of the products, nutritional properties, shelf-life, country of origin or place of provenance, preparation and storage time (Bandara et al., 2016). Researchers, in numerous studies, have included other food labelling indicators that have emerged in the European food market, for example, food miles; location of origin; carbon footprint and improved animal welfare indicators (Gracia and de-Magistris, 2016). The indication of ingredients or technological auxiliaries which cause allergies or intolerances used in food manufacturing or preparation is mandatory, thus the indication of allergenic substances must be clearly included in the ingredients list. The name of the substance should be highlighted through clear spelling which differentiates it from the remaining list of ingredients. It is also mandatory to include ingredients or substances used which may endanger the health of consumers, such as glycyrrhizic acid or its ammonium salt. Consequently, the food product should be marked immediately after the list of ingredients. The Nutritional Declaration includes information on the average nutritional values per 100 g/ml, namely energy, amount of lipids (saturated acids), carbohydrates (sugars), proteins and sodium. These indications may be

accompanied by other nutrients such as monounsaturated and polyunsaturated fatty acids, polyols, starch, fibre and vitamins or minerals. The nutritional declaration should appear in a tabular or linear format, according to the available space (Gracia and de-Magistris, 2016). Bandara et al. (2016) referred that consumers consider the name of the food as being the most important mandatory labelling information, scoring at 85.56%, followed by date of minimum durability, list of ingredients, quantity of certain ingredients, special storage information or conditions and instruction for use, weight and/or volume, manufacturer contact details and product origin (Bandara et al., 2016).

1.4 Factors Related to Awareness, Knowledge and Use of Food Labelling Information among Consumers

1.4.1. Internal Factors

Currently, there is an increase in the consumption of ready-to-eat (RTE) meals and take-away food due to lifestyle habits. Consumers consider the nutritional value of food at moment of purchase to be important. Researchers have referred to several factors related to socio-demographic characteristics which can affect the interest and willingness of consumers in making use of labelled information, such as gender, civil status, educational level, income, time constraints, label format and nutritional knowledge (Cavaliere et al., 2017). Besler et al. (2012) considered that female consumers, married people and those with higher educational levels showed higher interest in nutritional labels while older consumers were more concerned about labelling due to increased health issues. (Besler et al., 2012). Kim et al. (2016) mentioned that the different ages of consumers confirmed differences between awareness and usage. Nutritional labelling can have an influence on the health and welfare of the population so as to promote healthy food product choices (Kim et al., 2016).

1.4.2. External Factors

The assessment of food products is influenced by a great number of cognitive, behavioural and attitudinal factors relating to nutritional label use, for example, remuneration, employment, health, diet-health awareness and/ or special diet, nutritional knowledge, meal planners and organic and certified food buyers (Besler et al., 2012). The factors that can influence consumer assessment is the trust in food manufactures and producers or any other actor in the food domain, the knowledge of the nutritional value of products and preference for food naturalness (Hartmann et al.). Miller and Cassady (2015) have shown that nutritional knowledge is

associated with a higher frequency of food label use, for example, nutritional labels, ingredient lists, and health and nutritional claims. However, there are doubts about whether the information on labels is well understood by consumers (Miller and Cassady, 2015).

1.5. Consumers Perception on Food Labelling Information

Consumers have many different reasons for examining food labels, for example, a new product, food allergies or intolerance, health consciousness, product variants, product origin, instructions for use, suitability for vegetarians/ vegan, organic food and religious (Bandara et al., 2016). It is important to be reassured that the information on labels truly identifies the products one wants to purchase. Many consumers have expressed their uncertainty on the reliability of food labels concerning mislabelling of organic, kosher, halal and/or other types of products (Huck et al., 2016a). However, consumers have reasons to distrust food labelling since a lot of information is much stricter or non-existent. Thus, consumers perceive the information on labels to be misleading (Fenko et al., 2016). Brand loyalty, lack of time and/or understanding, excess information and mistrust concerning food labels are good enough reasons for consumers not to read labels (Bandara et al., 2016). Bandara et al. (2016) referred that the major reasons for consumers to examine food labels were to: assess the suitability of the food product for vegetarians/ vegans (89%); avoid diseases related to food (85%), religious reasons (83%), and check whether the food is organically grown or not. For consumers, there are pertinent problems with labels, including misunderstanding, confusion, inappropriate font size, brand loyalty and credibility, lack of time, insufficient accuracy of information provided to accompany health claims. Ninety-eight percent of the respondents mentioned the habit of examining the labels on food products and 41% of respondents considered labels as a legal requirement (Bandara et al., 2016), nonetheless, Besler et al. (2010) referred that most participants (76.5%) reported they read food labels. The level of interest was higher when consumers considered purchasing a product they had not used before (78.3%), but lower when consumers purchased products of known brands (70.4%) (Besler et al., 2012). However, consumers believed to a lesser extent that food labels provided useful information but to a greater extent that there was too much information which was not easy to understood (Gracia and de-Magistris, 2016a).

1.6. Food Labelling Requirements or Food labelling policy

1.6.1. Nutritional Labelling

Today, there is an increase in the consumption of RTE meals and take-away food due to new lifestyle habits. Consumers consider the nutritional value of food upon purchasing to be important. The variations of interest of European consumers in nutritional labelling is due to education, cultural differences and the history of health policies. Factors such as region, age and gender, women, parents of children living at home as well as older consumers induce interest regarding nutritional labelling (Hieke et al., 2018). The different ages of consumers showed differences between awareness and usage. Nutritional labelling can have an influence on the health and welfare of the population, To promote healthy choices, nutritional labelling is an important educational tool (Kim et al., 2016). The directive 2000/13/EC indicates that consumers are obliged to have essential information, such as, composition, manufacturer, methods of storage and preparation and the existence of substances known as allergens. Decree-Law nº167/2004 establishes norms of nutritional labelling of food to inform the final consumer. The regulations on nutritional labelling of food products regarding recommended daily consumptions and energy conversion factors are defined in Decree-Law nº 54/201. The nutritional indication on labels is optional however, it is mandatory when a nutritional claim is declared or there is additional vitamins or minerals (Mnerie et al., 2015). When food has certain beneficial properties in food, packaging can have a type of claim that will be 'source of', 'free of', 'high', 'low' or 'reduced' in calories or in a particular nutrient (Mnerie et al., 2015).The European Regulation (EC) nº 1924/2006 mentions nutritional and health claims regarding food (Mnerie et al., 2015). Nutritional labels contain much information about the level of calories and daily amounts of macronutrients, (carbohydrate, protein and fat) and micronutrients (vitamins, minerals and fibres) (Miller and Cassady, 2015). Consumers, upon selecting food, give value to nutrients such as vitamins defined as qualifying nutrients and reject fat, salt and sugar also known as disqualifying nutrients (Kim et al., 2016). The legislation which covers nutritional and health claims prohibits all information which is false, ambiguous or misleading to protect the consumer. Cliona et al. (2018) in their study on the influence of nutritional labelling concluded that consumers were most likely to use nutritional labels where nutrition composition is heterogeneous and frequently ambiguous (Ni Mhurchu et al., 2018).

1.6.2. Quality Labelling

Consumers are increasingly interested in choosing products with higher quality, originality, and of traditional production. These want to know the country origin, quality label and price. Moreover, in agricultural policy, several quality attributes are considered important such as geographic origin, special ingredients, specific production and traditions, environmental and animal welfare standards, processing, preparation, presentation and labelling (Košičiarová et al., 2016). There is an increasing concern regarding extrinsic quality attributes that constitute an opportunity for food producers. The European Commission (EC) adopted three schemes of quality assurance to award products with certain conditions. These schemes are used to help consumers at the time of purchase, encourage agricultural production, and protect product names (Grunert and Aachmann, 2016). Protect geographic indications (PGIs) are a distinctive sign which defends food product closely linked to a country and/ or region, this is, where specific quality is related to phases of production, processing or preparation of these locally. The Protected Designation of Origin (PDO) is a distinctive sign that protects food products with specific qualities and/ or characteristics which are obtained in the production process through natural and human factors which derive from a particular region or country (Bernabéu et al., 2018). The distinctive sign, Traditional Speciality Guaranteed (TSG) in food products ensures that the production and composition of food has traditional characteristics (Košičiarová et al., 2016). The production of food products in a specific geographic region and under the influence of human factors can be used with higher quality indications so consumers prefer organically produced food for reasons related to health, environment, and animal welfare. All organic products have a mandatory EU logo introduced by regulation (EC) nº 271/2010 making it easier for consumers to recognise these products in the market. To obtain the organic product logo consumers must comply with Regulation (EC) nº 834/2007 where the applicable principles of organic production in agriculture, processing of organic food for animals and the rules of production are expressed. Food is considered organic when produced without the use of pesticides and fertilizers, growth hormones, irradiation or antibiotics and/ or any presence of genetic modified organisms (Lee et al., 2018). Organic labelling can encourage increased consumption of these food products (Janssen and Hamm, 2014). The quality and diversity of agricultural and fishery products is an important point that gives a contribution to the European cultural heritage and gastronomy. The European Commission (EC) has encouraged the use of labelling to identify products through the

commission Regulation (EU) nº 1151/2012 applied to agricultural products for human consumption. This regulation which replaced regulation (EC) nº 510/2006 covers the protection of geographic indications and designations of origin for agricultural products and food products while regulation (EC) nº 1898/2006 lays down detailed rules of its implementation.

1.6.3. The European Union Law

A new food policy and regulatory framework was established based on risk assessment, control, management and communication aimed at providing food products with greater safety and health standards. In 1960, the Codex Alimentarius Commission was established by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) to protect the health of consumers and ensure fair practices in food trade. The Codex Alimentarius are international food standards, guidelines and codes of practice which contribute to the safety, quality and fairness of international food trade. Consumers can confide in the safety and quality of the food products they buy and importers can trust the food they have ordered in accordance with their specifications (Stankovic, 2016). The Directive 2000/13/EC establishes the labelling of foodstuffs and certain aspects relating to the presentation and advertising thereof. Regulation (EC) nº 178/2002 defines and specifies the general principles, requirements and procedures that underpin decision making relating to issues of food safety laws, covering all stages of food and feeding production and distribution (2002). Consequently, EFSA was created to implement general principles of food regulations becoming a basis for consumers to make informed choices about the food they consumed and, consequently preventing any practices that may mislead consumers. In 2004, the new "hygiene package" was created and introduced; Regulation (EC) nº 852/2004 relates to food; Regulation (EC) nº 853/2004 defines rules for the food of animal origin; Regulation (EC) nº 854/2004 outlines rules for the organization of official controls on products of animal origin intended for human consumption. Additionally, Directive nº 2004/41/EC establishes food hygiene and health rules for the production and marketing of certain products of animal origin intended for human consumption. ISO 22000:2005 constitutes specific requirements about food safety which controls hazards in the food chain to ensure that a food is safe at the time of human consumption. The principles of the Hazard Analysis and Critical Control Point (HACCP) were implemented in this standard (Soman and Raman, 2016). The amendments to the Regulation (EC) nº 1924/2006 are part of the Regulation (EC) nº 1169/2011 that provides food information and outlines the means to guarantee the right of consumers to

specific food information. Regulation (EC) nº 1337/2013 covers the indication of country of origin or place of provenance for fresh, chilled and frozen meat of swine, sheep, goat and poultry. In Portugal, Decree-law nº 194/2012 has indicated the food and economic safety authority (ASAE) responsible for verifying compliance standards so the information of food labelling is not false, ambiguous or misleading and does not raise any doubt about food safety.

2. FOOD FRAUD/ADULTERATION

2.1. Outlines and Types of Adulteration

Food adulteration can be described as the actions that are carried out when deleterious or expired food is mixed with or substituted by another food item, an adulterated food item is purposely mislabelled, or has toxic substances added to it (Peng et al., 2017). Mislabelling can appear in different forms as well as at any stage during the production process thus changes can occur while the ingredients listed on the labels might continue the same. In some cases, the country of origin has also been changed or allergens have not been reported, which is also a special case of mislabelling (Huck et al., 2016a). Economically motivated adulteration (EMA) has been described as "The fraudulent, intentional substitution or addition of a substance in a product for the purpose of increasing the apparent value of the product or reducing the cost of its production for example, economic gain."(Manning and Soon, 2014). Adulteration of food products has become a serious issue throughout the world. There are different types of fresh meat and fish product adulteration this is, partial or total substitution of commercial valuable species by cheaper products, as adding proteins from several origins, substituting fresh meat by frozen-thawed meat, adding food additives or not describing genetically modified organisms (GMO).

2.2. Historical Perspective and Notable Incidents

Consumer perception showed that increasingly globalized markets and greater processing in the food chain has contributed to a perceived distance and knowledge gap between people and food manufacturers, for example, how food is produced or where it is produced (Asioli et al., 2017). Food scandals provide opportunities for the public to scrutinize actions of those within the agricultural and food sector (Tonkin et al., 2016). Consumers are increasingly concerned about the apparent lack of control and safety of agriculture and food industries. The last two decades, have witnessed multiple outbreaks: bovine spongiform encephalopathy and dioxin contamination; heavy use of pesticides in conventional and intensive agricultural practices;

phenylbutazone and nitrofuran contamination; use of artificial ingredients, additives or colorants; horsemeat and Chinese milk scandal; false labelling on halal products and the adoption of controversial food technologies like genetically modified organisms (Asioli et al., 2017). Consequently, the European policy and food chain have changed substantially.

2.3. Factors Related to the Occurrence of Food Fraud or Adulteration

Fraudsters are often connected to the food supply chain and when they perceive an opportunity for economic gain, they commit fraud. These can be legitimate companies or employees who have no regard for the health or interests of consumers (Spink et al., 2016b). Factors such as opportunity (time and place) and motivation (cultural and behavioural) influence fraud nonetheless, technical and managerial control measures inhibit the susceptibility of fraud vulnerability.

2.3.1. Economic Factors

The production of sufficient, safe and nutritional food is a global challenge faced by the actors operating in the production chain. Fraud in the supply chain can arise as a result of misrepresentation associated with product and process integrity (authenticity). Therefore, personal integrity can be described as the honesty and principles exhibited by an individual while data integrity relates to the information accompanying the food item throughout the food supply chain which is the consistency and accuracy of data through the food product life-cycle. (Manning, 2016). The Rapid alert system for food and feed (RASFF) however determines six types of fraud; improper, fraudulent, missing or absent health certificates; illegal importation; adulteration; improper, expired, fraudulent or missing common entry documents or import declarations; expiration date, and mislabelling (Bouzembrak and Marvin, 2016). Mislabelling can appear in different forms as well as at any stage during the production process. Changes can occur during the production process, nevertheless the ingredients listed on labels might stay the same. In some cases, the country of origin has also been changed so allergens are sometimes not reported, which is a special case of mislabelling. Authenticating products is imperative for consumers, official bodies in charge of labelling and industries where incoming batches of raw materials and finished products must be tested. (Huck et al., 2016a). The reasons for the growth of food counterfeiting can be attributed to the increase in world trade and emerging novel markets. Fraudsters usually demonstrate technological expertise so as to actively to avoid detection. These substitute specific ingredients or even whole products in order to have greater

yield profit, consequently inadequate legislation in different countries has encouraged incorrect labelling, often resulting in consumer fraud (Huck et al., 2016). There are differences between food fraud vulnerability, threat, and risk. Food fraud vulnerability is the susceptibility of a system, for example bovine meat products are not tested for other species of meat. Food fraud threat is the cause of an event, for example, fraudster can mix bovine meat with horse meat and then sell it to a deceived consumer. Finally, food fraud risk is the combined likelihood and consequence – that considers threat and vulnerability – of food fraud (Spink et al., 2017). Fraud causes an increased lack of trust in consumers towards food products and harm to human health depending on the nature of the fraud (Manning, 2016). Several economic factors affect the increase of fraud: supply of raw materials; addition of valuable attributes; health of businesses; competition and financial strains forced on suppliers and price differences due to diverse regulations in countries (van Ruth et al., 2017). Relatively to the supply of raw materials, factors can be described as the substitution of a higher quality product with one or more lower quality ones. And when There is regional or global supply shortage or few physical product availability, prices can shift, causing an increase in fraud vulnerability (Huck et al., 2016). Regarding the addition of valuable attributes to food products, one can refer that the assignment of quality labels in food products can augment prices compared to similar products. Another situation is, producers can increase the artificial content of markers with certain specific characteristics, for example, the addition of protein and fat contents of milk, motivations/decisions that can increase fraud vulnerability. Economic motivation influences the vulnerability towards fraud and can have different forms; profit maximization or loss minimization; economic health businesses and level of competition; severe weather conditions. There are several ways to cut costs, this is, removing traceability, eliminating controls and buying ingredients or products from cheaper or unknown suppliers. Comparatively to financial strains, when powerful consumers require large quantities of specific products and the supplier is unable to meet the demand, suppliers feel the need to fill the gap with alternative products that can be fraudulent (van Ruth et al., 2018).

2.3.2. Cultural and Behavioural Factors

The factors that encourage cultural and behavioural aspects of fraud vulnerability are the business strategy, previous criminal offences, and ethical business culture of organizations, international and national corruption levels in which organizations operate and are victimized. Business strategies can refer that some organizations have hard-to-reach financial goals so the

potential fraudsters namely employees, for the sake of saving themselves from being fired, are influenced by the business strategy of their organization to commit fraud (van Ruth et al., 2017). The ethical business culture, the agro-industry with a culture described by employee demotivation, distrust and discontent can be a cause for unethical behaviour. Besides, unethical business cultures produce processes of the normalization of fraud, which support fraudulent activity in the long term. The industries which have a history of criminal offences, have a higher risk of committing new crimes. Therefore, previous criminal offences of organizations and individuals potentially increase the vulnerability to fraud. The level of corruption of a country can also influence the risk of food fraud of a company, hence, it is possible to state that the company can choose illegal or immoral means to gain profits due to the (inter) national corruption level (van Ruth et al., 2018). The victimization of companies may fall upon food fraud by activities of others in the industrial chain or sector and imminent fraud through recurrent victimization due to factors which were already present and not mitigated after the first victimization. Counterfeit products can affect the brand of a defined region and the symbolic value of product negatively (Huck et al., 2016).

3. PREVENTION OF FOOD FRAUD OR ADULTERATION

Currently, truthfulness and authentication of food labels represents a major concern for the food industry, regulators and consumers. Fraud in the food supply chain can arise as a result of misrepresentation associated to product integrity (authenticity); process integrity; people integrity which can be described as the honesty and principles exhibited by an individual and/or data integrity of information accompanying the food item throughout the supply chain which is the consistency and accuracy of data through the food product life-cycle (Manning, 2016). Food fraud is committed by complex food supply chains, local and niche food activities and to implement this, it is necessary to demonstrate product and process authenticity. The factors which will mitigate fraud include: appropriate legislation, technology, inventory management (Manning, 2016) .

3.1 Statutory and Regulatory Authorities

There are strict protocols in industry at different levels of the food chain however, it is necessary that food regulators play a special role in monitoring and enforcing existing regulation because they have an important impact on consumer trust. (Huck et al., 2016). There are different organizations whose objective is the identification of emerging risks in the food chain. This

function is essential to protect consumer health and interests of the food industry, too. The World Health Organization (WHO) has a leadership role in promoting food safety worldwide. International Food Safety Authorities Network (INFOSAN) is an organization which assists member states in managing food safety risks, and ensures quick sharing of information during food safety emergencies to stop the spread of contaminated food from one country to another (Schlundt, 2014). The aim of this organization is to transmit important information in the interest of global food safety, promote partnerships and collaboration among countries, between networks and help countries to strengthen their capacity in managing food safety emergency. The European Food Safety Authority (EFSA) has an important role in emerging risk activities in areas such as food and feeding safety, animal and plant health and providing a mechanism whose information can be shared and communicated on a scientific basis (Costa et al., 2017). According to the definition adopted by the Scientific Committee of the EFSA in 2007, "an emerging risk to human, animal and/or plant health is understood as a risk resulting from a newly identified hazard to which a significant exposure may occur or from an unexpected new or increased significant exposure and/or susceptibility to a known hazard "(EFSA, 2007). The European Commission created the Food Fraud Network whose objective is sharing information in matters of suspected, intentional and economically motivated violation incidents of food fraud. RASFF ensures that food is safe for consumers and the disclosure of information allows quick rapid reaction when there are risks for public health in the food chain. Additionally, this organization allowed that many food safety risks had been averted before they could have been harmful to consumers. Through RASTFF, vital information is exchanged which can lead to products being recalled immediately from the market. Between 2000 and 2013, RASTFF reported the existence of 749 notifications under the hazard category "adulteration/fraud" (Bouzembrak and Marvin, 2016a). In Portugal, the ASAE states its mission as the supervision and prevention of compliance with legislation regulating the exercise of economic activities, in the food and non-food sectors, as well as the evaluation and communication of risks in the food chain, being the national body of connection with its counterparts, at a European and international level. ASAE is governed by the principles of scientific independence, precaution, credibility, transparency and confidentiality (Mil-Homens et al., 2016). In the U.S., Food and Drug Administration (FDA) emits regulations and guidance for the food industry, so it plays an important role concerning education so that consumers were provided with accurate and useful information on labels. This organization has the responsibility to uphold the development of

more nutritious food, and to protect consumers from the exposure to harmful food constituents (Mayne and Spungen, 2017). In 2015, the US Food Safety Modernization Act (FSMA) published FSMA Preventative Controls rule (FSMA-PC). This rule covers the mentions "economically motivated adulteration", but it did not mention Food Fraud. This organization aims at the prevention of all food safety hazards regardless of their nature. The FSMA-PC does not assure the compliance with all USFDA regulation scanned and referred, US law, or even with all FSMA.

3.2 Industry

The reasons for growth of food counterfeiting can be attributed to the increase in world trade and emerging novel markets. Fraudsters usually demonstrate technological expertise and actively seek to avoid detection. These substitute specific ingredients or even whole products to change and yield greater profit. There is inadequate legislation in different countries encouraging incorrect labelling, often resulting in fraud by the consumer (Huck et al., 2016). In industry, the product is considered 'authentic' when it complies with the ingredient composition, production protocols, labelling regulation, genetic identity and certain practices and technologies necessary for production (Huck et al., 2016). The Global Food Safety Initiative (GFSI) aims at harmonizing the Food Safety Management System, food chain and the strategic direction of GFSI around the world in providing groups of retailers, manufacturers and food service operators. This organization created a Food Fraud Think Tank (FFTT) to further advance the food fraud mitigation topic. The Safe Supply of Affordable Food Everywhere (SSAFE) is an industrial group that has the objective to improve food protection systems and standards and is based on the GFSI parameters with several methodology for each individual product (Spink et al., 2016)

3.3 Scientific community

EFSA works with the information which is provided by Scientific Committees, namely, methodologies on scientific matters of a horizontal nature and strategic scientific advice. The Scientific Committees have the function to provide scientific advice and draw its attention to new and emerging problems.

3.4 Consumers / End-users

Most consumers would unknowingly and unintentionally purchase counterfeit products because product counterfeits consist of reproducing original products. The authentication of product is important for consumer, official bodies in charge of labelling and for industries where incoming batches of raw materials and finished products must be tested (Huck et al., 2016a). Fraud causes an increased lack consumer trust in food product and has the potential, depending on the nature of the fraud, to harm human health (Manning, 2016). Several prototypes of devices for self-authentication of labels have been developed to detect food adulteration so consumers can test the origins and ingredients in food products. The species-specific DNA-based tags permit authenticating food however, they are quite time-consuming and expensive. The self-authentication of food products is important due to the lack of governmental action on food adulteration (Huck et al., 2016). Recently emerged, spectroscopic and chromatographic techniques combined with the use of multivariate and chemometrics methods where determination of product integrity can be verified. Currently, there is the smallest NIR spectrometer weighing proximally 60g that permit the analysis of micro-electro-mechanical systems to produce optical-mechanical functions in the near infrared region (4000–12000 cm^{-1}) and linear variable filters in combination with ATR MIR (400–4000 cm^{-1}) spectroscopy.

4. Analytical Methods for Detection of Food Adulteration in Products of Animal Origin

Currently, detecting food adulteration, quick, reliable and competent analytical methods are required to tackle authentication challenges and ensure product quality for consumers (Lohumi et al., 2015). There are several methods in detecting low levels of adulteration: biochemical fingerprinting and/or DNA fingerprinting; protein electrophoresis; isoenzyme electrophoresis namely, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), pulsed-field gel electrophoresis (PFGE); and chromatographic fingerprinting; gas chromatography (GC) and high performance liquid chromatography (HPLC) (Manning and Soon, 2014). Protein-based methods represent techniques that use proteins as specific markers, which include

electrophoretic methods, such as isoelectric focussing (IEF), chromatography, immunological techniques like Western-Blotting, enzyme-linked immunosorbent assay (ELISA), and

proteomics.(Rahmati et al., 2016). However, these methods are arduous, technically demanding, slow, invasive, expensive, destructive, and require sophisticated laboratory procedures and highly qualified employees. Moreover, they are not suitable for real-time applications.

4.1. Isoelectric-Focusing Methods

Electrophoresis is a promising technique to detect food product adulteration and authentication. Some methods based on protein analysis, such as sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE), isoelectric focusing electrophoresis (IEF) and two-dimensional gel electrophoresis (2-DE) have been applied in some different animal species (Trimboli et al., 2017). Isoelectric focusing consists of separating proteins based on their differences in the isoelectric point (PI). This method has certain advantages, for example, cheaper, faster, and easier to apply since it controls laboratories and uses less sophisticated technology than the DNA-based techniques for specie identification (Ortea et al., 2010). Ortea et al., (2010) stated that by using isoelectric focusing, he was able to identify commercial prawn and shrimp species as a food interest and referred that this was a simple method and fast technique to be used by fisheries to prevent mislabelling and fraudulent practices (Ortea et al., 2010). The capillary electrophoresis (CE) is a technique which helps one understand protein separation due to excellent resolution power, high speed and easy automation (Trimboli et al., 2017). Trimboli et al., (2017) used CE to quantify ewe milk adulteration with cow milk and revealed that this method is a rapid, inexpensive and robust screening approach to identify fraudulent samples (Trimboli et al., 2017). However, electrophoretic methodologies have some disadvantages such as high procedure complexity, degradation of proteins because the food is processed and false results arise due to mixing several species and closely related species compared to the proteomic methods (Ortea et al., 2016).

4.2. Immunological Methods

Immunological tests have several advantages as specificity, sensitivity, easy implementation for non- specialised employees, the possibility to process a high number of samples in a short

time and low-costs making them available for routine use in food control laboratories (Sentandreu and Sentandreu, 2014). Several immunological tests namely the Precipitin Ring Test, the Hemagglutination Inhibition Test, Agar Gel Immunodiffusion (AGID), Radioimmunoassay (RIA), Lateral Flow (non-enzymatic chromatographic immunoassays), and enzyme-linked immunosorbent assay (ELISA) have been applied for meat specie identification. These tests consist of the capacity of antibodies to specifically detect the protein characteristic of an individual animal species or meat adulterant. However, in cases of closely related species. If antibodies are not specific for individual species, they can give false-positive results or underestimated results of the ingredient subjected to quantification. The problem arises with highly-processed meat product investigation, especially in production conditions which can lead to protein denaturation. Hence, they have other limitations as there is a need for specific antibodies. Within the immunological methods, ELISA is an optimal method for obtaining quantitative data and the most widely used in meat products and processed meat authentication (Abbas et al., 2018). Jiang et al., (2018) showed that the quantification of porcine haemoglobin in meat products after validation has high sensitivity, good precision, reliable and satisfactory performance (Jiang et al.). Another study, Zvereva et al. (2015) used a sandwich ELISA to characterize the skeletal muscle protein troponin I and species-specific biomarker of mammalian muscle tissues in raw meat. This method was suitable to detect TnI in mammalian meat and thus distinguished these samples from poultry meat (Zvereva et al., 2015). Nonetheless, Perestam et al. (2017) compared real-time PCR and ELISA-based methods for pork and beef species and concluded that the real-time PCR was a more sensitive, reliable and less expensive method. ELISA requires less time to consume and is easier to perform (Perestam et al., 2017).

4.3. Proteomic Methods

The proteomics method is used on specific-species or specific-component peptides which are more stable during thermal processing than in DNA. The heating processes applied to meat can cause denaturation and insolubilisation of native sarcoplasm proteins however, these techniques are suitable for identifying proteins in processed meat. The determination levels of specific proteins in the samples enables the identification of the origin of the species meat. Colorants, aromas, preservatives, stabilizers can be added to meat products to increase fresh meat appearance, flavour and shelf-life. Smoke aroma can be untruthfully used instead of natural

meat smoking. There are chromatographic methods such as liquid (LC) and gas (GC) chromatography which enables the identification of a specific ingredient in food products. The advantage of this method is the capacity of separating many compounds. GC is an essential technique in the separation and differentiation of natural products due to its high separation efficiency therefor it can be applied to determine food composition and detect and trace the amount of food additives and pesticide residues. Regarding the determination of organic compounds added to meat products, GC may be appropriate (Rohman et al., 2012), seeing that it is more suitable for detecting volatile and semi-volatile compounds compared to LC (Luykx et al., 2018). The mass spectrometry (MS) method has an important role in the identification of proteins being considered an alternative for meat specie identification. The method for several food matrices has the correct selectivity, sensitivity and discriminating capacity and can be used coupled with chromatography originating liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) (Herrero et al., 2012). These techniques permit the determination of fatty acids as target analytes for the specie identification, while the percentage of saturated, monounsaturated and polyunsaturated fatty acids can be used as an indicator of animal species (Luykx et al., 2018). Ultra-high performance liquid chromatography (UHPLC) has several advantages such as high efficiency, good resolution, relatively short analysis time and the use of flow rates fully compatible with mass spectrometry (MS) to detect food safety, quality and traceability (Prandi et al., 2017). Li et al. (2018) have applied ultra-performance liquid chromatography (UPLC)-MS/MS determination of fatty acids to identify species or to verify the percentage of saturated, monounsaturated and polyunsaturated fatty acids of animal species in food. However, it can have less reliable results when it comes to specie identification since the authenticity of food, claims not only a method to determine heat stable peptides for eight animal and plant species in meat products but also concludes that this method is accurate, sensitive, simple, and can be used to identify different types of adulteration (Li et al., 2018). Prandi et al. (2017) have used mass spectrometry to quantify beef and pork in highly processed food. These researchers refer that this method is a very promising tool to detect a few amounts of meat species in highly processed food (Prandi et al., 2017). In another study, the HPLC-MS/MS screening method was used to detect lupine, pea, and soy proteins in meat products. One also concluded that the method has high potential for the development of the simultaneous detection of additional sources of foreign proteins (Luykx et al., 2018).

4.4. Molecular Methods

DNA is a macromolecule with genetic information that is an excellent source to food. A single DNA molecule contains coding information for a high number of genes consequently only a single sample of specific genes is analysed using what is called polymerase chain reaction (PCR). DNA techniques are reliable due to high specificity, sensitivity, quickness and cost effectivity. DNA-based methods are linked either to the PCR, electrophoresis in agarose or polyacrylamide gel. PCR consist of the exponential amplification of specific DNA fragments, generating an amount of DNA fragments sufficient for analysis. Currently, there was an increase of DNA-based methods over protein-based techniques due to the capacities such as stability against high temperature, pressure, and chemical treatment. Electrophoretic methods enable one to analyse the amplified DNA fragments to determine the adulteration of meat and fish food products. These methods can include the restriction fragment length polymorphisms (RFLPs) (Ali et al., 2018a), random amplified polymorphic DNA (RAPD) (Koh et al., 1998), single-strand conformation pattern (SSCP) (Tisza et al., 2016a), real-time PCR (Druml et al., 2015a), and multiplex PCR (Ali et al., 2015). PCR techniques are a highly sensitive and specific test which helps identify species or breeds in a quick and reliable manner compared to protein-based methods. As well as appropriate in the identification of degraded DNA with apomorphic sites. The RFLP method can be used to determine inheritance patterns, identify specific mutation, recognise change in genetic sequence and provide correct detection, even if several species are present in the food product. This method can authenticate the amplified PCR product through restrictive digestion using restriction enzymes (Ali et al., 2018a). However, PCR-RFLP or conventional PCR is suitable for obtaining quantitative information. Ali et al. (2018) showed that the PCR-RFLP method was suitable for discriminating several species namely rabbit, rat and squirrel in frankfurter products. The assay's detection limit was tested until 0.1% adulteration (Ali et al., 2018). The SSCP has a cheaper, quicker and more sensitive method superior to the RFLP analysis so it is an appropriate tool for fraud detection. Moreover, it is founded on the electrophoretic mobility of the single-stranded DNA molecule under denaturing conditions in a polyacrylamide gel, where mobility depends on DNA conformation, allowing the detection of point mutations at multiple positions in DNA fragments and in other DNA polymorphisms. The PCR-sequencing method obtains precise information of the nucleotide sequence within a DNA molecule. And for authentication of animal species, the development of DNA sequence database was suggested. Currently, it is difficult to apply sequencing with

mixtures of several species (Tisza et al., 2016a). The PCR-based capillary electrophoresis (CE) applications have several advantages, such as automation, higher resolution, speed and reproducibility. The cost of this method is better for PCR-SSCP compared to CE-SSCP. In a study, Tisza et al. (2016) compared PCR-SSCP and CE-SSCP methods to detect poultry species where a reliable detection limit was 0.5% DNA for both applications, reflecting 0.75 ng DNA (Tisza et al., 2016).The multiplex PCR amplifies numerous different DNA targets simultaneously, thus helping a fast detection of multiple species in a short time compared to a simple PCR. This method is economical and less arduous resulting in reduced time and work however, it can have problems such as the formation of misprimed PCR products or "primer dimers", and the amplification discrimination of long DNA fragments. The multiplex PCR method uses species-specific primers that are greatly promising since it offers multiple target detections in a single assay, reducing cost and time compared to conventional single-species PCR (Ali et al., 2015). Prusakova et al. (2018) in a study showed that multiplex PCR assay can detect ten meat species thus the assay is accurate, specific, robust, simple, has high sensitivity and can be easily implemented in a routine laboratory analysis without specific and sophisticated equipment (Prusakova et al., 2018). The real time PCR method is based on the polymerase chain reaction, which is used to determine the nucleic acids data in real time, while the reaction proceeds. This method used specific specie primers and probes. Nevertheless, this method is more expensive, can produce false-negative results, and needs suitable equipment to carry out the analysis (Druml et al., 2015a). Ren et al. (2017) used the real-time PCR method to quantify mammalian and poultry species in food and concluded that this method is sensible, accurate and specific for detection and quantification of mammalian and poultry species (Ren et al., 2017).

4.5. Spectroscopic Methods

Several vibrational spectroscopic techniques have been developed over the past decades because they offer several advantages such as enabling complex chemical information to be determined whenever samples are being scanned (Lohumi et al., 2015). Emerging non-destructive mapping technologies for authentication, determination of adulteration and traceability include nuclear magnetic resonance (NMR) imaging, fluorescence (FS), visible (VIS) (Ferreiro-González et al., 2017), near infrared (NIR) (Rady and Adedeji, 2018), mid infrared (MIR) (Zhao et al., 2014), and Raman spectroscopy (RS) (Zając et al., 2014) sometimes

coupled with Fourier transform technique (FTIR), and multispectral (MSI) and hyperspectral (HIS) imaging (Feng et al., 2018). These are simple, non-destructive, non-invasive, low-cost, which allow real-time analysis. All spectroscopic techniques require small samples and no further preparation is necessary. They are powerful tools for conducting adulteration tests (Abbas et al., 2018). The methods presented might be used as a complement or even constitute an alternative to PCR based DNA (Lohumi et al., 2015, Manning and Soon, 2014).

4.5.1. Fourier Transform Infrared (FTIR)

Recently, Fourier transform infrared (FTIR) spectroscopy has been considered an ideal analytical method for accurate studies because it is quick, non-destructive and does not involve arduous sample preparation (Rahmania et al., 2015a). The FTIR is considered a quantitative method because it is a "fingerprint" technique, in which the band intensities are proportional to the concentrations of their respective functional groups. This technique uses an interferometer and exploits the mathematical process called Fourier transformation (Bell, 2012). By using the Fourier transform, easy manipulation of the data after analog-digital conversion is possible. Furthermore, it achieves a high signal-to-noise ratio and consequently a high resolution. In FTIR, transmission or attenuated total reflection (ATR) techniques are commonly used to obtain the absorption spectrum while the ATR devices have multiple configurations with different characteristics (Mirabella Jr, 1985). The FTIR spectroscopy combined with a powerful chemometric technique is an emerging and reliable analytical method to identify adulteration of fresh food products.

4.7. Chemometric Methods

The spectroscopic methods associated to chemometric methods are tools to identify species and foodstuffs that are not on the label. These methods are used to improve the understanding of chemical information and to correlate quality parameters or physical properties for analytical instrumental data. The application of chemometrics is required to amplify the information of interest and lessen the undesirable information in the spectra. There are several chemometric methods applied in spectroscopy, namely PCA, partial least squares discriminant analysis (PLS-DA), partial least squares regression (PLS-R), Naive-Bayes, soft independent modelling of analogy class (SIMCA), k-nearest neighbour (KNN) and probabilistic neural networks (PNN). PLS-DA is a prevailing and commonly used method for discriminant classification, which is

particularly well appropriate to deal with collinear data matrices like in the case of infrared spectroscopic data (Callao and Ruisánchez, 2018).

5. OBJECTIVES OF THE PRESENT WORK

The main objectives of the present work were:

i) To assess and to understand the usefulness of the information provided for consumers in the label of foodstuffs, through the accomplishment of a consumer survey. The current study also pretends to assess the consumer's opinion and confidence in food labelling and to evaluate the consumers´ knowledge about food labelling and food fraud.

ii) To investigate the potential of the use of Fourier transform infrared (FTIR) spectroscopy coupled with chemometric to detect the adulteration of wild fallow deer meat (*Dama dama*) with goat meat (*Capra aegagrus hircus*) and *Atlantic salmon* with *Onconrhynchus mykiss* along the storage period, as a quick and accurate analysis of food constitution to avoid fresh food fraud or adulteration.

CHAPTER III. MATERIALS AND METHODS

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1. SURVEY FOR CONSUMERS PERCEPTION ON THE FOOD LABELLING INFORMATION

1.1. Survey Design and Collection

1.1.1. Study Design

An exploratory study was carried out on labelling to allow for probe and adjusting initially proposed objectives, acquiring knowledge about research method. The study design used was analytical cross-sectional. The design was selected that it allowed analysis of factors related to awareness of food labelling information and use of such information in purchasing foods products.

1.1.2. Study Area, Population and Sampling Procedure

The survey was applied in a random to all regions of the country, in consumers with different age and civil status. Simple random selection was used to get selected a minimum of 308 respondents were questioned in order to attain the estimated sample size (300). Respondents were selected using convenient – quota sampling method because of unavailability of sampling frame due to the nature of the study population and site.

1.1.3. Data Collection

For the purposes of the investigation, information was collected about labelling, implying the use of documentary research and inquiries already applied to the consumer. Through the documentary research in particular, legislation, research articles, descriptive information was collected regarding, to the constitution of the label, their reading and consumer opinion, crises of consumer confidence, fraudulent labelling and confidence in food product.

In relation to surveys, it was studied type of questions that could be applied (open response, Likert scale, among others) and works which was investigated and published in journal academic. The aim of survey was provide new data information to food industry and give to know the confidence level consumers have in food products. The elaboration of this survey also in the preparation of training that could be given to consumers to better interpret the labels.

The survey was conducted close ended questions and in Likert 1-5 format who was used to collect information on the study variables (socio-demographic characteristics, awareness and opinion of food labelling information (format and language) and food product attributes.

After the investigation was completed, this was validated through the application of the pre-test a small sample of 20 consumers with similar characteristics to the population with the purpose of validating the questionnaire, in order to detect potential errors, difficulties of interpretation, time spent in response, response instructions or other important aspects that could unintentionally influence the understanding of the issues, in order to make the final questionnaire more effective and valid. Pre-test of the data collection tool (survey) was done prior the study, at the gym and supermarkets.

Consumers were asked about food labelling and whether they are used to read or not food labels. Level of awareness on label was obtained by asking respondents to express their familiarity with the 9 standard information which is supposed can be found on pre-packaged food labels. Respondents were asked to explain their perception on the importance of food labelling information and difficulties they encounter in reading and understanding food labels. On the other hand, respondents who do not read food labels were asked to briefly explain the reasons for not doing that.

1.1.4. Study Variables

The dependent variable was gender, age, education level, civil status, remuneration, physical activity and lifestyle habits health.

Independent variables included social demographic characteristics of respondents, awareness of food labelling information, food labelling information (format and language) and product attributes such as price and appearance product.

1.1.5. Ethical considerations

Participants were given brief information on the nature of the study and requested to sign a consent form for their participation. However, based on the nature of the field environment (supermarkets and gyms). The respondents did not sign consent forms and therefore verbal consent was used.

1.1.6. Survey

The survey which involved 308 respondents in Portugal was carried out between September 2016 and October 2017. Answers were available on google forms online but participants were interviewed face to face. The survey was developed by professionals of food science and comprised three sections: Section A: designed to establish the sociodemographic profile of interviewees and their health concerns; Section B: aimed at understanding if the constitution of labels (yes or no) are read, trusted or easy to visualize and consumer opinion about important factors upon purchasing They had to use a 5-point Likert scale (1 = never; 2 = rarely, 3 = sometimes, 4 = frequently, 5 = always); Section C: related to the reasons why consumers read or not the label and the Section D: knowledge about the frequency of reading food labels, trust in the constitution of food employing; and the consequence of food adulteration/ fraud (5-point Likert scale (1 = weightless; 5 = extremely serious). The final survey was scrutinized by experts in consumer science and statistics for content validity **(Annex I).**

1.2. Data Analysis

1.2.1. Descriptive Statistics and Cronbach's Coefficient Alpha

At the end of the digital data collection, the data were imported and the entire statistical component was treated into SPSS (Statistical Package for the Social Sciences) software. Acclamations were created to identify the issues, then the existence of errors in the transcription of the data was verified through the verification of outliers and missing values. Subsequently, the characterization of the sample was made through the descriptive analysis. The descriptive statistics also known as frequencies were performed to understand consumer knowledge and opinion. The Factorial Exploratory analysis of the data matrix was performed to ascertain the reliability of the factorial structure, having been elaborated by the calculation of Cronbach's Alpha, to estimate the reliability of the questionnaire and the consistency of the factors. Cronbach's alpha evaluates the level of correlation of the items of a data matrix with each other. The last stage of the investigation corresponds to the presentation of the conclusions, which show the results of consumer opinion regarding food labelling and the consequences of adulteration by the food industry. Internal reliability of extracted factors was determined through Cronbach's coefficient alpha and mean inter-item correlations (Peterson, 1994). Mean factor scores were calculated from the mean of all the questions contributing to it, so that factor scores could be interpreted on the original 5-point Likert scale. To determine inter-item

reliability, cross-tabulations between similar in meaning, or opposite items were determined. Spearman's correlation coefficients ($P < 0.01$) were calculated in order to study the relationship between variables (Hauke and Kossowski, 2011). These statistical tests were done with IBM® SPSS® version 22.

1.2.2. Kruskal–Wallis Test

The Kruskal–Wallis test (KWt) was used to indicate differences between respondents according to sociodemographic variables and lifestyle options and the consumer health variable. KWt was performed to assess statistical significance between the demographic characteristics of respondents and awareness and use of food labelling information in decision making during purchase of pre-packaged foods. Frequencies for information mostly sought by respondents when reading food labels, motivations to read food labelling information, perceived importance of food labelling information, circumstances in which respondents purchase pre-packaged foods without reading labelling information and difficulties encountered in reading food labelling information were determined. Values of $p < 0.05$ were considered as statistically significant (Ruxton and Beauchamp, 2008).

1.2.3. Principal Components Analysis (PCA)

PCA was performed to obtain a smaller number of uncorrelated factors (principal components, PCs), and thus reducing the size of the data set. The decision to use PCA was confirmed by Bartlett's sphericity test ($P < 0.0001$). The number of components retained in the final solution was based on the Kaiser-Meyer-Olkin criterion (>0.8) for the analysis of eigenvalues (>1) and the proportion of variance retained (>65%), usually seen as the minimum needed to make the model suitable for explaining the original data (Polyak and Khlebnikov, 2017).

2. DETERMINATION OF ADULTERATION IN FRESH FOODS

2.1. Sampling

2.1.1. Meat Adulteration

Fallow deer (D) and goat (G) meat were excised from carcasses 24 hours *post mortem*, cut in smaller pieces and later minced. Four batches of fallow deer adulterated with goat were prepared and transformed into mini burgers (samples). Five formulations, with the values of 0, 25, 50, 75 and 100 %w/w of goat were considered. The samples, weighing approximately 30 g, were packed in air and overwrapped with polyethylene film. Following packaging, samples were stored at 3 ºC and examined after 12, 24, 36, 48, 72, 96, 120, 144, 168, 216, 264, 312, 360 and 432 h. At each sampling point (including t=0), the samples were analysed for three parameters; spectroscopic, pH and microbiological analysis. The microorganism's results were totally mesophilic (TVC) and psychrotrophs. Four batches were assessed and each one was constituted by 75 samples (15 sampling points and 5 formulations), therefore, 300 samples were analysed.

2.1.2. Fish Adulteration

SS and OM fish were eviscerated, the removal of the skin was carried out hygienically and muscle was crushed separately in a mincer with hygienic conditions, previously sterilized.

Samples of adulterated SS with OM in phases of 10%, samples were obtained from 100% SS to 100% OM. Samples were obtained with the following percentages: 100%SS/0%OM, 10%SS/90%OM, 20%SS/80%OM, 30SS/70%OM, 40%SS/60%OM, 50%SS/50%OM, 40%SS/60%OM, 30%SS/70%OM, 20%SS/80%SS, 10%SS/90%OM and 0%SS/100%OM.

These samples were produced in duplicate, one for fat extraction and another for microbiological analysis.

Mini burgers weighing approximately 15g were packed in air overwrapped polyethylene film. Following packaging, samples were stored at 3ºC and examined at intervals of 0, 72, 160 and 240h. The experiment was repeated 4 times over a period of a few months, a total of 352 mini burgers corresponded to 11 percent adulteration and 4 storage times. At each sampling point, two samples were analysed for different parameters, specifically, spectroscopic, physical-chemical, microbiological. The microorganisms analysed were totally mesophilic (TVC) and psychrotrophic (TP).

2.2. Microbial Analysis

Samples were homogenized with tryptone salt (tryptone 0.1% and NaCl 0.85 %) in a stomacher for 90s. Serial decimal dilutions were prepared in the same solution for microbiological determinations. TVC (ISO4833, (2003)) and TP (2307:1987, (1987)) populations were obtained after incubation on PCA) (Oxoid CM0325, England) at 30 ºC for 3 days and 7 ºC for 10 days, respectively.

2.3. Physical-Chemical Measurements

2.3.1. pH Evaluation

pH was measured directly in the muscle using a penetration electrode with a pH meter (Crison instruments, Spain) and was immediately assessed in duplicate after opening the packages (International Organization for Standardization, 2004).

2.3.2. Determination of Moisture Content

The mixture content consisted of dehydration samples, placed in the oven at 100°C with petri uncovered dishes to facilitate evaporation. The weight of the samples was controlled at 60 minute intervals on an analytical scale with a resolution of 0.001g, until the mass of the last two were weighed, and separated for 60 minutes, did not differ by more than 0.1%. Before proceeding to the final weighing phase, the samples were cooled to room temperature in a desiccator with silica (Rahayu et al., 2018).

2.3.3. Determination of Free Fat Content/ Fat Extraction (Soxhlet Extraction)

Fat extraction is done by n-hexane in dehydrated samples. The samples were produced by the method of determining moisture content. The dried sample was placed in a extraction thimble, removing traces of the sample on the petri dish, using cotton wool moistened with n-hexane which was also transferred to the extraction thimble.

Posteriorly, the extraction thimble was placed in the extraction tubes together with n-hexane, adapting a flask to the extractor apparatus.

The extraction process occurred for 8h, then the flask was placed in a water bath at 90°C to remove n-hexane, practically leaving only fat. After this process, the flask was placed in the drying oven for 1h at 103°C to remove n-hexane residues. These drying, cooling and weighing

operations were repeated until the results of the two successive were weighed, separated 1h before heating, and did not differ by more than 0.1% (Aloglu and Harrington, 2018).

2.3.4. Fourier Transform Infrared Measurement

Infrared spectra was collected in a FTIR spectrometer (Mattson, Unicam Research Series, USA) equipped with a single reflection ATR module (Golden Gate, U.K.), a DLaTGS detector and a KBr beamspliter. The device was connected to a computer and controlled by the LabSolution IR of Shimadzu, with the purpose of correcting baseline (auto baseline). The sample of fish fat was placed on top of the ATR crystal whose temperature was ~35°C. The collection time for each sample spectrum was approximately 2min. Spectrum was recorded in the region between 4000 and 500cm -1 with a resolution of 4cm -1 and 32 scans. The ATR method consists of the penetration of a radiation beam into the crystal that undergoes total internal reflection when the incident angle at the interface between the sample and the crystal is higher than the critical angle, which is a function of the refractive indices of two surfaces (Beasley et al., 2014). The ATR base was carefully cleaned in situ by scrubbing with pure ethanol (Sigma Aldrich, Germany) before measuring the next sample. For each sample two spectra were collected and the average was calculated.

2.4. Mathematical Treatment

2.4.1. Factorial Analysis of Variance

A factorial analysis of variance (ANOVA) was performed on the pH and microbiological data at which the effect of time and the effect of the mixtures of fish samples was tested. For this, the following was considered: no significant effect (ns) when $p \geq 0.05$; significant effect (*) when $p < 0.05$; very significant effect (**) when $p < 0.01$; highly significant effect (***) when $p < 0.001$. This was determined by the Tukey HSD test ("honestly significant different").

2.4.2. Principal Component Analysis and Discriminant Analysis

Spectral data collected between 500 and 4000 cm^{-1} was divided into two ranges, from 650 to 1850 and from 2800 to 3050 cm^{-1} initially submitted to smoothing based on the Savitzky-Golay algorithm (Savitzky and Golay, 1964). Afterwards, mean-centered and standardized spectra were subjected to a PCA to inspect differences between samples. The PCA transformed the large number of potentially correlated factors into a smaller number of uncorrelated factors (principal components, PCs), and thus reduced the size of the data set (Abdi

and Williams, 2010) or qualitative analysis, principal components contributing to the variance of the data set were subjected to discriminant analysis (DA) in an attempt to predict the likelihood of a sample belonging to a previously defined group. Since the raw spectral data could not be used because of the strong correlation between the wave numbers, uncorrelated PCs resulting from PCA were employed. DA is a statistical method used to find a linear combination of structures which characterize or separate classes of objects or observations (McLachlan, 2004). The resulting arrangement may be used as a linear classifier or a reduction dimension priori to classification.

2.4.3. Partial Least Squares Regression

For quantitative analysis, the measured microbial and physical-chemical, factors which considerably contribute to the variance of the data set, were regressed using PLS-R onto the referred variables (Liang and Kvalheim, 1996, Wentzell and Montoto, 2003). This multivariate calibration technique, sometimes called factor analysis, transformed the original variables (FTIR spectra absorbances) into the new ones (known as latent variables), which were linear combinations of original variables (Miller and Miller, 2005). The method relied on two phases, the so-called calibration and cross-validation phases. In the calibration phase, a mathematical model was built to establish a correlation between the matrix of FTIR spectra (predictor variables, X) and the concentration of the analytics of interest (response variables, Y) and used a set of observations usually named calibration set. In the cross-validation phase, the developed calibration model was used to calculate the concentration of samples not used to set up the model (De Luca et al., 2009). The dependent variable (Y) was either the measured pH or bacterial counts for PLS-R analysis.

The relative performance of the established model was accessed by the root mean square error of calibration (RMSEC), root mean square error of cross-validation (RMSECV), and multiple coefficient of determination or regression coefficient (R2) (Divya and Mishra, 2007). The model selected was then used to determine the concentration of the samples in an independent prediction set. The predictive ability of the model was evaluated from the root mean square of prediction (RMSEP). The lower the RMSEP value, the higher the degree of accuracy of the prediction result provided by the calibration model (Corgozinho et al., 2008). PCA, DA, and PLS-R calculations were performed using the Excel-based XLSTAT V2006.06 package

(Addinsoft, Inc, NY, USA) and statistical software Unscrambler V9.6 package (Camo, Oslo, Norway).

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CHAPTER IV. EXPERIMENTAL RESULTS

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PART A - CONSUMERS PERCEPTION

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PAPER I - MANUSCRIPT "EVALUATION OF FOOD LABELLING USEFULNESS FOR CONSUMERS". INTERNATIONAL JOURNAL CONSUMER STUDIES. 2019; 00:1–8. DOI - 10.1111/IJCS.12511 (ANNEX II)

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EVALUATION OF FOOD LABELLING USEFULNESS FOR CONSUMERS

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Statement of originality

Consumer is concerned with food labelling information due to healthy lifestyles, practice of sport, allergies or diseases. Food labelling constitution through mandatory information allows to know the characteristics of food products and to take a consumer decision in moment of purchase. The objective of this study was to assess consumer opinion about reasons for read label and usefulness of label variables. Principal Component analysis lets perceive associations through variables of this study.

Abstract

Food labelling is a means of communication between food business operators and consumers, representing an important factor in consumer purchasing decisions. The enforcement of the new food labelling policy is aimed to improve food safety and public health through the mandatory indication of information and nutritional values. To understand the usefulness of the information provided for consumers, a survey was carried out to assess the efficacy of the information presented in food labelling. Principal component analysis was performed to obtain a smaller number of uncorrelated factors regarding the usefulness of food labelling. Results showed consumers usually do not read food labels due to lack of time and excessive information.

Additionally, food labelling was observed to be more useful for specific consumer groups, such as, athletes, consumers with health conditions or consumers concerned with a healthy lifestyle. The results of the present study highlight the need of information campaigns by public health authorities to show the importance and advantages of reading food labels as well as the development of essential information which should be quickly and clearly seen and understood by consumers.

Keywords: Behaviour, Consumer, Food labelling, Food purchase, Principal component analysis

1. Introduction

Food labelling is the main means of communicating between food business operators and consumers and this may often influence the consumer's option to purchasing (Wandel, 1997). Since food labelling provides information about the characteristics of the product, the correct interpretation of all mentions is essential when the appropriate food is chosen according to consumer preference, lifestyle and health conditions (Cecchini and Warin, 2016). In the last few years, consumers are concerned about the type of food they consume so demand more transparent labelling mentions such as full ingredients list which includes additives, nutritional values or real health benefits among others (Röhr et al., 2005, Weaver et al., 2014).
Improve consumer rights regarding proper use of foods and choosing appropriately according to their dietary needs, the publication of Regulation (EU) No 1169/2011 on the provision of food information to consumers has harmonized food labelling across Europe.

Although this regulation aim at transparency and confidence of consumers and public health, however, all these efforts are useless if consumers do not have the habit of reading it. Consumers usually do not read food labels due to a lack of confidence, education or lifestyle. Thus, increasing consumer perception associated to the new mandatory mentions displayed on food labels should be assessed since it depends on factors, such as literacy and/or lifestyle (Himmelsbach, Allen, & Francas, 2014).

Most studies on food labelling assess consumer perception of specific food labelling characteristics, such as nutritional composition, design and/or label layout, indication of premium products or local products, among others (Feldmann and Hamm, 2015, Gregori et al., 2014, Pettigrew et al., 2016). However, the usefulness of the information provided to consumers is scarcely assessed (Grunert and Wills, 2007). Therefore, the current study assesses the usefulness of consumer perceptions about food labelling.

2. Material and methods

2.1. Survey design and data collection

To assess the usefulness of food labelling information for consumers, a specific online questionnaire was designed on google forms and it comprised of 37 questions divided into 6 groups based on the European food safety policy and scientific literature reviews regarding food labelling and food product choices.

The survey distribution was mainly performed by email invitation and social media for a period of 12 months (September, 2016–October, 2017). Appropriate information was provided to survey participants, allowing them to decide their participation in this research study. All questions were measured on a 5-point Liker scale (1 = never; 2 = rarely, 3 = sometimes, 4 = frequently 5 = always). Questions concerning socio-demographic characteristics, such as sex, age, civil status, economic status, lifestyle and health of respondents were also included.

2.2. Data analysis

Once data were collected, registered into a SPSS 22.0 database (SPSS, IBM, New York, USA) and carefully checked, it was immediately available as an SPSS data set. Cronbach's alpha coefficient was calculated to assess the consistency of the survey. The influence of the socio-demographic characteristics on the use and understanding of the information displayed in food product labelling was assessed by the Kruskal–Wallis test. Socio-demographic characteristics with $p < 0.05$ were considered as statistically significant and further subjected to Principal Component Analysis (PCA). The appropriateness to perform PCA was confirmed by Bartlett's sphericity test ($p < 0.001$). The number of components retained in the final solution was based on the Kaiser–Meyer–Olkin criterion (>0.8) for the analysis of eigenvalues (>1) and the proportion of variance retained (>65%), usually seen as the minimum required to make the model suitable for explaining the original data (Polyak and Khlebnikov, 2017). The statistical analysis was done by using IBM® SPSS® version 22.

3. Results

3.1. Socio-demographic characteristics of consumers

A total of 308 consumers answered the online survey. The sample set consisted of 83 men (26.9%) and 225 women (73.1%). 195 respondents (63.3%) were single, 21 (6.8%) married and 92 (29.9%) divorced. According to age, 23.4% were under 25, 62.0% ranged from 25 to 45 and 14.3% were older than 45. Respondent salary was under 500€ (31.2%), 500€–900€ (33.4%), 900€–1,500€ (21.1%) and over 1,500€ (14.4%). Regarding respondent education and lifestyle, 81.2% were graduates, 95.8% of whom declared having a healthy lifestyle and 41.3% practised sport regularly. Additionally, 116 (37.7%) respondents declared some dietary restriction and only 9 (2.9%) were vegetarians.

3.2. Evaluation of usefulness and perceptions food labelling

Results from the internal consistency test based on the Cronbach alpha coefficient was 0.802, indicating a good internal reliability. The results (Table 1) of the online survey indicated that consumers do not usually read food labels. However, an increase in the frequency of reading was observed when a new product is present at the time of purchase or if it has a new preparation condition or intended use. Moreover, consumers scarcely read the allergenic ingredients or the suitability of food products for vegetarians.

Table 1. Consumer perceptions and usefulness about food labelling.

	Never	Rarely	Sometimes	Frequently	Always
Reasons why consumers do not read food labels					
Food product brand confidence	31.8	20.5	22.1	19.5	5.8
Lack of time	20.8	18.5	33.1	23.7	3.8
Information displayed in the food product labelling is difficult to understand	24.7	28.2	35.1	10.7	1.3
Food product labelling provided excessive information	23.1	27.3	33.8	13.0	2.9
Lack of consumer confidence on information displayed on the food product labelling	27.3	34.1	28.2	7.5	2.9
Reasons why consumers read food labels					
New food product	0	0	100	0	0
Consumer presented some food intolerance or allergies	62.0	17.2	7.5	6.8	6.2
It is aware of existence of healthy products	2.6	6.8	23.1	36.4	31.2
Interest of country of manufacture / origin of the foodstuff	2.9	14.0	29.2	29.5	24.4
Comparison to similar products	3.2	7.8	29.5	44.8	14.6
Interest on the instructions for use	2.3	12.3	26.3	39.9	19.2
Appropriate for vegetarians	62.0	17.2	10.7	3.9	6.2
Verification of organic food product	20.8	24.4	28.9	15.9	10.1
Existence of certification	10.7	19.8	31.5	22.7	15.3
Factors affecting consumer buying decision					
Brand	6.5	27.3	44.8	19.5	1.6
Price	-	4.2	25.6	39.3	30.5
Appearance	1.6	10.4	25.3	39.9	22.4
Country of origin	14.0	21.1	26.9	28.2	9.4
Shelf life	1.6	4.9	19.5	19.5	54.2
Nutritional value	6.8	15.6	28.2	29.9	19.2
Ingredients	4.9	11.7	29.5	32.1	21.4
Usefulness of food labelling					
Product constitution	1.9	7.1	19.2	40.9	30.8
Nutritional value	1.9	10.7	21.4	34.4	31.2

Shelf life	0.3	6.8	10.7	25.3	56.8
Presence of preservatives / additives	2.6	12.0	18.8	34.1	32.5
Weight or volume	3.2	13.6	29.5	34.1	19.5
Product name	2.3	15.9	33.4	33.1	15.3
How to use	2.3	11.7	30.5	37.0	18.5
Name or business name and address	13.0	27.9	28.9	18.8	11.4
Place / country of production	6.8	16.6	26.6	26.3	23.4
Reading frequency of food labels					
Meat and meat products	11.4	21.1	22.4	30.5	14.6
Fish and fish products	11.7	19.5	22.1	30.8	15.9
Milk and dairy products	5.2	13.6	27.9	34.1	19.2
Frozen products	3.9	12.3	22.4	35.7	25.6
Perception of food mislabelling					
Risk to public health	61.4	23.1	13.3	2.3	0
Loss of consumer confidence	33.1	39.9	24.4	0.6	1.9
Benefit for the food business operator	41.9	33.1	20.1	2.6	2.3

The reasons why consumers mentioned not reading food labels showed that over 50% declared "lack of time", almost 45% considered they have excessive information and about 50% trusted the brand name. The factors which influenced consumers at the time of purchase were price, presentation, product shelf-life and ingredients.

Curiously, only 20% of consumers indicated the brand name as an important factor when purchasing, 75% considered the food label information important, although the name of the food manufacturer was not relevant for 40% of consumers.

Moreover, the perception of mislabelling showed that 85% of consumers did not consider this practice as a risk for public health and about 75% indicated that mislabelling is not associated to an economic income for food business operators.

3.3 Influence factors of usefulness and perceptions of food labelling

The study of the factors which influence food labelling usefulness and the perceptions (Table 2) of mislabelling revealed that consumers who regularly practice sports and those who declared a healthy lifestyle considered the information displayed on food labels helpful.

Regarding the reasons of reading food labels, consumers with dietary restrictions paid more attention to the food composition.

CHAPTER V. EXPERIMENTAL RESULTS

PART A - CONSUMERS PERCEPTION – PAPER I

Consumers with more schooling and healthy habits had greater perception of healthy products. In addition, the verification of the usefulness of instructions was related to age, education and sports practitioners. It was observed that age, practicing sports and/or the existence of food restrictions influenced the evaluation at the time of purchase, in respect to appearance, product origin or the list of ingredients. Moreover, there were no differences in the usefulness and perceptions of compulsory mentions of food labelling among the different socio-demographic characteristics of the consumers surveyed.

Table 2. Socio-demographical characteristics that influence the food labelling perceptions by consumers.

	Age	Education	Civil status	Salary	Dietary restrictions	Healthy lifestyle	Vegan lifestyle	Practice of sport
Reasons why consumers read food labels								
a Consumer presented some food intolerance or allergies	-	-	-	-	P<0.05	-	-	-
It is aware of the existence of healthy products	-	P<0.01	-	-	-	-	-	-
Interest of the country of manufacture / origin of the food product	-	-	P<0.05	-	-	-	-	P<0.05
Interest on the instructions for use	P<0.05	P<0.05	-	-	-	-	-	P<0.05
Verification of organic food product	-	-	-	-	-	P<0.05	P<0.05	-
Existence of certification	-	-	-	P<0.05	-	-	P<0.05	-
Factors affecting consumer buying decision								
Appearance	-	-	-	P<0.05	P<0.05	-	-	P<0.05
Country of origin	P<0.05	-	P<0.05	-	-	-	-	P<0.05
Nutritional value	-	-	-	-	-	P<0.01	-	P<0.05
Ingredients	P<0.05	-	-	-	P<0.05	-	P<0.05	P<0.001
Usefulness of food labelling								
Product constitution	-	-	-	-	-	-	P<0.05	P<0.05
Nutritional value	-	-	-	-	-	P<0.01	-	-
Weight or volume	-	P<0.05	-	-	-	-	-	-
Product name	-	-	-	-	-	P<0.05	-	-
How to use	-	P<0.05	-	-	-	-	-	-
Place / country of production	-	-	-	-	-	-	-	P<0.05
Perception of food mislabelling								
Risk to public health	-	-	-	-	-	-	-	P<0.05
Benefit for the food business operator	-	-	-	-	-	-	-	P<0.05

n.s: not significant

3.4. Principal component analysis (PCA)

Loadings of each principal components (PC) after varimax normalized rotation and communalities from de PCs are represented in Table 3. Figure 1 shows the projection of the 27 original variables on the two-dimensional space defined by two PCs. The first and second principal components together (PC1–PC2) accounted for 40.57% of data variance. The first component specified the reasons why consumers read food labels, usefulness of food labelling and perception of food mislabelling variables. The second component is characterized by factors affecting variables related to consumers buying decision. A significant association between shelf-life, certification, product constitution and place/ country origin of production could be observed. The variables with the greatest partial contributions for the variability were, in decreasing order, shelf life (FL = 0.76), existence of certification (FL = 0.75), product constitution (FL = 0.72), place/country origin of production (FL = 0.70), awareness of the existence of healthy products (FL = 0.61) and nutritional value (FL = 0.61) for the positive dimension PC1. Contrarily, the variables, risk to public health (FL =−0.44), loss of consumer confidence (FL = −0.44) and benefit for food business operators (FL = −0.44) were presented in the negative dimension. PC2 showed that ingredients are associated nutritional value. In decreasing order and in positive dimension of PC2, the variables, ingredients (FL = 0.86), nutritional value (FL = 0.83), country of origin (FL = 0.547) and shelf-life (FL = 0.501) were observed.

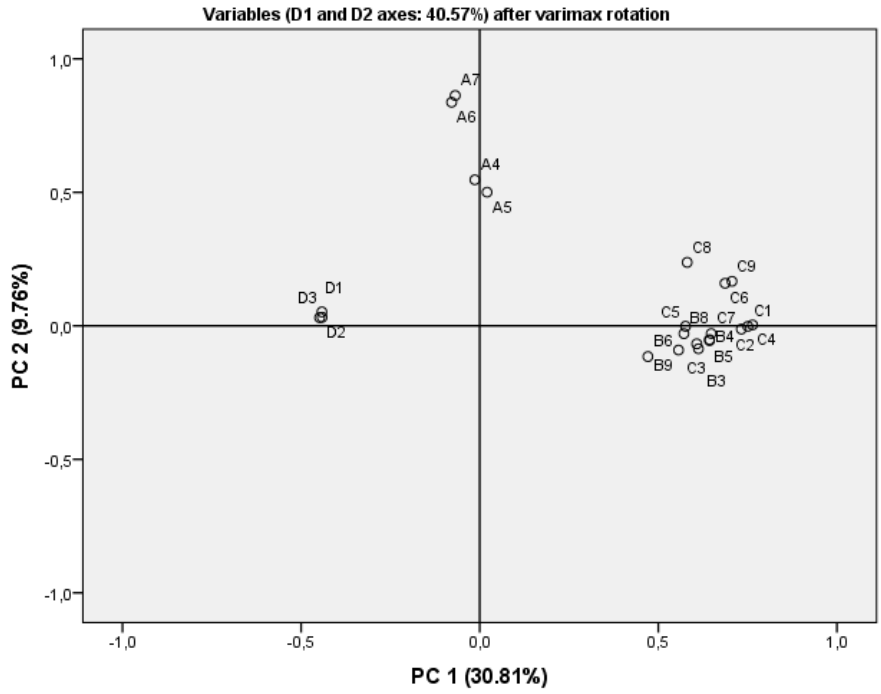

Figure 1. Loadings for the PC1–PC2 dimensions of the 20 variables selected to a principal components analysis, after varimax normalized rotation: A4 - Country of origin; A5 - Shelf life; A6 - Nutritional value; A7 - Ingredients; B3 - It is aware of the existence of healthy products; B4 - Interest of the country of manufacture / origin of the food products; B5 - Comparison to similar products; B6 - Interest on the instructions for use; B8 - Verification of organic food products; B9 - Existence of certification; C1 - Product constitution; C2 - Nutritional value; C3 - Shelf life; C4 - Weight or volume; C5- Product name; C6 - Product name; C7 - How to use; C8 - Name or business name and address; C9 - Place / country of production; D1- Risk to public health; D2 - Loss of consumer confidence; D3 - Benefit for the food business operator.

Table 3. Factor loadings and communalities of variables in the first two components (PC1 and PC2) after varimax normalized rotation.

Variable	Factor loading		CM
	PC1	PC2	
Bartlett's test of sphericity	P< 0.001; df =231; X2= 2939.14		
KMO[c] measure	0.845		
Country of origin	-0.014	0.547	0.299
Shelf life	0.021	0.501	0.251
Nutritional value	-0.079	0.837	0.707
Ingredients	-0.069	0.863	0.749
It is aware of the existence of healthy products	0.612	-0.086	0.382
Interest of the country of manufacture/origin of the food products	0.643	-0.051	0.416
Comparison to similar products	0.643	-0.055	0.417
Interest on the instructions for use	0.471	-0.115	0.235
Verification of organic food products	0.572	-0.029	0.328
Existence of certification	0.557	-0.091	0.318
Product constitution	0.751	-0.002	0.564
Nutritional value	0.733	-0.012	0.537
Shelf life	0.608	-0.067	0.374
Presence of preservatives/additives	0.764	0.004	0.583
Weight or volume	0.576	-0.001	0.332
Product name	0.687	0.160	0.497
How to use	0.648	-0.029	0.421
Name or business name and address	0.581	0.238	0.394
Place / country of production	0.706	0.167	0.527
Risk to public health	-0.448	0.031	0.202
Loss of consumer confidence	-0.441	0.032	0.196
Benefit for the food business operator	-0.442	0.052	0.198

CM - communality; PC - principal component; KMO - Kaiser-Meyer-Olkin.

4. Discussion

Food labelling laws ensure consumers get vital information about the food they consume. In the EU, the introduction of Regulation nº 1169/2011 (2011) set standards to create common ground for diffusing food information across member states and aimed to provide consumers more safety, clarity and transparency of information, thus, also improving food safety and public health (Regulation, (EU) nº. 1169/2011).

Studies about consumer perceptions concerning food labelling are mainly aimed at assessing the perceptions regarding nutritional composition and the way information is presented (Huang and Lu, 2016). Despite the effort developed by the authorities to improve a healthy lifestyle and further food safety and public health, the current study showed most consumers do not read food labels (FSAI, 2009). Consequently, lack of time and excess of information have been referred as the most important factors answered by respondents. This behaviour may explain the higher frequency of reading the product shelf-life (Vemula et al., 2014).

In general, respondents considered all the compulsory mentions of food labelling important. Since no studies assess consumer perception of compulsory food labelling information, hence the results can be associated to the literacy of the respondents. Nutritional value has been referred as an important factor at the time of purchase (Volkova and Mhurchu, 2015), however, our results indicated that price and/or appearance showed the same importance, indicating that the usefulness of nutrition labels in food purchasing is currently low(Gomes, 2017). Despite brand name and country of origin are also described as a purchase factor (Berry et al., 2015), our respondents curiously did not consider the food manufacturer or its location as relevant factors.

Regarding consumer characteristics which may affect the choice of food at the time of purchase, the current study showed that respondents who lead a healthy life, practised sports or declared some health condition, that is, food allergies, more attention to nutritional value, product appearance, allergens, intended use or knowledge of healthy food.

Although the present study does not show differences among gender (Lassen et al., 2016), showed that women are more concerned about nutritional value while men considered the price as the principal factor at purchase.

Regarding mislabelling, the low perception of risk to public health or the economic benefit of food fraud displayed by respondents is difficult to explain but it can be associated to the belief that popular brands of food are made from recognized food business operators selling food products with accurate labels (Drescher et al., 2012).

5. Conclusion

Food labelling is how food business operators and consumers communicate and interact. Also, it may influence the consumer's buying decision. The enforcing of the food labelling policy by the implementation of new policy was aimed to improve guarantee the food safety and public health with new mandatory information and nutritional values concerning each product.

However, the effort undertaken by food and health authorities can be compromised since consumers do not read the food label as observed in the present study. Thus, lack of time and excessive information was referred as the main factors of absence of food labelling reading. Furthermore, it was observed that food labelling is more useful for specific consumers groups, such as athletes, consumers with health conditions or consumers concerned with a healthy lifestyle. The results of the present study highlight the need of information campaigns by public health authorities to show the importance and advantages of reading food labels as well as ensuring food labels with essential information which are not only quickly and clearly seen but also understood by consumers.

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PAPER II - MANUSCRIPT MOREIRA, M. J.1, GARCÍA-DÍEZ, J1., DE ALMEIDA, J. M. M. M., SARAIVA, C., "CONSUMER KNOWLEDGE ABOUT FOOD LABELLING AND FRAUD " SUBMITTED TO FOOD CONTROL

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CONSUMER KNOWLEDGE ABOUT FOOD LABELLING AND FRAUD

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Abstract

Food labelling influence consumers´ preferences at the time of purchase, then consumers are advised to read the information displayed on labels to verify if the products meet their preferences and needs. As almost every type of food fraud involves mislabelling, the objective of the present work is to assess the consumers´ knowledge about food labelling and food fraud. To understand the usefulness of food labelling information and perception of food fraud a survey was carried out to assess the perceptions about foods labelling and fraud. Principal component analysis was performed to obtain a smaller number of uncorrelated factors regarding the usefulness and confidence of information displayed in food label and perception of food fraud. Data showed respondents consider that information displayed in food label is useful, but the way the information is presented may decrease the consumer interest and increases its understand. Regarding respondents´ confidence on foodstuffs, over half of them stated that information provided in food label is reliable. However, a lack of confidence on food composition is observed in those processed foodstuffs such as meat products. Food fraud is recognized by over half of respondents with a higher perception of those practices that implies a risk to public health than those related to economic motivation. Age and consumers' education revealed the most important socio-demographic factors regarding food label perception, confidence on its information and also knowledge about food fraud. Implementation of training programs to increase consumer knowledge about food labelling and food fraud is essential. As scarce research is available about consumer perceptions of food label information and food fraud, the respondents´ perceptions observed in the current work can be used as guidelines for food industry to improve food label design to enhance the consumer understand and usefulness. Also, addition of enough information in a clear way may enhance the consumer trust in a period of loss of confidence in the food industry due to the recent food fraud scandals.

Keywords: Consumer, food confidence, food labelling, food fraud.

1. Introduction

Traditionally, the aim of food safety is the control of chemical, biological and physical hazards. However, new consumers´ issues related to food safety have emerged in the last years mainly associated with health conditions such as weight control, diabetes, blood pressure or cancer. Thus, aspects as ingredients, allergens, chemical additives, food processes or health impact associated to long-term consumption become new food safety trends for food processors (King et al., 2017). Food labelling, compulsory by law (Regulation 1169/2011), has an important role in food safety. The indication of specific mentions such as ingredients, intended use, batch number, shelf-life and storage conditions are essential to guarantee food safety. Since food labelling influence consumers´ preferences at the time of purchase (Bandara et al., 2016), consumers are advised to read the information displayed on labels to verify if the products meet their preferences and/or are adapted to specific nutrition programs (i. e. vegetarians) or adapted to specific health conditions (i. e. diabetic) (Tonkin et al., 2016). Regarding food labelling, some studies assess the consumers´ perception about nutritional labelling (Findling et al., 2018, Lundeberg et al., 2018). However, scarce research addresses the opinion and perception of consumers about the compulsory mentions displayed in food packages (Hawley et al., 2018). Some studies indicated that most important characteristics for consumers is that the food labelling is clear and easy to understand. It has been indicated that consumers pay great attention to expiry date, list of ingredients and nutritional values (Samant and Seo, 2016). However, many of them do not assess the perception or understanding of consumers (Nocella and Kennedy et al., 2012). Despite the effort made by food industry to improve consumer information and adapt to the changes in food labelling established in Regulation 1169/2011, further research about the real perception of consumers´ regarding the clarity and information understanding is still needed. Food fraud is the act of purposely altering, misrepresenting, mislabelling, substituting or tampering with any food product at any point along the food chain. Since food fraud damage the food industry and decrease the consumers´ confidence, some studies have assessed the changes in the purchase behaviour (Agnoli et al., 2016, Charlesbois et al., 2016). Since some of the recent food fraud scandals are associated with mislabelling practices, this may lead to believe, although difficult to demonstrate, that there is an advantage of the low perception of consumers regarding food fraud and the scarce test of foods for materials that are not expected to be present by food authorities. Thus, the objective of the present work is evaluate the consumers´ knowledge about food labelling and food fraud.

2. Material and methods

2.1. Survey design and data collection

An exploratory study was carried out on labelling to allow for probe and adjusting initially proposed objectives, acquiring knowledge about research method. Through the documentary research in particular, legislation, research articles, descriptive information was collected regarding, to the constitution of the label, their reading and consumer opinion, crises of consumer confidence, fraudulent labelling and confidence in food product.

The design was selected that it allowed analysis of factors related to awareness of food labelling information and use of such information in purchasing foods products. After the investigation was completed, this was validated through the application of the pre-test a small sample of 20 respondents with similar characteristics to the population with the purpose of validating the survey, in order to detect potential errors, difficulties of interpretation, time spent in response, response instructions or other important aspects that could unintentionally influence the understanding of the issues, in order to make the final survey more effective and valid. Pre-test of the data collection tool (survey) was done prior the study, at the supermarkets.

To assess the usefulness of food labelling information for consumers, a specific online questionnaire was designed on google forms and it comprised of 42 questions divided into 5 groups (respondent opinion about the usefulness of information displayed in food label, respondent confidence about information displayed in food label, respondent confidence regarding the constitution of food, respondent knowledge about consequences derived from food mislabelling, respondent knowledge about food fraud) based on the European food safety policy and a scientific literature review on food safety. The survey distribution was mainly performed by email invitation and social media for a period of 12 months (September, 2016 to October, 2017). Appropriate information was provided to survey participants, allowing them to decide their participation in this research study. All questions for each group were measured as closed questions. Also, to increase the performance of respondents´ opinion and knowledge, socio-demographic characteristics such as gender, age, civil status, economic status, education, lifestyle and health of respondents were also included.

2.2. Data analysis

Once data were collected, registered into a SPSS 22.0 database (SPSS, IBM, New York, USA) and carefully checked, results were immediately available as an SPSS dataset. The Factorial Exploratory analysis of the data matrix was performed to ascertain the reliability of the factorial structure, having been elaborated by the calculation of Cronbach's Alpha, to estimate the reliability of the survey and the consistency of the factors. Cronbach's alpha evaluates the level of correlation of the items of a data matrix with each other. The influence of the socio demographic characteristics on understanding the food label information was assessed by the Kruskal–Wallis test. The Principal Component Analysis (PCA) was carried out to obtain a smaller number of uncorrelated factors (principal components, PCs), consequently, reducing the size of the data or qualitative set. The appropriateness to perform PCA was confirmed by Bartlett's sphericity test ($P < 0.0001$). The number of components retained in the final solution was based on the Kaiser-Meyer-Olkin criterion (>0.8) for the analysis of eigenvalues (>1) and the proportion of variance retained (>65%), usually seen as the minimum needed to make the model suitable for explaining the original data (Polyak and Khlebnikov, 2017).

3. Results

3.1. Socio-demographic characteristics of consumers

A total of 308 respondents answered the online survey. The sample set consisted of 83 men (26.9%) and 225 women (73.1%). 195 respondents (63.3%) were single, 21 (6.8%) divorced and 92 (29.9%) married. According to age, 23.4% were under 25, 62.0% ranged from 25 to 45 and 14.3% were over 45. Respondent salary was under 500€ (31.2%), 500€-900€ (33.4%), 900€-1500€ (21.1%) and over 1500€ (14.4%). Regarding respondent education and lifestyle, 81.2% were graduates, 95.8% declared a healthy lifestyle and 41.3% practised sport regularly. In addition, 116 (37.7%) respondents declared some dietary restriction while only 9 (2.9%) said they were vegetarians.

3.2. Assessment of the opinion and perceptions about food labelling and food fraud

In overall (Table 1), over 65% of respondents considered food label information not useful and almost 65% declared some difficult to understand the information displayed on it. In addition, some label characteristics such as font size, symbols or label design were considered negatively. With respect to consumer confidence regarding the information displayed on food labelling, only 52% of respondents stated that the information provided is reliable. However, over 60% of respondents declared that information indicated in food labels neither prevents food fraud not guarantee the traceability. In addition, respondents indicated that information not provides enough information regarding religious or ethics aspects. As regards of food constitution, over 55% of respondents declared distrust in the information provided by food manufacturers. Among the different kinds of products, respondents showed more confidence in less processed products such as milk, oils or frozen products than in those subjected to more process such meat products, pre-cooked foods or ready-to-eat food products. However, consumers declared better confidence in foodstuffs labelled with protected designation of origin or protected geographical indication in the labelling. The evaluation of the knowledge about food fraud showed that over 75% of respondents not considered the food fraud derived from mislabelling neither a health risk nor economic benefit for food industry. Chronbach α values for all factors were larger than 0.87 and the internal consistency test based on the Cronbach α coefficient was 0.88, indicating good internal reliability.

Table 4. Consumers´ opinions and perceptions (%) about food labelling information and food fraud

	Not useful	Useful	Chro α
Respondent opinion about the usefulness of information displayed in food label			
Compulsory information displayed in food label is useful	34.3	65.6	0.88
Information displayed in food label is easy to understand	63.6	36.4	0.88
Food label design helps sell product	65.9	34.1	0.88
Information displayed in food label provide information about quality	61.7	38.3	0.88
Information displayed on front-of-the package is useful	45.7	54.3	0.88
Information displayed on back-of-the package is useful	53.0	47.0	0.88
Food label displayed enough information about the food	68.2	31.8	0.87
Food product is correctly described in the label information	59.8	40.5	0.88
Information about intended use and preparation mode is clear	48.7	51.3	0.88
Symbols displayed in food label provide useful information	58.8	41.2	0.88
Font size of is adequate	81.9	18.1	0.88
Food label design is appropriate	74.7	25.3	0.88
	Not provides confidence	Provides confidence	
Respondent confidence about information displayed in food label			
Information displayed in food label ensures food quality	43.9	56.1	0.87
Information displayed in food label ensures food safety	41.5	58.5	0.88
Information displayed in food label is helpful to choose healthy foods	26.9	73.1	0.87
Information displayed in food label ensures nutritional quality	37.3	62.7	0.88
Information displayed in food label allows consumption according to ethics	52.3	47.7	0.88
Information displayed in food label prevents food fraud	60.0	40.0	0.88
Information displayed in food label respects religious beliefs	70.5	29.5	0.88
Information displayed in food label ensures traceability	48.0	52.0	0.88
	Not provides confidence	Provides confidence	
Respondent confidence regarding the constitution of food			
Meat and meat products	71.7	28.3	0.88
Fish and fishery products	56.1	43.9	0.88
Milk and dairy products	40.6	59.4	0.88
Ready-to-eat products	75.3	24.7	0.88
Pre-cooked products	76.9	23.1	0.88
Frozen products	50.3	49.7	0.88
Olive oil and other oils	45.5	54.5	0.88
Foodstuffs with quality labels (PDO, PGI)	38.0	62.0	0.88
	w/o cons	Hard cons	
Respondent knowledge about consequences derived from food mislabelling			
Mislabelling implies a risk to public health	84.5	15.5	0.89
Mislabelling increase the consumer distrust	73.0	27.0	0.89
Mislabelling implies economic benefits for food company	75.0	25.0	0.89

W/O: without; cons: consequences; PDO: protected designation of origin; PGI: protected geographical indication; Chro α: Chronbac α

3.3. Influence of socio-demographic characteristics on food labelling information

The influence of socio-demographic characteristics regarding food labelling and fraud perception are presented in table 2. Age and education were the main factors influenced ($p<0.05$) the respondents´ perceptions about food labelling and fraud. Other socio-demographic characteristics such us sex, civil status, salary or life style were not significant ($p>0.05$). With regard of the usefulness of information displayed on food labels, education influenced ($p<0.05$) the usefulness of information provided in food label while age ($p<0.05$) was associated with the font size of mention displayed on the label. The confidence of the information of food labelling was influenced by the age, mainly associated to aspects related to health and nutritional characteristics. Confidence about food fraud aspects was influenced both by age and education while education was the main factor that influenced the knowledge about the food constitution. In contrast, no socio-demographic characteristics influenced the knowledge about consequences of food mislabelling.

Table 5. Influence of socio-demographic characteristics in food label information, food label confidence and trust in food constitution (results p<0.05 are statistically significant)

	Age	Education
Respondent opinion about the usefulness of information displayed in food label		
Overall information displayed in food label is useful	ns	p<0.05
Information displayed in food label is easy to understand	ns	ns
Information displayed in food label contains enough information about food product	ns	p<0.05
Food label design helps sell product	ns	ns
Information displayed in food label provide information about quality	ns	ns
Information displayed on front-of-the package is useful	ns	ns
Information displayed on back-of-the package is useful	ns	ns
Food label displayed enough information about the food	ns	ns
Food product is correctly described in the label information	ns	ns
Information about the intended use and preparation mode is clear	ns	ns
Symbols displayed in food label provide useful information	ns	ns
Font size of is adequate	p<0.05	ns
Food label design is appropriate	ns	ns
Consumer confidence about food label information		
Information displayed in food label ensures food quality	ns	ns
Information displayed in food label ensures food safety	ns	ns
Information displayed in food label is helpful to choose healthy foods	p<0.05	p<0.05
Information displayed in food label ensures nutritional quality	p<0.01	ns
Information displayed in food label allows consumption according to ethics	ns	ns
Information displayed in food label prevents food fraud	p<0.05	p<0.05
Information displayed in food label respects religious beliefs	ns	ns
Information displayed in food label ensures traceability	p<0.01	ns

Consumer confidence regarding the constitution of food		
Meat and meat products	p<0.01	ns
Fish and fishery products	ns	ns
Milk and dairy products	ns	p<0.05
Ready-to-eat products	ns	p<0.05
Pre-cooked products	ns	ns
Frozen products	ns	p<0.05
Olive oil and other oils	ns	ns
Foodstuffs with quality labels (GDP, POD)	ns	p<0.01

ns: not significant

3.4. Respondents´ knowledge about food fraud and socio-demographic factors influenced the knowledge

Results of respondents´ knowledge about food fraud according to the consequences for consumers are presented in table 3. In overall, 55% of respondents showed some knowledge about food fraud although perception was higher for those practices that imply a risk to public health (61.0%) than those related for economic gain of food companies (51.8%). Regarding of food fraud as a public health risk, knowledge of food fraud, practices that imply the addition of unauthorized substances (ingredients or additives) was the most indicated. Respondents´ knowledge about practices to increase the economic benefit was mainly associated to those that imply the substitution of ingredients. As previously observed for respondents´ perceptions about food label information, age and education were also the most important factors influenced the knowledge of food fraud ($p<0.05$). However, knowledge of about food fraud is more perceived for an economic increased for food industry than a threat for public health. In addition, those consumers that declared dietary restrictions or presented a healthy life style are more concerned with mislabelling of food ingredients.

Table 6. Respondents´ knowledge about food fraud and socio-demographic factors influenced the knowledge

	Knowledge		p			
	Known	Unknown	Age	Education	Dietary restriction	Physical activity
Risk to public health						
Presence of chemical hazards derived from food processes	60.4	39.6	ns	ns	p<0.05	ns
Addition of unauthorized additives/preservatives	81.8	18.2	ns	ns	ns	p<0.05
Addition of food additives/preservatives not declared on food label	65.6	34.4	ns	p<0.05	ns	ns
Use of food approved additives/preservatives over the maximum levels defined by law	54.9	45.1	ns	ns	ns	ns
Presence of genetically modified organisms not declared on food label	42.5	57.5	p<0.05	ns	ns	ns
Economic gain						
Partial/total substitution of an ingredient/substance	65.9	34.1	p<0.05	ns	p<0.05	p<0.05
Addition of unauthorized ingredient/substance	58.8	41.2	ns	p<0.05	ns	ns
Use of authorized ingredient/substance over the maximum level defined by law	51.9	48.1	ns	p<0.05	ns	ns
Sold of frozen-thawed foods fresh products	44.5	55.5	ns	ns	ns	ns
Adulteration of geographical origin of foodstuffs	52.9	47.1	ns	ns	ns	ns
Use of unauthorized food practices or processes	36.7	63.3	p<0.05	p<0.05	ns	ns

Ns: not significant

3.5. Principal component analysis

Loadings of each principal component (PC) after the varimax and normalized rotation and communalities from the PCs are presented in table 4. Figure 1 shows the projection of the 20 original variables on a two-dimensional space defined by the two PCs. The first and second PC together (PC1-PC2) accounted for 48.1% of the data variance. A total of variance was approximately 66.5%, achieved by using 5 PCs. The first component set the opinion regarding label information and perception of food mislabelling variables. The second component was characterized by the importance of food labelling application variables. There was a significant association between the following variables: the information written on the labels is useful, Information displayed on front-of- the pack is useful and Information displayed in food label is easy to understand. The variables which had the greatest partial contributions for variability were, in decreasing order, information displayed on front-of-the pack is useful (factor load (FL) = 0.77), food product is correctly described in the label information (FL=0.77), overall information displayed in food label is useful (FL = 0.74) and information displayed in food label is easy to understand (FL = 0.74), and for the positive dimension PC1. In contrast, mislabelling implies a risk to public health (FL = -0.33), mislabelling increase the consumer distrust (FL = -0.32) and mislabelling implies economic benefits for food company (FL = -0.23) are located in a negative dimension.

PC2 found that food safety is associated with labelling and allows one to choose healthy foods, ensures nutritional quality and prevents fraud. In decreasing order, in a positive dimension of PC2, the variables information displayed in food label ensures food safety (FL = 0.80), information displayed in food label ensures nutritional quality (FL = 0.77), information displayed in food label prevents food fraud (FL=0.74) and information displayed in food label is helpful to choose healthy foods (FL= 0.71).

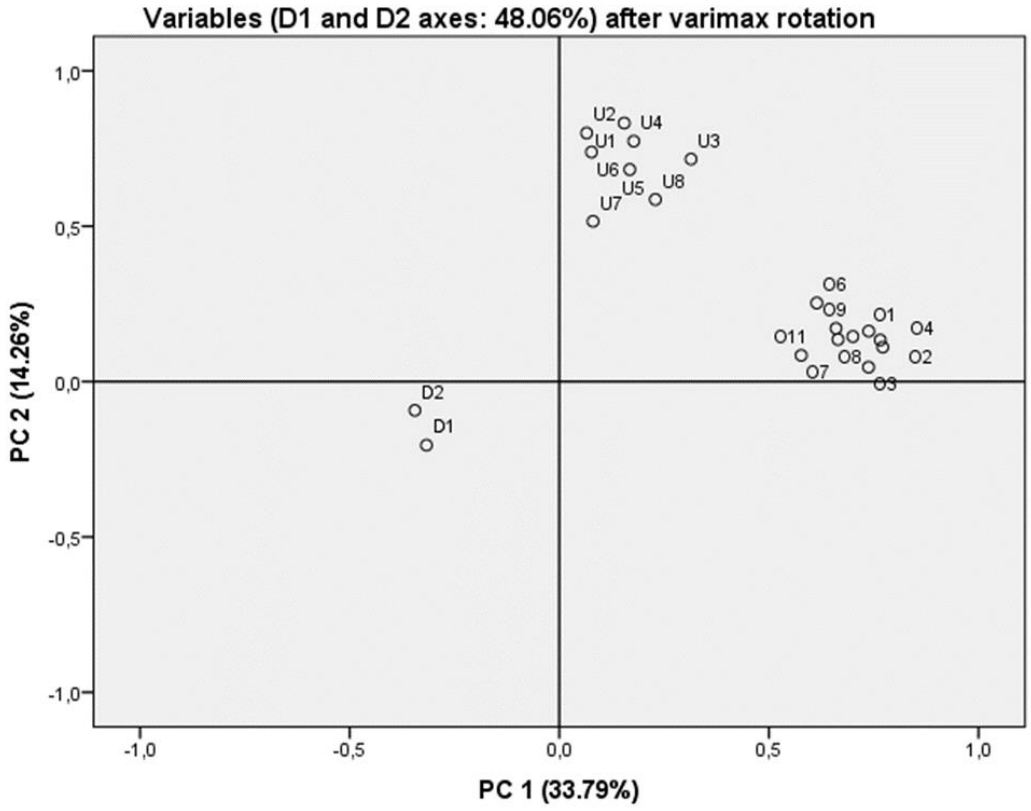

Figure 2. Loadings for the PC1–PC2 dimensions of the 20 variables selected to a principal components analysis after varimax normalized rotation: 01- information written on label is useful; 02- good source of information; 03 –easy to understand information on label; 04 - label has information about food product; 06 - label gives information about quality of product; 07 –information on the back of packaging is very useful; 08- food label contain enough information; 09 - symbols provide useful information; 011 - label is appropriate; U1 - ensures food quality; U2 - ensures food safety; U3- helps one choose healthy foods; U4 - ensures nutritional quality; U5- allows consumption according to ethics; U6 - prevents food fraud; U7- respects religious beliefs; U8- ensures traceability; D1- risk to public health; D2 – loss of consumer confidence.

Table 7. Factor loadings and communalities of variables in the first two components (PC1 and PC2) after varimax normalized rotation

Variables	Factors loading		
Bartlett's test of sphericity	P< 0.001; df =190; X^2= 2954.64		
KMO measure	0.857		
	PC[b]1	PC[b]2	CM[a]
Compulsory information displayed in food label is useful	0.74	0.16	0.76
Information displayed on front-of- the pack is useful	0.77	0.14	0.74
Information displayed in food label is easy to understand	0.74	0.05	0.62
Food product is correctly described in the label information	0.77	0.13	0.66
Information displayed in food label provide information about quality	0.61	0.26	0.53
Information displayed on back-of-the package is useful	0.59	0.09	0.41
Information about intended use and preparation mode is clear	0.70	0.16	0.73
Symbols displayed in food label provide useful information	0.65	0.16	0.56
Food label design helps sell product	0.67	0.14	0.60
Information displayed in food label ensures food quality	0.17	0.83	0.84
Information displayed in food label ensures food safety	0.09	0.80	0.79
Information displayed in food label is helpful to choose healthy foods	0.32	0.71	0.70
Information displayed in food label ensures nutritional quality	0.18	0.77	0.66
Information displayed in food label allows consumption according to ethics values	0.19	0.69	0.60
Information displayed in food label prevents food fraud	0.10	0.74	0.60
Information displayed in food label Respects religious beliefs	0.09	0.51	0.78
Information displayed in food label ensures traceability	0.25	0.59	0.60
Mislabelling implies a risk to public health	-0.33	-0.20	0.52
Mislabelling increase the consumer distrust	-0.32	-0.08	0.72
Mislabelling implies economic benefits for food company	-0.23	-0.14	0.75

CM - communality; PC - principal component; KMO - Kaiser-Meyer-Olkin.

3.6. Spearman test

Variables with some significant Spearman's correlation coefficients ($p<0.01$) are presented in table 5. The risk to public health was positively correlated with mislabelling increase the consumer distrust ($r=0.362$, $p<0.001$) and negatively correlated with overall information displayed in food label is useful ($r = -0.291$, $P< 0.001$), information displayed on front-of- the pack is useful ($r = -0.271$, $P <0.001$), food product is correctly described in the label information ($r = -0.298$, $P<0.001$) and information displayed in food label ensures nutritional quality ($r = -0.208$, $P<0.001$). The loss of consumer confidence was mostly negatively correlated with information displayed on the back-of-the package is useful ($r = -0.236$, $P<0.001$). In general, all opinions on label information variables are positively correlated in the confidence provided by food labelling variables.

Table 8. Significant correlations of Spearman's rho.

Variables		O1	O2	O3	O4	O5	O6	O7	O8	O9	O10	O11	U1	U2	U3	U4	U5	U6	U7	U8	D1	D2
	P value																					
01		1	0.775	0.556	0.573	0.209	0.366	0.380	0.389	0.386	0.275	0.375	0.318	0.299	0.390	0.258	0.237	0.164	0.062	0.293	-0.291	-0.210
02		**0.000**	1	0.547	0.581	0.135	0.419	0.389	0.444	0.387	0.285	0.366	0.287	0.241	0.368	0.277	0.279	0.192	0.124	0.265	-0.271	-0.247
03		**0.000**	**0.000**	1	0.569	0.135	0.312	0.312	0.407	0.354	0.460	0.459	0.151	0.162	0.327	0.215	0.210	0.157	0.151	0.254	-0.199	-0.229
04		**0.000**	**0.000**	**0.000**	1	0.193	0.460	0.363	0.442	0.457	0.306	0.437	0.243	0.184	0.377	0.266	0.279	0.149	0.135	0.295	-0.298	-0.131
05		**0.000**	0.017	0.018	**0.001**	1	0.187	0.390	0.187	0.228	0.193	0.192	0.119	0.034	0.058	0.093	0.173	0.100	0.097	0.172	-0.096	-0.194
06		**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	1	0.358	0.470	0.463	0.301	0.429	0.342	0.223	0.276	0.309	0.298	0.243	0.231	0.241	-0.208	-0.138
07		**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	1	0.443	0.291	0.267	0.342	0.187	0.142	0.281	0.191	0.263	0.133	0.113	0.145	-0.152	-0.236
08		**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**	1	0.579	0.477	0.586	0.262	0.209	0.228	0.300	0.227	0.213	0.140	0.203	-0.142	-0.163
09		**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.000	1	0.407	0.449	0.233	0.171	0.260	0.242	0.222	0.214	0.171	0.290	-0.140	-0.123
010		**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**	**0.000**	**0.000**	1	0.693	0.111	0.099	0.200	0.189	0.151	0.223	0.189	0.278	-0.090	-0.059
011		**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	1	0.229	0.156	0.244	0.284	0.253	0.201	0.157	0.252	-0.125	-0.079
U1		**0.000**	**0.000**	**0.008**	**0.000**	0.036	**0.000**	**0.001**	**0.000**	**0.000**	0.053	**0.000**	1	0.839	0.623	0.628	0.505	0.539	0.257	0.369	-0.232	-0.175
U2		**0.000**	**0.000**	**0.004**	**0.001**	0.551	**0.000**	0.012	**0.000**	**0.003**	0.083	**0.006**	**0.000**	1	0.572	0.559	0.449	0.533	0.192	0.346	-0.234	-0.095
U3		**0.000**	**0.000**	**0.000**	**0.000**	0.310	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	1	0.729	0.517	0.409	0.208	0.399	-0.270	-0.194
U4		**0.000**	**0.000**	**0.000**	**0.000**	0.103	**0.000**	**0.001**	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**	**0.000**	**0.000**	1	0.530	0.461	0.257	0.434	-0.214	-0.133
U5		**0.000**	**0.000**	**0.000**	**0.000**	0.002	**0.000**	**0.000**	**0.000**	**0.000**	**0.008**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	1	0.494	0.472	0.411	-0.144	-0.032
U6		**0.004**	**0.001**	**0.006**	**0.009**	0.081	**0.000**	0.020	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	1	0.421	0.464	-0.104	-0.107
U7		0.276	0.030	**0.008**	0.018	0.088	**0.000**	0.048	0.014	**0.003**	**0.001**	**0.006**	**0.000**	**0.001**	**0.000**	**0.000**	**0.000**	**0.000**	1	0.424	-0.047	-0.081
U8		**0.000**	**0.000**	**0.000**	**0.000**	0.002	**0.000**	0.011	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	1	-0.260	-0.079
D1		**0.000**	**0.000**	**0.000**	**0.000**	0.091	**0.000**	0.007	0.013	0.014	0.116	0.029	**0.000**	**0.000**	**0.000**	**0.000**	0.011	0.068	0.408	**0.000**	1	0.362
D2		**0.000**	**0.000**	**0.000**	0.021	**0.001**	0.015	**0.000**	**0.004**	0.032	0.301	0.168	0.002	0.097	**0.001**	0.019	0.581	0.060	0.157	0.169	**0.000**	1

01- information written on label is useful; 02 - good source of information; 03 – easy to understand information on label; 04 - label has information about food product; 05 - label serves to sell product.; 06 - label gives information about quality of product; 07 –information on the back of packaging is very useful; 08- label contains enough information; 09 - symbols provide useful information.; 010 - font size is adequate; 011 - label is appropriate; U1 - ensures food quality; U2 - ensures food safety; U3- helps one choose healthy food; U4 - ensures nutritional quality; U5- allows consumption according to ethics; U6 - prevents food fraud; U7- respects religious beliefs; U8- ensures traceability; D1- risk to public health; D2 – loss of consumer confidence. Significant correlations (P <0.01) and correspondent r values were presented in **bold**.

4. Discussion

The new food policy framework established in the European Union is aimed not only to guarantee food safety but also improve public health. Since consumer demands regarding healthy and safer products have increased in the last years, the importance of food labelling and the information provided has gained a huge importance in the present days (Aung and Chang, 2014). Also, the recent food fraud scandals, due to mislabelling practices, have increased the consumer distrust in the food industry since information displayed on food labels does not reflect the real characteristics declared (Barnett et al., 2016). Food labelling can be considered as communication tool between food industry and consumers. Although some reports highlighted the influence of food label information and purchase behaviour (Liu and Niyongira, 2017), the lack of interest of consumers in food label reading have recently discussed (Dörnyei & Gyulavári, 2016) mainly associated to the lack of time (Moreira et al., 2019). Our study showed that overall information is useful for respondents although specific mentions such as information located in the back-of-the package, symbols or proper description of food products seems to be not useful enough. Although the difficulty in understanding the information displayed on food label was not affected by socio-demographic characteristics in the present study, other works indicated age and education as critical factors about food label comprehension (Jackey, Cotugna & Orsega-Smith, 2017). In addition, reduced font size may contribute to undermine the opinion of respondents regarding food industry, also increasing the mistrust in the own food product. The importance of food label design, such as font colors and graphics, may affect consumer choice decision (Shen, Shi and Gao, 2018). The lack of usefulness declared by respondents could be associated to factors as text blocks at right angles, separation of the nutrition facts table from the list of ingredients, inadequate spacing between lines or words placed over illustrations as indicated by Mackey and Metz (2009).

Consumers´ confidence regarding food fraud has been affected in the last years due to the recent food scandals (Montanari, Varallo and Pisanello, 2016) such as horse meat scandal. This situation is compatible with the results observed in the present work in which meat and meat products showed the worse confidence among respondents. In addition, it seems that lack of confidence is directly proportional to the degree of food processing. Thus, olive oil has been considered as one of the foodstuffs that generates more confidence among respondents. However, olive oil together with honey, are one of the most falsified foodstuffs today (Conte el

at 2019, JCR, 2016). With regard of quality labelling, foodstuffs under protected designation of origin or protected geographical indication schemes displayed better confidence among the respondents, in accordance with Gracia and de-Magistris (2016), probably associated with a greater perception of control by food authorities.

Food fraud is an old practice that has always existed but recently become much more of an issue for consumers, thanks to viral news articles and social media (Shears, 2010). Although slightly more than half of the respondents recognize some food fraud practices, implementation of education programs to increase consumer knowledge is essential since education, according to the results, seems to be a critical factor (Spink et al., 2016). Also, Charlebois et al. (2016) suggests that highly educated consumers are more likely to distrust the information on food labels, and are more willing to use a device to validate food label content. In addition, it seems that respondents recognize better food fraud practices that imply a public health risk than those related to economically motivated adulterations. The recent food frauds carried out by the food industry have been related to mislabelling practices due to the use of lower value ingredients not declared on the label (Christiansen et al., 2018, Di Pinto et al, 2015). Although it is difficult to explain, these practices carried out by the food industry could have taken advantage of factors such as lack of knowledge of consumers, as previously indicated, together the lack of control mechanisms of food authorities. Research about consumers´ perception about food fraud are mainly focused on indication of the country of origin (Chousou, Tsakiridou, & Mattas, 2018) traceability information (Bitzios et al., 2017) or implementation of new food safety strategies such as food defence plans throughout the food chain (Davidson et al., 2017). However, no research available evaluates the consumers´ knowledge about food fraud. Thus, the respondents´ perceptions about food labelling and fraud observed in the current work could be use as guidelines by food industry to improve the food label design to enhance the consumer understand and usefulness. Also, addition of enough information in a clear way may enhance the consumer trust in a in a period of loss of confidence in the food industry due to the recent food fraud scandals.

5. Conclusion

The recent food fraud scandals, due to mislabelling practices, have increased the consumer distrust in the food industry since information displayed on food labels does not reflect the real characteristics declared. The present study showed that compulsory information displayed in food label is useful however the way the information is presented may decrease the consumer

interest and also difficult its understand. Regarding consumers´ confidence about the information displayed on food labelling, over half of respondents stated that information provided is reliable. However, respondents showed a lack of confidence on food composition that still decrease in processed foodstuffs such as meat products. Food fraud is recognized slightly over half of respondents with a higher perception of those practices that imply a risk to public health than those related to economic motivation. Age and education revealed the most important socio-demographic factors regarding food label perception, confidence on its information and also knowledge about food fraud. Thus, implementation of education programs to increase consumer knowledge about food labelling and fraud is essential. Since scarce research is available about consumer perceptions about food label information and food fraud, the respondents´ perceptions observed in the current work could be use as guidelines by food industry to improve food label design to enhance the consumer understand and usefulness. Also, addition of enough information in a clear way may enhance the consumer trust a in a period of loss of confidence in the food industry due to the recent food fraud scandals.

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PART B - ADULTERATION OF FOODS FROM ANIMAL ORIGIN

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PAPER III - BOOK CHAPTER - MOREIRA, MARIA J.; SARAIVA, CRISTINA; ALMEIDA, JOSÉ M. M. M. 2017. SPECTROSCOPIC METHODS FOR FRESH FOOD AUTHENTICATION: AN OVERVIEW. IN TRENDS IN FOOD SAFETY AND PROTECTION, ED. V RAVISHANKAR RAI, JAMUNA A BAI, 131 - 166. ISBN: 9781138070912. INDIA: CRC PRESS, TAYLOR & FRANCIS GROUP. (ANNEX III)

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SPECTROSCOPIC METHODS FOR FRESH FOOD AUTHENTICATION: AN OVERVIEW

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Highlights

- Application of spectroscopic methods to detect fraud in fresh food.

- Quantification of adulteration with chemometric methods.

- Spectroscopic/chemometric methods for detection of adulteration applied at food industry.

Statement of originality

There is an increase of consumption of meat and fish from wild game species produced without addition of supplements in the feed or during the manufacturing process.

There is also an increase of fraud in fresh meat and fish such as partial substitution by species with lower value.

This work aims to provide an up-to-date review of spectroscopic and chemometric methods suitable for the detection of adulteration or fraud of fresh meat and fish.

Keywords

Fresh food authentication; Fresh food adulteration; Meat and fish products; Spectroscopic methods, Chemometrics

CHAPTER V. EXPERIMENTAL RESULTS

PART B - ADULTERATION OF FOODS FROM ANIMAL ORIGIN - PAPER III

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Summary

Authentication of food is acquiring interest in the food industry, among retailers and consumers and in the food authority's area due to the increased food shortages. To take the correct decision in cases of food fraud, it is necessary to have knowledge about species replacement and foodstuffs identification, discrimination between fresh and frozen-then-thawed products and geographical origin recognition of food products. The presence or absence of food additives and of genetically modified organisms can also be susceptible to fraud.

Various techniques have been developed for the determination of adulteration and authentication of foodstuffs. For example, classical analytical methods such as enzymatic methods and polymerase chain reaction have been used with satisfactory results, but these techniques have drawbacks. There are alternative methods such as vibrational spectroscopy and mass spectrometry that have been applied in fraud detection and authentication of food. Methods based on absorption spectroscopy in the visible, near infrared and mid infrared, Raman spectroscopy, nuclear magnetic resonance imaging, multispectral and hyperspectral imaging have great advantages since they are rapid, non-destructive and very powerful methods. However, it requires complex data analysis.

In this work it will be presented a review on the most relevant spectroscopic techniques developed for authentication and fraud detection in fresh food.

1. Introduction

Consumers recognize the worth of the information contained in the labelling, including the description of the ingredients and the information about the production processes applied to the final product by the industry or in the commercial areas. The food consumer's choice reflects lifestyle and religious issues and awareness of the nutritional properties and health. In fact, the identification and authentication of food play an important role in a healthy diet. The verification and reporting of food products components is needed to prevent the practice of adulteration (Huck et al., 2010)

Consumers have become more demanding on the patterns of meat and fish consumption, in terms of quality, safety and origin of products. The lately reports of food fraud occurrence suggests that it is required an effective identification of the species as part of foods authentication (Andrée et al., 2010, Ballin, 2010, Standal et al., 2010a, Lin et al., 2014a).

The animal products, particularly meat and fish, can be targets of adulteration, such as the substitution or removal of ingredients, addition of other proteins from various origins and the addition of food additives and genetically modified organism (GMO) not described in the label, often contributing to increase financial profits. The authentication and determination of quality of meat and fish is of great importance to prevent fraud and causes economic problems in the food industry and health problems for the consumer (Meza-Márquez et al., 2010, Cawthorn et al., 2013). For example, the substitution of fresh meat and fish for frozen-then-thawed products is a typical commercial fraud which may cause economic losses and food safety and quality issues to consumers. These products are characterized by an increased susceptibility to microbial grow and colour changes. Temperature fluctuations can result in formation of ice crystals (Cozzolino and Murray, 2004, Ballin and Lametsch, 2008, Fajardo et al., 2010, Standal et al., 2010a, Alamprese et al., 2013, Ottavian et al., 2013, Lin et al., 2014a) due to the migration of water vapour from the product to the surface, causing a poor quality of food products. This defect is recurrently found in frozen foods which have been inadequately controlled.

Food authentication depend on establishment of databases that contain information about origin of foods including biological and geographic origin, species, production methods, and other critical information. There is a need for reliable analytical methods that can verify geographic

origin of foods apart from their biological origin. Un-targeted spectroscopy approaches combined with chemometric analysis were investigated for its potential to classify the geographical origin and predict values of meat (Sun et al., 2012a). An overview of analytical methods for determining the geographical origin of food products can be found in (Luykx and van Ruth, 2008).

Modern food inspection is under an ever increasing demand for efficiency in the use of resources, either human or materials and for achievement of purposes through optimal inspection planning and the use of new methodologies. Spectroscopy based in analytical technology tools in combination with dynamic predictive models may bring these goals closer to reality (Thygesen, 2012). Dynamic chemometric methods were used in food inspection for quality monitoring in food processing industries (Singh and Jayas, 2013). The authors present a discussion on three broad categories of optical sensing techniques, namely, spectroscopic, fibre optic and imaging. In their work they described working principles, instrumentation, advantages, disadvantages, and limitations of these techniques. For instance, an ultra-low field magnetic resonance imaging (MRI) system using a high-temperature superconducting quantum interference device (HTS-SQUID) for food inspection was reported in (Kawagoe et al.).

There are several methods for the detection of low levels of adulteration (Ballin and Lametsch, 2008). Replacement of products are often similar to the main material from a biochemical point of view, therefore, adulterant identification can be particularly difficult (Ghovvati et al., 2009). Recently researchers have applied various analytical techniques for the detection of foodstuffs fraud. The protein-based methods (Al Ebrahim et al., 2013a, Mamani-Linares et al., 2012a), the deoxyribonucleic acid (DNA) based methods (Ali et al., 2012, Cammà et al., 2012, Mamani-Linares et al., 2012a, Sakaridis et al., 2013, Zhang, 2013, Karabasanavar et al., 2014, Lin et al., 2014a), the real time polymerase chain reaction (PCR) techniques and analysis of triacylglycerol (Kesmen et al., 2009, Fajardo et al., 2010, Soares et al., 2010, Druml et al., 2015a) and methods based on fat (Abbas et al., 2009, Rohman et al., 2011b), have become increasingly important. However, these methods are laborious, are technically demanding, slow, invasive, expensive, destructive, require sophisticated laboratory procedures and highly qualified employees. Moreover, they are not suitable for real-time applications (Damez and Clerjon, 2008).

The various multidimensional analytical approaches that permit authentication of food can be divided in targeted and un-targeted methods. The classical authenticity assessment of food is usually based on the analysis of specific marker compounds, which are indicative for a certain property of the product (Herrero et al., 2012). Given that most adulterants are unknown; they are difficult to recognize using the targeted screening approaches typically used in food laboratories. The industry needs non-targeted methods to analyse samples for adulterants to provide a proof of origin or to prevent deliberate or accidental undeclared admixture to food samples (García-Cañas et al., 2012). Food fingerprinting approaches are based on a high-throughput screening of samples with the purpose of a differentiation or classification of samples. The investigation of food fingerprints provides high potential with regard to the characterization and verification of food identity. These approaches are usually based on spectroscopic and spectrometric data, providing the ability for a comprehensive characterization of the investigated matrices. The aim is to differentiate various food fingerprints in terms of, for instance, possible adulterations or their botanical or geographical origin (Esslinger et al., 2014).

There is a growing interest in methods based on spectroscopic techniques because they offer several advantages. Emerging non-destructive mapping technologies for authentication and traceability include nuclear magnetic resonance (NMR) imaging, fluorescence (FS), visible (VIS), near infrared (NIR), mid infrared (MIR) and Raman (RS) spectroscopy, sometimes coupled with Fourier transform technique, and multispectral (MSI) and hyperspectral (HIS) imaging. These are simple, non-destructive, non-invasive, low-cost, and allow real time analysis (Lohumi et al., 2015). All spectroscopic techniques require small samples and no further preparation is necessary. They are powerful tools for conducting adulteration tests (Mamani-Linares et al., 2012a). The methods presented in this work might be used as a complement or even constitute an alternative to PCR based DNA (Schmutzler et al., 2015).

The techniques NIR and MIR combined with Fourier transform (the latter also called FTIR - Fourier transform infrared) (Cozzolino and Murray, 2004, Ortiz-Somovilla et al., 2005, Rodriguez-Saona and Allendorf, 2011, Mamani-Linares et al., 2012a, Alamprese et al., 2013, Morsy and Sun, 2013a, Rohman et al., 2011b, Meza-Márquez et al., 2010), RS (Abbas et al., 2009, Boyaci et al., 2014, Zając et al., 2014, Zhao et al., 2015) and NMR (Rezzi et al., 2007,

Aursand et al., 2009, Standal et al., 2010a) combined with multivariate statistical methods were largely applied in the authentication of foodstuffs.

In addition, ultraviolet (UV) based spectroscopic methods were used in meat and fish adulteration studies (Alamprese et al., 2013). In recent years, these techniques have received much attention for safety inspection and the quality of food and meat and related products (Kamruzzaman et al., 2013, Barbin et al., 2013, Kamruzzaman et al., 2016, Kamruzzaman et al., 2012, Ma et al., 2015).

This work is an up-to-date literature revision applied to the detection of fresh meat and fish adulteration using spectroscopic methods. This work can be important for future research and to develop equipment and methods to be applied in commercial area, allowing detection of adulteration analysis obtained in short time.

2. Methods of vibrational spectroscopy

2.1 Visible and near infrared spectroscopy

The VIS and NIR spectroscopies offer a number of important advantages when compared to traditional chemical methods. These methods deal with the VIS and the NIR region of the electromagnetic spectrum, from about 750 to 2500 nm, corresponding to a photon energy from 4000-13000 cm^{-1} (Huck, 2015). When using the NIR region, the spectra can be recorded in reflection or transmission. The interaction of the radiation with matter provides information about the presence of functional groups (Lohumi et al., 2015, Huck, 2015, Porep et al., 2015).

In comparison with other vibrational spectroscopic methods, NIR is considered a time consuming procedure and the detector is often a source of noise. The use of an interferometer reduces the time of analysis through the single output signal (spectrum) which has all the infrared frequencies encoded therein. A Fourier transform in necessary to extract the information from the spectrum. The interferometry coupled to Fourier transform started to received great attention for its use in the quantitative analysis of edible fats and oils (Gouvinhas et al., 2015). The attenuated total reflectance (ATR), diffuse reflectance, high-yield transmission and diffuse transmission cells are measuring methods used together with Fourier transform (Lohumi et al., 2015).

The diffuse reflectance or trans-reflectance spectroscopy gained attention in the control process of fraud in the food industries, however, adequate overall reflectance is the most widely adopted method for analysing the quality and authenticity of the final food product (Porep et al., 2015). Reflectance infrared spectroscopy allowed to discriminate fresh meat and fish products from frozen-then-thawed and mixtures of species that are not permitted in the final product (Mamani-Linares et al., 2012a).

NIR spectroscopy was applied in industrial online setups using a fibre optic probe and in the laboratory to detect different veal meat adulteration with pork parts (Schmutzler et al., 2015). Samples of veal meat with different percentages of pork parts were analysed using chemometric methods. Control samples without adulteration (100% veal) and samples with increasing level of adulteration were prepared in 10% steps from original until to obtain the composition of 50%

veal and 50% pork parts. Figure 1 shows the second derivative of spectra from 6200 to 5480 cm^{-1} measured with the laboratory setup as a function of the adulteration of the veal meat with pork. Close connection between the signal intensity and the level of adulteration from genuine (no adulteration) up to 50% were found at 5940 cm^{-1} (1683 nm), 5908 cm^{-1} (1693 nm), 5892 cm^{-1} (1697 nm), 5868 cm^{-1} (1704 nm), 5776 cm^{-1} (1731 nm), 5756 cm^{-1} (1737 nm), 5668 cm^{-1} (1764 nm), 5648 cm^{-1} (1770 nm) and 5492 cm^{-1} (1821 nm). Principal component analysis (PCA) were used to obtain a three-dimensional projection of samples and to observe the relation between the genuine product and samples with adulteration. It was possible to notice absence of associations from 20 and 50 % of adulteration, with one and two principal components (PC), for laboratory and the industrial setups, respectively. However, three PC were necessary for models applied to the on-site setup. Data from PCA were used as input for classification and validation using support vector machines (SVM). The SVM allowed correct calibration values of discrimination of 94.4 % for the laboratory, 91.7 % for the industrial and 77.8 % for the on-site analyses to be achieved (Schmutzler et al., 2015).

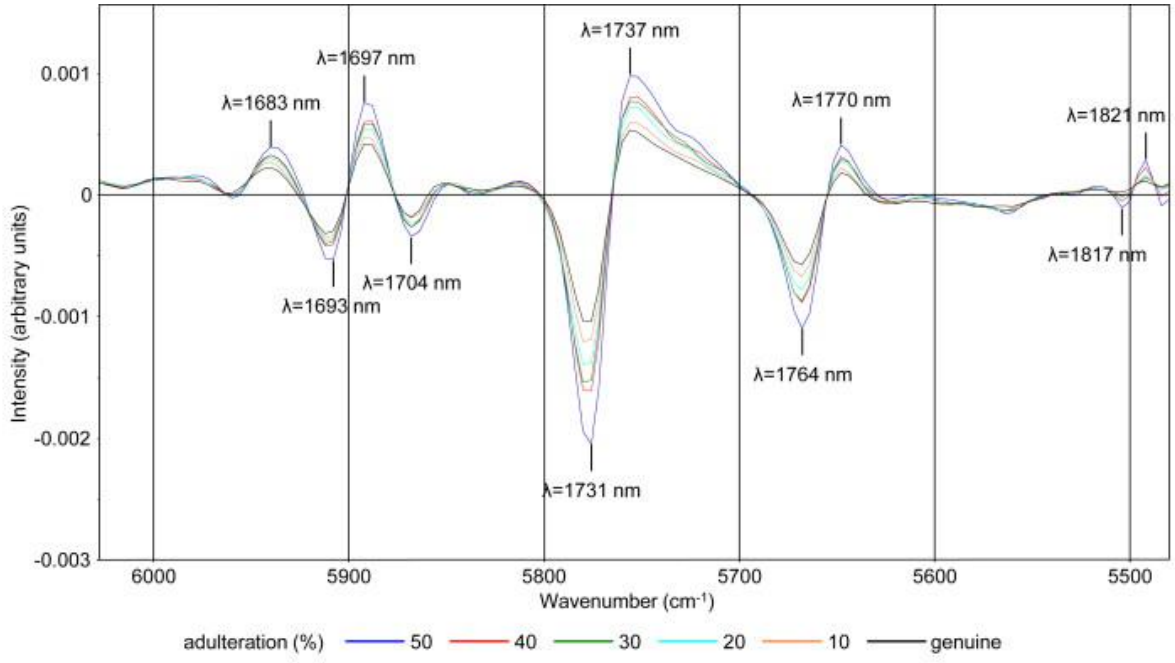

Figure 3. Second derivative of spectra (from 6200 to 5480 cm^{-1}) measured with the laboratory setup as a function of the adulteration of the veal meat with pork. Adulteration levels from genuine (no adulteration) up to 50%, in 10% steps. (Reprinted with permission from (Schmutzler et al., 2015).)

In a study conducted by Mamani-Linares et al. (Mamani-Linares et al., 2012a), VIS and NIR reflectance spectroscopy or trans-reflectance methods were used to discriminate meat and meat juices from three livestock species. Meat samples from beef, llamas and horses were purchased from different butcher shops and supermarkets. The 79 samples of *Longissimus lumborum*, 500 g each, were used: 31 of beef, 21 from llama and 27 from horse were thawed at 4 °C for 24 h and stored for 4 to 6 h before measuring the spectra. Others 58 samples of the same muscle (20 of beef, 19 from llama and 19 from horse) were used for obtain the juice. They concluded that the VIS-NIR spectroscopy coupled to PCA, and with partial least squares regression (PLS-R), is a useful tool to discriminate different species. In addition, it is useful to discriminate the geographical origin and the production system (Mamani-Linares et al., 2012a).

The potential of UV-VIS, NIR and MIR spectroscopies coupled with the chemometric techniques PCA, PLS-R and linear discriminant analysis (LDA) enabled the detection of minced beef adulteration with turkey meat (Alamprese et al., 2013). Each batch was separately minced and then used to prepare (in duplicate) 7 mixtures of bovine meat added with different percentages of turkey meat: 5 to 50 %, in 5 % steps. With NIR, two PCs explained 98 % of the total variance and for MIR the first two PCs explained 82.3 %. LDA allowed to classify correctly 78.6 % in the UV-VIS, 88.3 % in the NIR and 84.8 % in the MIR. PLS-R allowed construction of models with the root mean square error of cross-validation (RMSECV) and the root mean square error of prediction (RMSEP) slightly smaller than for NIR (Alamprese et al., 2013).

NIR has a potential for detecting and quantifying different adulterants in fresh and frozen-then-thawed minced beef. In addition to the pure beef and pork, fat trimming and offal samples, a series of mixed samples in the range of 10-90 % (w/w) from pork (n=144) and 10-80% of fat trimming (n=112) was prepared (Morsy and Sun, 2013b). The mixtures of samples adulterated with offal were prepared in the range of 2.5-30 %. The PLS-R had a determination coefficients (R^2) of 0.96, 0.94 and 0.95 with standard error of prediction (SEP) of 5.4, 5.1 and 2.1 %. Models based on PLS-R/DA and LDA distinguished between the unadulterated and adulterated classes with a classification of 100% (Morsy and Sun, 2013a).

The NIR combined with chemometric analysis was used for data analysis to classify the geographical origins of lamb meat (Sun et al., 2012). The δ 13 C and δ 15 N values of defatted

lamb meat (Alxa League (37º53'N, 105º23'E, n = 20), Xilin Gol League (42º21'N,115º08'E, n = 19) Chongqing City (30º50'N, 108º24'E, n = 20) and Heze City (34º48'N, 116º04'E, n = 20) and Hulunbuir City (49º06'N, 119º40'E, n = 20) were determined by isotopic ratio mass spectrometry (IRMS). The analytical precision was both lower than 0.2‰. FDA/PLS-R and LDA gave a total correct classification of 88.9% and 75% to the five individual region samples, respectively. The PLS-R/DA and LDA classified correctly 100 % of samples from pastoral and agricultural regions. For PLS-R calibration models the obtained R^2 value was 0.76 and 0.87 for predicting δ 13 C and δ 15 N, respectively. The first three PCs explained 98% of the total variance.

The adulteration of beef with pork and chicken was studied by (Bilge et al., 2016). The beef samples were adulterated with pork and chicken (concentrations between 10 and 50%). The PLS-R method was used for evaluating laser-induced breakdown spectroscopy (LIBS) spectral data and RMSEC and R^2 values of 2.67 and 0.994 were obtained for beef adulterated with pork. A 83.4% correct discrimination rate between beef, pork and chicken was achieved by PCA (Bilge et al., 2016).

The samples (43 adulterated and 12 controls) originated from dismantled criminal networks by the Brazilian Police were analysed using chemical parameters and ATR in conjunction with FTIR spectroscopy (Nunes et al., 2016a). This fraud consisted of injecting aqueous solutions of non-meat ingredients (NaCl, phosphates, carrageenan, maltodextrin, and collagen) in bovine meat in nature. The PCA model of ATR-FTIR spectroscopy data was obtained with 4 latent variables (LV), accounting for 95.7 and 26.7% of variance in X and Y blocks, respectively. The PLS-R/DA model correctly detected 91.0% of the adulterated samples (Nunes et al., 2016a).

Several strategies have been proposed for the determination of substitution of fresh fish with frozen-then-thawed fish (Ottavian et al., 2013). One of the first strategies consists on using the PLS-R/DA method to classify the fresh and frozen-then-thawed status of each sample considering the species altogether. In another approach, a two-level cascade arrangement of PLS-R/DA was developed: in the first level, a PLS-R/DA was used to classify the samples according to their species and in the second level, a different PLS-R/DA discriminated between fresh and frozen-then-thawed samples. In a third strategy, an orthogonal PLS-R/DA was used to remove the information from the spectral data which is not related to the fresh and frozen-

then-thawed status of the samples. Depending on the strategy, the obtained overall calibration accuracies ranged between 80 and 91 %. The PCA explained 97 % of the total variability (Ottavian et al., 2013).

The NIR and VIS-NIR spectroscopy was used for distinguish fresh from frozen-then-thawed swordfish cutlets (Fasolato et al., 2012). Thirty specimens of swordfish were caught using traditional hooks and fishing. The relevant data were recorded to maintain sample traceability. The samples were vacuum-packed in polyethylene bags and three of them were refrigerated at 2 ºC. The remaining were frozen and stored: 30 samples at -18 ºC and the remaining 30 at -10 ºC for 30 days. Before analysis, the frozen samples were thawed overnight in the lab at 2 ºC. The first three PCs of the PCA explained 87.2 % of the variability and with milling treatment the first three PCs explained a higher value (94.8 %). The samples were classified using VIS-NIR spectroscopy with a correct classification of 96.7 %, whereas this value for NIR was higher than 90.0 %.

In another study to distinguish fresh from frozen-thawed, *Pagrus major* fish were divided into 2 equal groups and used for further evaluation (Uddin et al., 2005). For fresh or frozen-then-thawed fish, 54 samples were used soon after being killed, whereas the second lot of 54 fish was kept at -40 °C. After 30 days fish were removed and thawed then evaluated as frozen-then-thawed samples. The fresh or frozen-then-thawed status was investigated and discrimination was carried out by soft independent modelling of class analogy (SIMCA), LDA based on PCA. The investigators obtained a classification of 100 %. However, the high percentage of water in the fish is a major limitation for the analysis of samples with this application.

Real time measurement and noise reduction using NIR spectroscopy requires Fourier transform. The methodology allows detection of small molecules, however, water interference is a major drawback. This method allows reading through glass or polypropylene containers.

2.2 Mid-infrared spectroscopy

The MIR spectroscopy it is also fast, non-destructive and do not involve laborious sample preparation. It is an attractive option to identify and quantify the adulteration and chemical composition (Rohman and Man, 2011). The absorption bands in the MIR region are

characteristic of functional groups of molecules (Meza-Márquez et al., 2010, Zhao et al., 2014). The MIR region can be divided in the functional group region, from 4000 to 1500 cm $^{-1}$, and fingerprint region, from 1500 to 500 cm $^{-1}$ (Lohumi et al., 2015). The spectroscopy in MIR associated with FTIR spectroscopy and multivariate analysis require low sample volume and is environmentally friendly. FTIR spectroscopy in combination with PLS-R regression technique and PCA are powerful tools for quantification and classification of adulterants (Rahmania et al., 2015a).

These methods are fast and effective for detection of contaminants and adulterants (Meza-Márquez et al., 2010, Rodriguez-Saona and Allendorf, 2011). Some countries make regulations to determine whether foods are safe, authentic and protect consumers of Halal. Investigators have been conducted studies for detection of adulterants in this type of food (Kurniawati et al., 2014).

In a research on the investigation of the adulteration of Gamo meat (*Dama dama*) with different percentages of Goat meat (0, 25, 50, 75 and 100%). Samples were stored at 3 °C for periods of time of 12 to 432 h. The methods used were microbiological analysis, measurement of colour, lipid oxidation based on the thiobarbituric acid reactive substances method (TBARS), FTIR, sensory analysis and statistical methods of multivariate analysis. In Figure 2 can be observed the average FTIR spectrum of different mixture proportions stored at 3 °C for 0h between 2000 and 900 cm^{-1}. It can also be observed a peak at approximately 1639 cm^{-1} due to the presence of water (O-H stretch) with simultaneous contribution of amide I (C=O). A second peak at 1550 cm^{-1} can be associated with the amide II (N-H, C-N). The peak at approximately 1460 cm^{-1} can be assigned to fat (ester CO). The absorptions in the region of 950-1200 cm^{-1} may reflect the content of carbohydrates, especially muscle glycogen. The amide content III can be viewed at about 1300 cm^{-1} and amino acid side chains between 1480 and 1800 cm^{-1}. Figure 3 shows the graph of observations of a LDA, were the discriminant factors F1 and F2 explain 96.63% of the total variance. With this analysis the authors obtained a clear distinction between each blending percentage. The accuracy and performance of the model that relates the current and estimated values obtained from FTIR spectra is shown in Figure 4 and at t = 27 and t = 0h 432h, respectively. The PLS-R was used in order to validate and calibrate the model used. The PLS-R model was conducted to determine the relationship between the predicted values and the

measured values of the mixtures. The R^2 coefficient shows high values and the RMSEC and RMSECV have low values which demonstrates that the PLS-R model has a good predictive accuracy and performance.

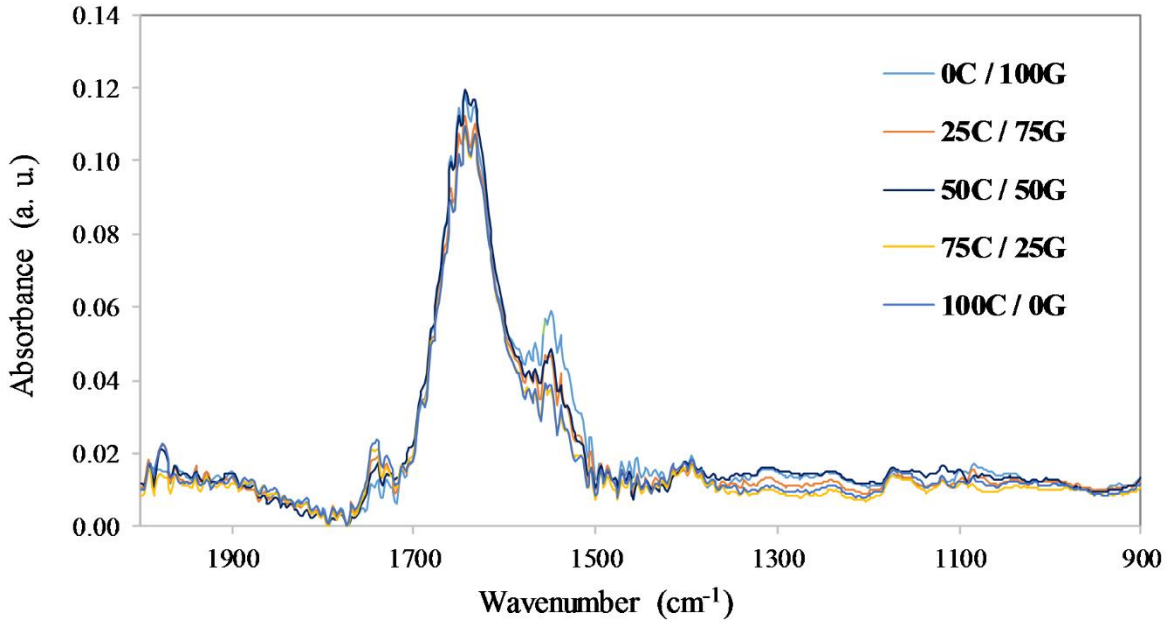

Figure 4. FTIR spectra of hamburgers containing different percentages of Gammo (G) and Goat (C) from 2000 to 900 cm $^{-1}$. (Reprinted from (Silva, 2014).)

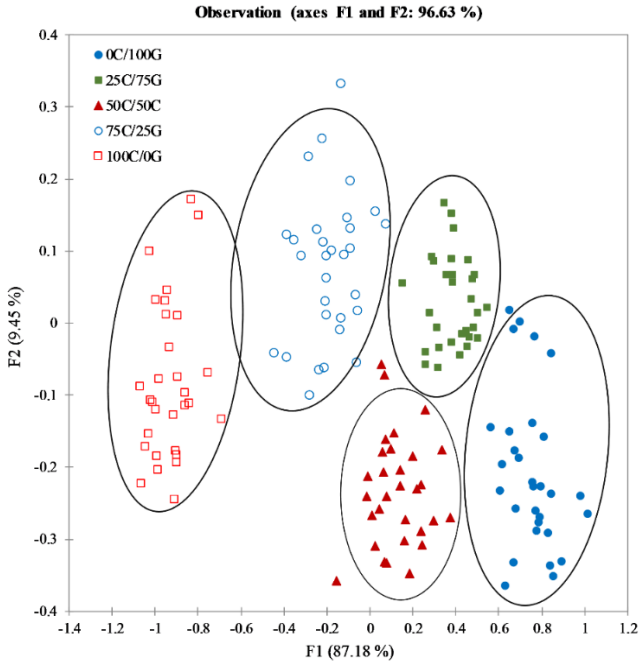

Figure 5. Projection of the samples according to the storage time (t0 to t14) and the type of meat to both two batches where G samples corresponding to Gamo, and C corresponding to samples of goat. (Reprinted from (Silva, 2014).)

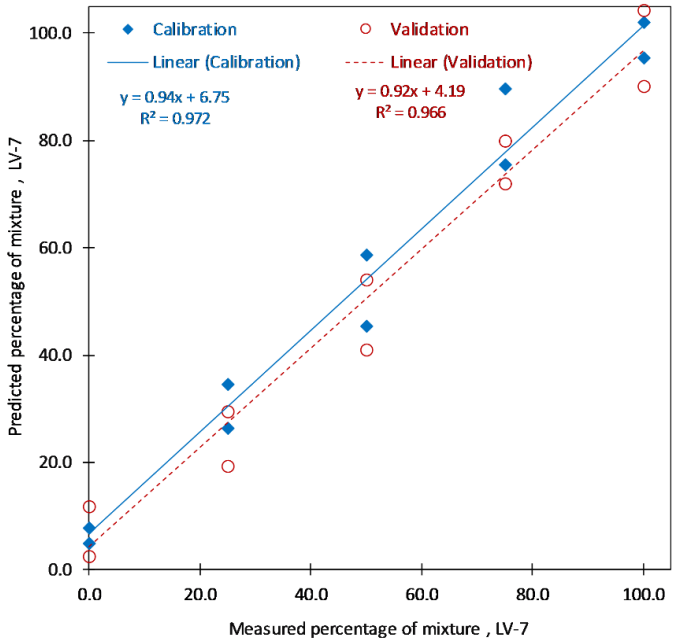

Figure 6. Illustration of the quality of the PLS-R model conducted to determine the relationship between the predicted values and the measured values of the mixtures. (Reprinted from (Silva, 2014).)

The adulteration of beef meatballs with meat of rat (*Rattus argentiventer*) was studied by (Rahmania et al., 2015a). Rat meat was obtained from farmers while beef was purchased from several local markets. During the preparation of calibration samples, rat meat and beef was prepared by mixing rat meat at concentration of 0, 10, 20, 35, 50, 65, 80, and 100 % in beef. The FTIR spectroscopy in combination with PLS-R and PCA multivariate calibrations were used for the differentiation between rat meat and beef meatballs. The frequency region from 750 to 1000 cm^{-1} was selected during PLS-R and a R^2 value of 0.993 and root mean square error of calibration (RMSEC) of 1.79% was obtained. The PCA modelling method classified correctly the meatball sample with 100 % rat meat and 100 % beef.

In a similar study, the investigators prepared oils of pig (lard), lamb, beef and chicken. FTIR and GC analyses were performed. For calibration, a training set of 30 samples consisting of lard, body fats of beef, chicken and mutton with certain concentrations were prepared. Each sample was subjected to FTIR analysis and gas chromatography (GC). PCA showed that PC1 accounted for 57% of the variation, while PC2 explained 25% of variation, and PC3 contributed to 13% of variation. These three first PCs can describe more than 95% of variation (Rohman et al., 2012).

The adulteration of beef meatball with pork was studied by (Rohman et al., 2011b). The calibration set was prepared by spiking pork to beef meatball in the concentrations of 1.0, 3.0, 5.0, 10.0 and 25.0%. Samples containing 100% beef and 100% pork were also made to observe the spectral differentiation. The adulteration was detected using FTIR spectroscopy and PLS-R. This regression method was used to develop a calibration model and a R^2 value of 0.999 was obtained.

In another similar research, pork fat (lard) and beef fat were obtained by rendering process of the corresponding animal (Kurniawati et al., 2014). The fatty acids composition of lard and beef fat was carried out using GC with flame ionization detector (GC-FID). A set of standards consisting of lard in beef fat was prepared by mixing both types of fat in the concentration range of 0 to 100 %. The FTIR spectroscopy in combination with PLS-R and PCA was used for the detection of the substitution of beef fat with lard. The PLS-R was characterized by a high R^2 value (0.998). The PCA was used successfully in the region from 1200 to 1000 cm^{-1}.

The adulteration of high-quality beef steak with horse meat, fat beef trimming and soybean protein was studied by (Meza-Márquez et al., 2010). The beef steak samples were minced using a food processor. Horse meat samples and fat beef trimming were minced separately in the same way as lean beef. Textured soybean protein was rehydrated according to instructions on the packet label. Samples of each type of adulterated mixture (minced lean beef-horse meat, minced lean beef-textured soybean, and minced lean beef-fat beef trimmings) ranging from 2 to 90% w/w adulterant concentration were prepared in increments of 2 %. A methodology using MIR spectroscopy in tandem with chemometrics was developed to discriminate between pure minced meat and adulterated samples. The results of the developed PLS-R models showed, in the region 1800 - 900 cm $^{-1}$, values of R^2 greater than 0.99. The SIMCA model showed 100% correct classification for minced beef and for beef adulterated with horse meat, fat beef trimmings or soy protein.

A common adulteration process is the substitution of fresh food by frozen-then-thawed. Fresh and frozen-then-thawed samples of offal-adulterated beef burger were analysed using ATR-FTIR technique and chemometrics methods (Zhao et al., 2014). The authentic beef burgers were produced in two groups, called lean and fat, which correspond to higher (lean) and lower (fat) quality levels. The beef burgers in each of the two groups were made on separate occasions beginning with the highest lean meat content and moving to the lowest. A total of 82 fresh beef burger samples (36 authentic + 46 adulterated) and 82 frozen-then-thawed beef burger samples (36 authentic + 46 adulterated) were prepared. The first three PCA components accounted for 72.9, 11.3 and 8.4 % of the variability. From the PLS-R models, 100 % accurate classification in calibration and in validation were obtained. The SIMCA efficiency values varied from 0.57 to 0.87 for fresh and from 0.62 to 0.91 for frozen-then-thawed beef burgers.

The MIR spectroscopy requires the preparation and dilution of samples. In this methodology the interference of water contained in the food may occurs. However, MIR with chemometric methods are a promising technique and allows detection of larger functional groups.

2.3 Fluorescence spectroscopy

Fluorescence is a physical process associated with the emission of photons upon molecular transition from the electronic excited state to the ground state. The emission of photons occurs

at a higher wavelength than the wavelength of the incident excitation source (Bridier et al., 2015). The FS involves the application of a light beam in the sample, causing excitation of electrons in molecules of certain compounds and the emission of low energy light. It's a fast, sensitive and non-destructive technique (Karoui et al., 2006, Damez and Clerjon, 2008). A stable fluorescent label is of crucial importance for the sensitivity of quantitative and qualitative detection as well as the contrast of fluorescent microscopic imaging. Covalently bound fluorescent labels would offer a promising tool to obtain highly stable fluoresce labelled particles for a considerable period of time. However, unneglectable leakage and low signal intensity have been also reported (Weiss et al., 2006).

The method combined with multivariate statistical analysis is an effective tool for discrimination of different beef muscles in relation to the age of the animal, chemical and mechanical properties make it possible to evaluate the quality and adulteration of food (Sahar et al., 2016). There are different applications of FS: heterocyclic particular aromatic amines (HAA), tryptophan fluorescence and nicotinamide adenine dinucleotide phosphate oxidase (NADPH) (Karoui et al., 2006). The method of NADPH fluorescence spectrum allows the differentiation of fresh from fresh-thawed fish. The simplicity of the method allows the extension of the VIS spectroscopy characterization efficiency of the fish (Karoui et al., 2006).

Fluorescence spectroscopy allows observation of aromatic acids of the amino acids. When using excitation at 250 nm the emission will be at 305-400 nm). For observation of proteins folding, tryptophan fluorescence can use excitation at 290 nm and emission at 305 - 400 nm (Albani, 2012).

The presence of fluorescent molecules such as tyrosine, phenylalanine and tryptophan in proteins, their environment and biological samples may be detected by this technique (Karoui et al., 2006). Adipose tissue contains fluorescent molecules that are specific for fat. Few studies have been conducted with this method in food. However, NADH/FS and tryptophan fluorescence in combination with chemometric methods allows to determine fresh and frozen-then-thawed fish fillets (Karoui and Blecker, 2011, Karoui et al., 2006). Regarding the PCA of tryptophan fluorescence spectra, the first two PCs explained 55.9 and 36.9 % of the total variance. On the other hand, the PCA of NADH fluorescence spectra led to 84.9 and 12.1 % of

variance for the first two PCs. Then PCA applied to the factorial discriminant analysis (FDA) method obtaining a 100 % accuracy when using the calibration set (Karoui et al., 2006).

The conventional and the synchronous fluorescence spectroscopic method of excitation-emission in combination with chemometric methods, namely predictive and descriptive methods, determines changes in foodstuffs during technological process and storage. The front-face FS has the potential to reduce the analysis time and costs compared to the enzymatic and biochemical methods (Karoui and Blecker, 2011).

2.4 Raman spectroscopy

Raman spectroscopy can be used to observe vibrational, rotational and other low-frequency modes in a molecule and/or a system. The vibrational modes provide a major contribution to knowledge of the chemical constitution of a specific analyte. Raman spectroscopy depends upon the inelastic scattering of monochromatic light, usually from a laser in the ultraviolet, visible or near infrared wavelengths (Li-Chan, 1996).

Raman spectroscopy was used to determine authentication and quality of foodstuffs (Lohumi et al., 2015). This technique allows the determination and provides specific information about lipids, proteins and carbohydrates, and it can be employed to classify microorganisms (Argyri et al., 2013). It has the capacity to provide information on the chemical structure of molecules without causing changes in the samples (Boyacı et al., 2014a). It is a very promising method and has high potential for evaluating the quality of foodstuffs during handling, processing and storage (Boyacı et al., 2014a, Zając et al., 2014).

There are techniques that can be used to improve the Raman signal, in particular scattering Raman spectroscopy (SRS) (Tipping et al., 2016), coherent anti-stokes Raman (CARS) (Roy et al., 2010), resonance Raman spectroscopy (RRS) (Wächtler et al., 2012), shifted excitation Raman difference spectroscopy (SERDS) (Sowoidnich and Kronfeldt, 2012) and surface-enhanced Raman scattering (SERS) (Hakonen et al., 2015). Reducing the Raleigh scattering allows to obtain high-quality spectra extension. The SERS is a powerful tool for characterizing a wide range of analytes, when combined with biologically relevant nanostructures (Shrestha et al., 2014). The combination of RS with Fourier transform provides high spectral resolution

with effective wavelength accuracy and allows estimating the degree of opening of fatty acids in foods (Lohumi et al., 2015).

The RS coupled with PCA was developed for the rapid determination of beef adulteration with different concentrations of horsemeat. The beef samples were provided from local supermarkets while horsemeat samples were bought from local markets. In the scope of study, beef samples containing 0, 25, 50, 75 and 100 % by weight of horsemeat were investigated (Boyaci et al., 2014). The PCA exhibited a first PC explaining 96.3 % and second PC explaining 3.2 % of the total variance. The developed model system was good enough to differentiate adulterated samples. This method has shown good results because of the short time analysis and simple preparation of sample (Boyacı et al., 2014a). Figure 5 illustrates the Raman spectra of meat samples collected between 200 and 2000 cm^{-1}. Raman bands at 555, 678, 815, 1032, 1265, 1392, 1611 and 1706 cm^{-1} were observed in the spectra of both horse and beef samples. The spectral difference between the samples arose from the unique bands of horse fat that were positioned at 919, 974 and 1215 cm^{-1}.

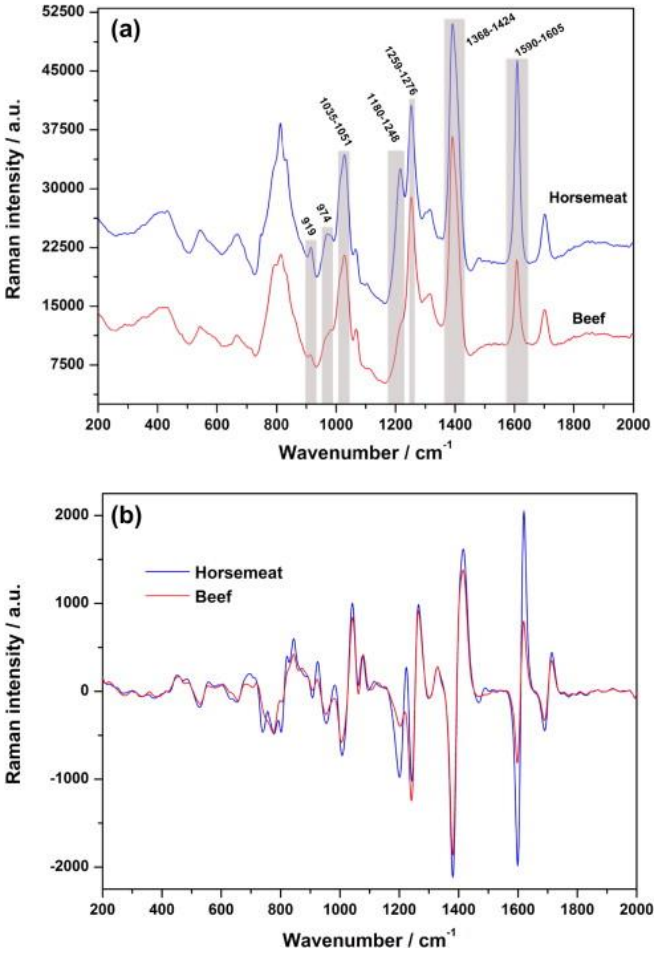

Figure 7. Original (a) and first derivative (b) Raman spectra of horsemeat and beef samples. (Reprinted with permission from (Boyacı et al., 2014).)

In other study, fresh meat species (cattle, sheep, goat, buffalo, pig, fish, chicken and turkey) were purchased from the local markets and slaughterhouses and kept at refrigerated conditions (Boyaci et al., 2014). These were utilized in the preparation of the salami products and fat was extracted from each meat sample. Raman spectroscopy coupled with PCA differentiated the origin of the meat and meat products. Principal components PC1 and PC2 explained 85.1 and 6.4 % of the variance, respectively. After, the third derivative be applied to the spectra, PC1 and PC2 explained the variance of 96.3 % and 2.2 %, respectively (Boyaci et al., 2014).

In a similar study, fresh samples of horse back muscles were purchased from a local butcher (Zając et al., 2014). The meat mixture was prepared from horse meat and beef of the

composition 1:4, 1:2, 3:4, respectively. The content of horse meat in the samples with beef was detected using the Raman bands at 937, 879, 856, 829 and 480 cm^{-1} (Zając et al., 2014).

Al Ebrahim, Swidnik et al. applied a 671 nm (50 mW) microsystem diode laser to study the applicability of the RS in the distinction of beef and horse (Al Ebrahim et al., 2013a). The fresh muscles of beef and horse were purchased from local butcher shops. The muscles were cut into 2 cm thick slices and packed separately. All slices were stored at 5 ºC for a period of 12 days in a laboratory refrigerator. The PCA method was applied for data evaluation and presented the PC1 and the PC2 explaining 79 and 18 % of the total variance, respectively. Raman spectroscopy showed changes in the spectra for proteins, lipids, water muscle meat.

The shifted excitation Raman difference spectroscopy (SERDS) were applied for separation of the meat samples into distinct groups (Sowoidnich and Kronfeldt, 2012). For each animal species, beef (rump steak), pork (loin chops), chicken (breast), and turkey (breast), 12 randomly chosen slices of fresh meat were bought in a local supermarket and measured at the day of purchase for separation of the meat species into four distinct groups with the PCA. The SERDS method has enormous potential and a quick breakdown for classification of different species of meat.

In a study conducted by Ellis, D.I., et al. RS was applied to the identification of meat and poultry-based products and showed potential for the rapid assessment of adulteration of food (Ellis et al., 2005). Samples of pre-packed meat (lamb, beef, pork) and poultry chicken (skinless breast fillets), turkey (skinless breast fillets) were acquired, and for the subsequent experiments, chicken (skinless breast fillets and legs with skin) and turkey (skinless breast fillets and legs with skin) were purchased from retail outlets and stored at 4 ºC. Raman spectra were collected using an infrared diode laser at 785 nm, using a Renishaw 2000 Raman probe system together with the Renishaw WiRE Grams software package and a CCD detector. Spectra were collected for 10 seconds and 1 accumulation over the wavenumber range 100 cm^{-1} to 3000 cm^{-1}. PCA and genetic algorithms multiple linear regression (GA-MLR) and discriminant multiple linear regression (D-MLR) were used.

The Fourier transform Raman spectroscopy (FT-RS) has been chosen for the discrimination of animal fat (Abbas et al., 2009). Four mixtures were analysed: Mixture 1 50% Bovine, 50%

ovine–porcine, Mixture 2 80% Bovine, 20% porcine, Mixture 3 50% Bovine, 50% ovine–porcine–avian–former food stuffs and Mixture 4 55% Bovine, 15% ovine, 30% porcine, traces of avian fat to assess the ability of the technique. PCA was applied and the first PC represents 67 % of the variance while the second one explains 24% of the variance. PLS-R/DA model allowed discrimination between poultry samples and other components (porcine, bovine, ovine fats, and fish oils) obtained a sensitivity and specificity of 0.917 and 1.000, respectively

Raman spectroscopy was used to classify adipose tissue from four different species (chicken, beef, lamb and pork) (Beattie et al., 2007). The samples used in this investigation were beef, lamb and pork, and from above the breast for chicken. In order to obtain a wide range of variation within each species the samples were obtained from a number of commercial and a wide variety of breeds and feeding regimes. Complementary fatty acid composition was determined by GC. PCA data reduction on the adipose Raman spectral data set was followed by LDA and allowed 97.6 % correct classification of the samples, while using the PLS-R/DA method further improved the correct classification rate to 99.6 %

Beef offal adulteration of beef burgers was studied using dispersive Raman spectroscopy and multivariate data analysis to explore the potential of these analytical tools for detection of adulterations in comminute meat products with complex formulations (Zhao et al., 2015). Fresh beef (brisket), beef offal (kidney, liver, lungs, and heart) and beef fat were purchased from local stores and stored overnight at 4 ºC. Authentic beef burgers comprised two groups, higher quality burgers contained only lean beef and beef fat; lean meat content was varied between 80 and 100 % of burger in 2.5 % increments, with fat accounting for the remainder and lower quality burgers contained rusk (5%) and water (20%) in addition to lean beef (45-65% in 2.5% increments) and beef fat (30-10% in 2.5% increments). Adulterated beef burgers were formulated with lean beef, beef fat, water, rusk, and offal (liver, lung, kidney, and heart). Multivariate data analysis methods of the DRS spectra comprised PLS-R/DA, SIMCA and PCA. In relation to the PCA, the first three PCs described 61, 34, and 3 % of variance, respectively, in the frozen-then-thawed beef burger spectral data set. PLS-DA models correctly classified 89-100% of authentic and 90-100% of adulterated samples. The SIMCA has a specificity of 0.64 to 0.89 and a sensitivity of 0.95 to 1. In comparison with other studies of these authors (Zhao et al., 2014) the model of PLS-R/DA, adulterated samples obtaining the

74-91 % value for NIR, 73-100 % for Fourier transform-NIR, and 81-100 % for RS. The SIMCA efficiency was 0.62 to 0.91 for NIR, 0.81 to 0.94 for Fourier transform-NIR and 0.88 to 0.97 for DRS.

Raman spectroscopy allows high rating of detection of adulteration compared to other spectroscopic methods. Water interference doesn't occur on RS technique, providing specific information of the matrices of the samples. Samples can be read through glass or polymer packaging. However, the heating from the laser radiation can destroy to samples or hide the Raman spectrum. This process requires only a small sample. It has the ability to supply information about the chemical structure of molecules without causing any alterations. This technique is a new approach to the determination of meat adulteration and showed reasonable results for the determination of fraud meat mixtures.

2.5 Nuclear magnetic resonance spectroscopy

The NMR is based on the emission and absorption of energy in the radiofrequency range of the electromagnetic spectrum. The most commonly measured nuclei are ^{1}H and ^{13}C, the first for proteins because they are rich in hydrogen and the second for larger proteins and lipids (triglycerides) (Aursand et al., 2009, Jakes et al., 2015). The shielding effect of electrons, which decreases resonance frequencies of nuclei, varies with the chemical environment and is, therefore, characteristic of specific structural fragments of organic compounds (e.g., methyl, methylene, or methine ^{1}H nuclei) and their substituents (e.g., OH, NH_2, NH, COOH, CONH) (Mlynárik).

This technique presents advantages compared with other spectroscopic methods for foods with high water percentage because the protons are easily detected. This method is expensive and time consuming (Aursand et al., 2009, Jakes et al., 2015, Santos et al., 2014). Low-resolution NMR or time-domain ^{1}H nuclear magnetic resonance (TD-NMR) is an excellent alternative to traditional methods because it is rapid, simple and has the potential for online and in situ measurements. Using permanent magnet technologies significantly reduces the overall system and running costs (Santos et al., 2014). The NMR is interesting for analysing the safety of food and so it has been an increase in their use in food industry (Damez and Clerjon, 2013). The NMR showed high sensitivity to demonstrate the differences between the structure of muscles

of wildlife and farmed (Standal et al., 2010a). The ^{1}H NMR is effective technology for analysis and quantification of triglyceride samples. The use of high resolution (HR) ^{13}C NMR in the analysis of lipids is increasing. The lipid analysis is a potential tool for authentication of the fish and marine oils (Standal et al., 2010a). There are few studies with NMR for authentication of meat or fish products.

The TD-NMR spectroscopy when combined with univariate and multivariate analysis provided a valuable tool for tracing the sex and bull race of beef samples (Santos et al., 2014). It has been demonstrated that NMR is a fast and accuracy method for measuring conjugated linoleic acid (CLA) content in beef samples (Manzano Maria et al., 2010). The beef samples were collected from calves (43 heifers and 56 steer) from different bull race (Angus, Bonsmara and Canchim) and cows (Simmental-Nellore and Angus- Nellore for cows). The calves were designated according to the bull race and sex, resulting in 14 Angus heifers, 21 Angus steer, 17 Bonsmaraheifers, 19 Bonsmarasteer , 12 Canchimheifers and 16 Canchimsteer. Carr-Purcell-Meiboom-Gill (CPMG) and Continuous Wave Free Precision (CWFP) pulse sequences were used to obtain time-domain ^{1}H NMR. The PLS-R/DA showed a correct classification higher than 79 % either for CPMG or CWFP decays (validation set). The k-nearest neighbour (KNN) showed a correct classification of 75 and 76 %, while SIMCA showed a correct classification 66 and 78 %, respectively, for the CWFP and CPMG dataset. SIMCA method obtained a best predictability for the CWFP dataset of correct classification between 85 and 89 % for beef samples. ^{1}H NMR coupled CPMG CWFP and with univariate and multivariate methods obtain a correct classification of more than 80 %. The ^{1}H TD-NMR method applied to meat allowed authentication and traceability.

The 60 MHz ^{1}H NMR method was used to differentiate samples of fresh beef and horse. Peak integration was sufficient to differentiate samples of fresh beef (76 extractions) and horse (62 extractions) using Naïve Bayes classification. Fresh meat samples were purchased from a variety of outlets. It was possible to obtain 100 % correct classification of the different samples of beef and horse, exploiting the differences in triglyceride compositions. In relation to the PCA, principal components 1 and 2 described 83 and 12 % for lab 1 and 81 and 13 % for lab 2, respectively, of variance in fresh beef and horse spectral data set (Jakes et al., 2015). The first two PC scores are plotted against one another in Fig. 6 (a) and (b), with symbols coded

according to species. In both cases, the first dimension contains most of the relevant information relating to the difference between the two species. Furthermore, regions of the loading corresponding to the olefinic and *bis*-allylic peaks are positively associated with horse samples, Fig. 6(c) and (d).

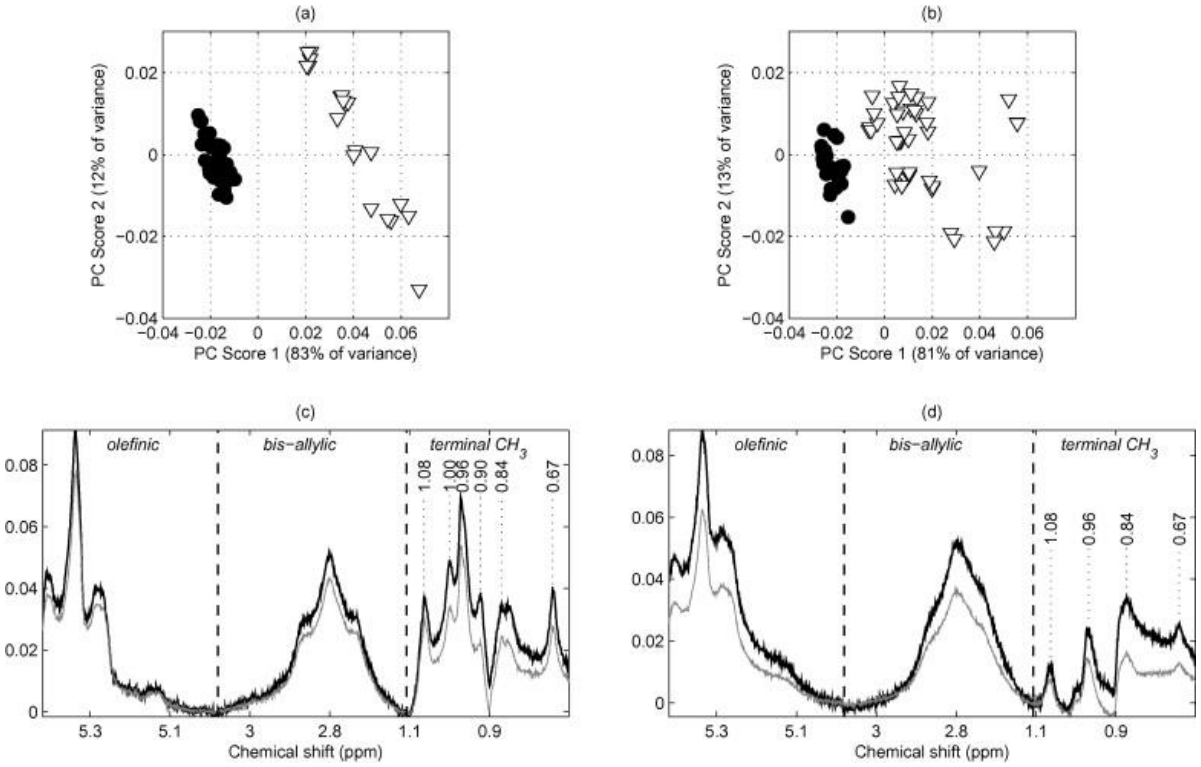

Figure 8. First versus second principal component scores plots for (a) Lab 1 Training Set data, and (b) Lab 2 Training Set data (black disks = beef, open triangles = horse). (c) and (d) Corresponding loadings plots (black trace), together with the covariance of each dataset with the group membership (grey trace) and peaks picked from the loadings in the CH_3 region. (Reprinted with permission from (Jakes et al., 2015).

The differences in quality and price between different species of fish are a reason for falsification, therefore it is necessary methods able to verify the traceability of the right information to protect consumer rights (Aursand et al., 2009).

The following species and stocks of lean fish were caught outside the coast of Vikna, Nord-Trondelag: Norway north-east arctic cod, Norwegian coastal cod (*G. morhua L.*), haddock (*M. aeglefinus*), saithe (*P. virens*), pollack (*P. pollachius*). Approximately 90 mg of the oil sample

was transferred to 5 mm NMR tubes and diluted with 0.6 mL deuterated chloroform ($CDCl_3$, 99.8% purity, Isotec Inc., Matheson). Lipid was extracted from white fish muscle under the back dorsal fin according the Bligh and Dyer method (Bligh and Dyer, 1959). Before analyzing the lipid extract by NMR, parts of the chloroform phase were removed by evaporation. The ^{13}C NMR spectroscopy coupled with chemometric methods PCA, LDA and Bayesian belief networks (BBN) authenticated different 5 gadoid fish species. With PCA obtained groupings and the first two principal components accounted for 36 and 9 % of the variance. PCA were used as input variables in the LDA. LDA with the 3PCs obtained 21/27 correct classifications (78 % correctly classified) and the Bayesian belief networks (BBN) showed 100 % correct classifications (Standal et al., 2010a).

The ^{13}C NMR in tandem with chemometrics methods classified the Atlantic salmon in relation to geographical origin and in relation to wild or farmed. The probabilistic neural networks (PNN) and support vector machines (SVM) showed an excellent breakdown of 98.5 and 100 %, to wild and farmed salmon. The geographical origin obtained correct classification of 82.2 to 99.3 % for PNN and SVM, respectively (Aursand et al., 2009).

A similar study with NMR and chemometrics methods tried to classify *Gilthead Pargo* in accordance with wild or farmed and geographical origin (Rezzi et al., 2007). The LDA and PCA made a clear distinction between wild and farmed samples. This method showed a rating of 100 % of the samples in accordance with wild or farmed and 85-97 % for the geographical origin.

The Fourier transform spectroscopy and micro ^{1}H NMR LF are used in studies of changes in the structure of proteins and secondary water distribution (Rezzi et al., 2007, Damez and Clerjon, 2013). The NMR technique permits an easy reading of the characteristics of foods with large amounts of water, high sensitivity, is expensive and time consuming. It is a method that can cause spectra with many peaks. However, TD-NMR is fast, simple and with potential for online measurement.

2.6 Multispectral and hyperspectral imaging

Currently many researchers are using hyperspectral imaging (HIS) methods because they are powerful techniques which can provide spectral data of an object with certain chemical characteristics in a spatially resolved manner (Pu et al., 2015). The HIS with the aid of image processing techniques that allow visualization, allow detection of attributes by spectral analysis of the samples. This method is a non-conventional analytical technique, non-destructive, using few reactants and it is fast.

This method allows simultaneous analysis of several samples and was introduced to integrate images and spectroscopy in a system for providing spectral and spatial information of an object (Ma et al., 2015, Lohumi et al., 2015). The images originate three-dimensional data sets that can be analysed to characterize the object in greater detail than the imaging or spectroscopy techniques (Kamruzzaman et al., 2016). The HIS are composed of hundreds of discrete spectral bands for each spatial position for the object (Kamruzzaman et al., 2012). The spectroscopy is used to detect or quantify the biological, chemical and physical properties of samples based on their spectral signature images and transform chemical information steps to viewing space (Kamruzzaman et al., 2013). The HIS can be used to know the number of certain attributes and where they are located in the sample. Spectra can be used to characterize, identify and discriminate classes and types of materials in the image. The most commonly used spectral bands are in NIR, VIS-NIR, VIS (Lohumi et al., 2015). The NIR HIS is involved in acquiring a spectrum for each image pixel in micro- and macroscopic scale (Kamruzzaman et al., 2016).

The identification of the adulteration was conducted using pure minced lamb meat and lamb meat mixed with potential adulterants including pork, heart, kidney and lungs in 20% proportions (Kamruzzaman et al., 2013). The lamb samples were adulterated by mixing pork in the range of 2 – 40 %. Both minced lamb (28% fat) and pork (15% fat) were acquired from a local supermarket and transported to the laboratory. The NIR HIS detected the level of adulteration of minced lamb using a PLS-R method. PCA was used to interpret and visualize the spectral data to high light their properties. The first PC represents 87,5 % of the variance while the second one explains 8%. (The two first PCs explained 95.7 % variation). With PLS-R prediction results was possible to detect adulteration in minced lamb with high performance in both calibration and cross-validation conditions using five latent variables (LV). The coefficient of determination in calibration (R^2_c) of 0.99, RMSEC of 1.08 %, coefficient of

determination in cross-validation (R^2_{cv}) of 0.99 and RMSECV of 1.37 % were obtained for PLS-R. The calibration model was also evaluated based on the ratio of percentage deviation (RPD), this value for adulterate detection was 8.51. The multiple linear regression (MLR) model was then built using the reduced spectral data and the results of MLR for predicting adulteration are R^2_c of 0.99, RMSEC of 1.25%, R^2_{cv} of 0.98, RMSECV of 1.45% and RPD of 8.04. The prediction ability of PLS-R with selected wavelengths was equivalent to the PLS-R with full spectra, with R^2_{cv} (0.99 vs. 0.99), RMSECV (1.42% vs. 1.37%) and RPD (8.51 vs. 8.21) (Kamruzzaman et al., 2013).

Meat samples originated from *longissimus dorsi* muscles of pork, beef and lamb were analysed in a study reported in (Kamruzzaman et al., 2012). The muscles were dissected and then sliced by a mechanical slicer. The slices were labelled and vacuum packed and transported under refrigerated conditions to the laboratory. The HIS with PCA and PLS-R/DA was used for identification and authentication of different red meat species. The first three PCs resulting from PCA explained 99.7 % of the variation among samples. The PC1 and PC2 were particularly representative and accounted for 98.9 % of the total variance (PC1 - 88.9 % and PC2 - 10.1 %). The PLS-R/DA showed a classification accuracy of 93.3, 98.7 and 97.3 % for pork, beef and lamb respectively.

The NIR HIS coupled with PLS-R/DA were used to distinguish between fresh and frozen-then-thawed samples (Barbin et al., 2013). Fresh samples of pork from the loin muscle were obtained from a commercial food retailer and transported to the laboratory for storage at 4 °C. After 24 h, each fresh sample was removed from cold storage and scanned in the NIR hyperspectral system. Pork samples were then vacuum packed and frozen at -18 °C. PLS-R/DA with full cross-validation had coefficients of prediction of 0.97 and 0.89 for R^2_C and R^2_{CV}, respectively, with standard error of calibration (SEC) of 0.23 and standard error of cross-validation (SECV) of 0.46. To verify the potential information carried by the selected wavelengths, frozen-then-thawed samples were correctly identified (sensitivity = 1.00), and no fresh sample was misclassified as frozen-then-thawed (specificity = 1.00). The overall correct classification for this method was 100 % to discriminate fresh from frozen-then-thawed samples. The classification of pork samples according to freezing treatment are the fresh samples (85.4 %), frozen once (77.9 %), frozen twice times (60 %), frozen three times (70 %) and frozen four

times (90 %). The fresh pork meat and frozen-then-thawed was detected with PLS-R/DA obtained 97.9 % of accuracy, and with colorimeter method 75 % accuracy. The discriminant model PLS-R/DA obtained a variance to LV1 of 58 % and LV2 of 39 % to identify of the fresh and frozen-then-thawed samples. This method can be applied for the benefit of the retail sector and the consumer. Figure 7 shows the main configuration of the push room NIR hyperspectral imaging system, reprinted from (Barbin et al., 2013).

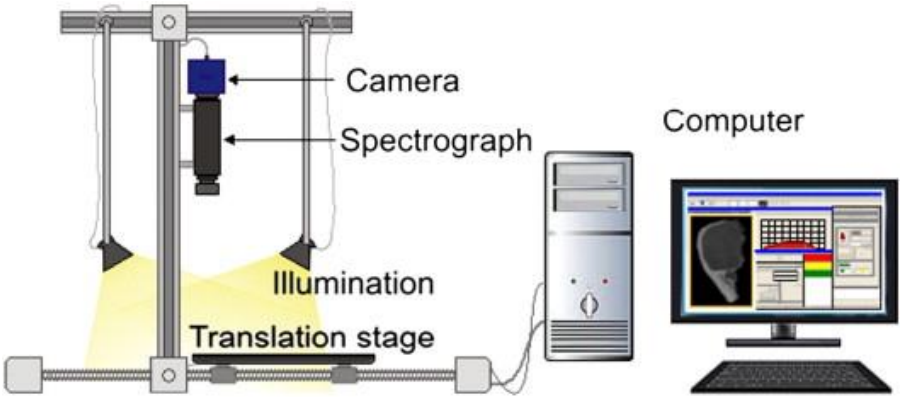

Figure 9. . Hyperspectral imaging system setup. (Reprinted with permission from (Barbin et al., 2013).)

In other research, the potential of VIS and NIR HIS with PNN was used for classification of fresh and frozen-then-thawed pork muscles (Pu et al., 2015). The animals with similar conditions (age, weight, feeding environment from the same farm) were obtained for the experiment. The pork samples were divided into three groups: the first group without any freezing treatment was designated as fresh pork meat, the second group was frozen-then-thawed-once, and the third group was frozen-then-thawed-twice. The PC images from HIS were obtained using histogram statistics (HS), gray level co-occurrence matrix (GLCM) and gray level-gradient co-occurrence matrix (GLGCM). PNN showed for fresh, frozen-then-thawed once and frozen-then-thawed twice meats a correct classification rate of 100 % and 97.73 % for calibration and validation sets, respectively. The successive projections algorithm (SPA) showed a correct classification rate of approximately 100 % for calibration and validation sets. The correct classification rate was reduced to 86.36 % and 86.36 % for calibration and validation sets, when six optimum wavelengths were used alone. The average classification

accuracy of PNN using optimum wavelengths (OW)-GLGCM was the highest (92.0 %), followed by OW-GLCM (91.3 %), OW-HS (91.3 %) and OW (86.4 %).

The multispectral imaging (MSI) coupled with PLS-R/DA and LDA was used for the detection of minced beef fraudulently substituted with pork (Ropodi et al., 2015). Different levels of adulteration of minced beef and pork were prepared, fillets were cut into smaller pieces and grinded separately one at a time, using a domestic meat-mincing machine. To achieve different levels of adulteration, ranging from 10 to 90% with a 10% increment, the appropriate amount of each type of meat was used and mixed in conditions that simulate industrial processing. The class of adulteration obtained an overall correct classification, mean per-class recall and precision of 83.3, 83.3 and 84.5 % respectively. The classification error for 98.48 % of the samples was at most 10 %, for LDA. The overall correct classification, mean per-class recall and precision of pure pork, adulterated and pure beef was over 94 % (mean recall, precision and overall correct classification was 94.4, 99.4 and 98.5 %, respectively). The PLS-R/DA showed a correct classification of 98.5 % using 12 PLS-R components after cross-validation.

The VIS-NIR HIS was used to classify the fresh and frozen-then-thawed pork meats (Ma et al., 2015). The pork *Longissimus Dorsi* muscles were obtained from a local market. The samples were divided in fresh samples without any freezing treatment, the second group of meat samples were frozen at -18 C for 24 h and then thawed at 20 ºC for 2 h, third group were frozen and then thawed twice. The correct classification rate was applied to assess the performance of the PLS-R/DA classifier for model establishment. The correct classification rate of 97.7 % was achieved, confirming the high potential of textures for fresh and frozen-then-thawed meat discrimination. The PCA with three components explained 99.9 % of variance and the first three PC images (the optimal GLGCM images) explained 98.1, 1.3 and 0.4 %, respectively. This method is a powerful tool and allows the analysis of the quality of food and its authenticity.

The chicken adulteration in minced beef was detected with VIS-NIR HIS (400 – 1000 nm) and HIS were acquired in the reflectance mode (RM) (Kamruzzaman et al., 2016). The pure minced beef and minced chicken were collected from a local supermarket. The minced beef samples were adulterated by mixing minced chicken in the range of 0% at 50%. Hyperspectral images were transformed into absorbance (A) and using the Kubelka Munk (KM) function (Nobbs, 1985). The performance of PLS-R developed using raw and pre-treatment spectra (1st

derivative, 2nd derivative, MSC, and SNV). The percentage of chicken adulteration in minced beef was predicted with R^2_c of 0.97, 0.97 and 0.95 with the corresponding RMSEC values of 2.5, 2.6 and 3.3% for RM-PLS-R, A-PLS-R and KM-PLS-R, respectively. When applied to an independent validation set, were capable of predicting adulteration with R^2_p of 0.97, 0.97 and 0.96 and the corresponding RMSEP of 2.67, 2.45, and 3.18 %, for RM-PLS-R, A-PLS-R and KM-PLS-R, respectively. The ratio of percentage deviation values obtained were 5.84, 6.24 and 4.81 for RM-PLS-R, A-PLS-R and KM-PLS-R, respectively.

Multispectral and hyperspectral imaging are quick technique that allows to analyse a large number of samples at the same time, provides spectral data on the chemical, biological and physical characteristics. However, the instrumentation is costly and data processing can limit the use of this method in real time.

3. Spectral data processing

Spectroscopic methods associated with chemometric methods are a tool for the identification of species and foodstuffs that are not on the label. However, the identifying of regions of interest and features in the spectrum, sometimes called regions of interest, of the tested substances is important for the optimization of the methodologies (De Jong, 1990).

Univariate methods that can be used, namely, the averages and standard deviations, descriptive statistics, box plots, analysis of variance (ANOVA), pairwise comparisons of mean values with Fisher's LSD test and t–test. These methods were applied, for example, by (Pillonel et al., 2005) in the study of geographic origin of European Emmental cheese. A comparison between univariate and multivariate methods was performed by (Moustafa et al., 2015) for evaluating the efficiency of spectral resolution when manipulating ratio spectra applied to ternary mixtures in common cold preparation. Multivariate regression techniques have been widely used to study food authentication. A comprehensive introduction and review on multivariate regression procedures can be found in (Higgins, 2005, van den Hout et al., 2007, Cruyff et al., 2016) and references therein.

The application pre-treatment may remove the effects of unsystematic spectral data and eliminate variations, light scattering, random noise, external factors and base line changes

(Rinnan et al., 2009). The most common pre-treatment methods are the standard normal variate (SNV) (Barnes et al., 1989), the multiplicative scatter correction (MSC) (Dhanoa et al., 1994), the Norris-Williams derivation (Rinnan et al., 2009) and the Savitzky-Golay 1st and 2nd derivatives (Savitzky and Golay, 1964b).

The SNV is an accurate and reliable method for ranking in spectroscopic field. The SNV is an ideal technique for classification and validation of the results of PCA (Alamprese et al., 2013, Schmutzler et al., 2015, Ropodi et al., 2015). The MSC method is a simple processing step that attempts to account for scaling effects and offset (baseline) effects. This correction is achieved by regressing a measured spectrum against a reference spectrum and then correcting the measured spectrum using the slope (and possibly intercept) of this fit (De Jong, 1990). The Norris-Williams derivation is a basic method developed to avoid the noise inflation in finite differences. This technique was elaborated on by Norris and Williams in 1984 as a way to calculate the derivative of NIR spectra. The NW derivation includes two steps, Smoothing of the spectra and first-order derivation (Norris and Williams, 1984). The Savitzky-Golay method reveals a larger structure of spectral data resulting in an easy interpretation of the chemical basis of the observed signals. The derivatives can also be used in conjunction with SNV (Press and Teukolsky, 1990).

After pre-treatment of the spectral data a few simple statistic methods allow extraction of information from the spectral data.

The PCA method is applied to spectral data to reduce the dimensionality, to classify samples and to identify outliers. The original variables are transformed into new uncorrelated variables called PC that are a linear combination of the original variables. The main components are linearly independent, represent variations in the dataset in descending order, PC1 describes the largest variance, PC2 the second largest variance, and so on.

The LDA method is a probabilistic classification technique that allows for maximum separation of samples between categories. The number of samples must be greater than the number of variables. This method allows the recognition of supervised patterns where the number of categories and the samples belonging to each category defined above is based on the assumption that samples of the same group are more similar the samples belonging to different groups.

Allows a linear transformation maximizing the variance between classes and minimizing the variance within the class (Morsy and Sun, 2013a, Uddin et al., 2005, Alamprese et al., 2013).

The PLS method permits associate a set of independent variables (predictors, X) to response variables (observations, Y) by reducing the original number of descriptors to a new set of data based on a reduced number of orthogonal factors called latent variables. The PLS-DA accounts for the maximum separation between the classes in the data were the variable is dependent and categorized (Morsy and Sun, 2013a). The PLS-R is used to reduce the original predictors to a new variable which has better predictive power (Sun et al., 2012a, Morsy and Sun, 2013a).

The SIMCA method provides a useful classification of high dimensional variations and incorporates PCA to reduce the dimensions of spectral information. The computing speed of SIMCA with PCA can be increased by calculating the covariance matrices and the indices. The MIR ATR spectroscopy with SIMCA made possible to successfully detect and quantify adulterants (Meza-Márquez et al., 2010, Zhao et al., 2015). The mean difference, standard deviation of difference, RMSECV and R^2 are used for validation. Generally, a good model should have high R^2 and RPD and low RMSEC and RMSEP.

The PNN method consists in establishing decision limits in feature space with distinct patterns belonging to different classes. This method improves the standards of classification and faster speed training (Cheng et al., 2015a). The PNN showed potential for the analysis of the NMR data technique. The PNN can be used as a classifier and find variables with the highest impact in classification (Standal et al., 2010a). For the MLR is necessary to establish the wavelength that can relate two or more explanatory variables and the response variable (Kamruzzaman et al., 2013). The GLCM method is an image processing method for resource collection textural analysing the relationship of levels and slope between 2 pixels (Karoui et al., 2006).

4. Discussion and conclusions

Authentication of foodstuffs is crucial due to design food with adulteration by substitution of species, geographical origin or freshness. The NIR spectroscopy detects the number of bands smaller molecules OH, CH and NH than that of mid-infrared spectroscopy which allows obtaining a greater of the molecules in the food matrix in more detail, which involves stretching, bending and shaking movements of functional groups, such as CC, CH, OH, C = O and NH (Mamani-Linares et al., 2012a, Alamprese et al., 2013, Zhao et al., 2014)

The MIR spectroscopy in conjunction with Fourier transform and chemometric methods proves to be a promising technique for the analytical determination of adulteration Halal food (Rahmania et al., 2015). However, the results of the ATR technique are affected by water content contained in food producing noise (signal). The method is fast, non-destructive and does not involve a lot of sample preparation, being sufficiently reliable results. MIR spectroscopy requires dilution of samples unlike NIR, however this technique has difficulty in reading of samples with large amounts of water, such as fish (Uddin et al., 2005, Zhao et al., 2014). The NIR spectroscopy is a powerful technique for rapid analysis in line applied to inspections of foodstuffs and discrimination linear and nonlinear allowing distinguish adulteration with ease (Morsy and Sun, 2013a). The FS reduces the time and cost of the measurements and analysis of enzymatic bio-analytic chemistry. This method allows to characterize fish and be adapted to detect online fresh and frozen-then-thawed fish (Karoui et al., 2006). Investigators used a simple, quick and inexpensive basic extraction with chloroform to obtain triglycerides in NMR spectra and found that 60 MHz ^{1}H NMR is a viable approach in high yield for the determination of adulteration meat (Jakes et al., 2015).

To obtain vibrational spectroscopic results is necessary to use chemometric models. The PCA, LDA, SIMCA and PLS-R/DA demonstrated that combined with spectroscopy methods are a useful tool for authentication, detection of adulteration food (Kamruzzaman et al., 2013, Meza-Márquez et al., 2010, Zhao et al., 2014). The RS is a promising technique owing to provide specific information on the identification of samples matrices based compounds (lipids, proteins, carbohydrates), it is sensitive to smaller components such as micro-organisms responsible for spoilage and provides information detailed on molecular vibrations and

chemical structure of the molecules without causing damage to the small sample required for analysis (Boyacı et al., 2014a, Argyri et al., 2013, Al Ebrahim et al., 2013a).

The RS is not interference occurs and results in water samples can be analysed by glass or packaging however the sample fluorescence spectrum can hide the impurities and heating the intense laser radiation can destroy the sample or hide the spectrum. However, the use of NIR laser reduces or prevents the fluorescence of the samples. It is a method with a high potential for identification purposes (Lohumi et al., 2015). Although NMR is a technique that allows the detection and analysis of different examples fat is an expensive technique can yield spectra with too many peaks and requires very concentrated solutions. NIR, MIR, RS are well established techniques however are based on a sample point, a relatively small area of a species which is not capable of providing the composition gradients yielding mean results compared with the multispectral image. The HIS and MS is important for food inspection, analysis is more convenient, fast and are analysed larger number of samples simultaneously. However the high initial costs and difficulties in data acquisition have limited the use of this real-time technology (Kamruzzaman et al., 2012).

The futures perspectives are application of multispectral image in food industry to many samples of a foodstuff while determining the quantity of contaminants and application of spectroscopy techniques to determine adulteration of food (Raman)

Table 9. Detection techniques of different types of meat and fish species.

Type of adulteration	Food products	Detection method	Chemometric method	References
Substitution or removal of ingredients	Halal and non-Halal Chinese Ham sausages	FTIR	PLS-R/DA and PCA	(Xu et al., 2012)
	Veal	Fourier transform –NIR	PCA	(Schmutzler et al., 2015)
	Lamb	NIR hyperspectral imaging	PCA and PLS-R	(Kamruzzaman et al., 2013)
	Iberian pork sausages	NIR	PCA and MLSD	(Ortiz-Somovilla et al., 2005)
	Beef or bovine meat	UV–VIS, NIR and MIR	PCA, LDA and PLS-R	(Alamprese et al., 2013)
		Raman	PCA	(Boyaci et al., 2014)
		Raman	PCA and PLS-R	(Ebrahim et al., 2013)
		60 MHz 1H NMR	Naïve Bayes classification model, PCA	(Jakes et al., 2015)
		FTIR	PLS-R and PCA	(Kurniawati et al., 2014)
		Mid-infrared	PLS-R and SIMCA	(Meza-Márquez et al., 2010)
		ATR-FTIR	PLS-R/DA	(Nunes et al., 2016)
		FTIR	PLS-R and PCA	(Rahmania et al., 2015)
		FTIR	PLS-R	(Rohman et al., 2011)
		Multispectral imaging	LDA and PLS-R/DA	(Ropodi et al., 2015)
		TD-NMR	SIMCA, KNN and PLS-R/DA	(Santos et al., 2014)
		Fourier transform -Raman		(Zając et al., 2014)
		Mid-infrared ATR	SIMCA, PCA and PLS-R/DA	

	Carp fish fillets	Hyperspectral imaging	SIMCA and PNN	(Cheng et al., 2015)
	Chicken	VIS NIR hyperspectral imaging	PLS-R	(Kamruzzaman et al., 2016)
Fresh vs. thawed meat	Porcine	NIR hyperspectral imaging	PLS-R/DA	(Barbin et al., 2013)
		VIS–NIR hyperspectral imaging	PCA	(Ma et al., 2015)
		Hyperspectral imaging		(Pu et al., 2015)
		VIS and NIR hyperspectral imaging	PNN	(Pu et al., 2015)
	Beef	NIR spectroscopy	LDA and PLS-R/DA	(Morsy and Sun, 2013)
		Mid-infrared ATR spectroscopy	PCA and LDA	(Zhao et al., 2014)
	Fish	Front-face fluorescence	PCA and FDA	(Karoui et al., 2006)
		NIR	PCA and PLS-R/DA	(Ottavian et al., 2013)
Classification of specie or origin	Gadoid fish species	13C NMR	PCA and BBN	(Standal et al., 2010)
	Lamb	NIR	LDA, PCA and PLS-R/DA	(Jakes et al., 2015)
	Different meat species	Raman spectroscopy	PCA	(Boyaci et al., 2014)
		VIS and NIR	PLS and PCA	(Cozzolino and Murray, 2004)
		Hyperspectral imaging	PCA and PLS-R/DA	(Kamruzzaman et al., 2012)
		VIS and NIR spectroscopy	PCA and PLS-R	(Mamani-Linares et al., 2012)
	Deer	Shifted excitation Raman difference spectroscopy	PCA	(Sowoidnich and Kronfeldt, 2012)

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PAPER IV – "PREDICTION OF ADULTERATION OF GAME MEAT USING FTIR AND CHEMOMETRICS", *NUTRITION & FOOD SCIENCE* 48, 2: 1 - 9.DOI: 10.1108/NFS-08-2017-0164 (ANNEX IV)

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Prediction of adulteration of game meat using FTIR and chemometrics

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Abstract

Background - Consumption of game meat is growing when compared to other meats. It is susceptible to adulteration due to its cost and availability. Spectroscopy may led to rapid methodologies for detecting adulteration.

Purpose - It is presented an investigation on Fourier transform infrared (FTIR) spectroscopy coupled with chemometric to detect the adulteration of wild fallow deer (Dama dama) meat with domestic goat (G) (Capra aegagrus hircus) meat, for samples stored for different periods of time.

Design/methodology/approach - Meat were cut and mixed in different percentages, transformed into mini burgers and stored at 3°C from 12 to 432 h and periodically examined for FTIR, pH and microbial analysis. Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA), were applied to detect adulteration.

Findings - The PCA model, applied to the spectral region from 1138 to 1180, 1314 to 1477, 1535 to 1556 and from 1728 to 1759 cm^{-1} describe the adulteration using four principal components which explained 95% of variance ($p<0.05$). For the levels of adulteration A1 (pure meat), A2 (25 and 50%w/w G) and A3 (75 and 100%w/w G) for an external set of samples, the correlation coefficients and the room mean square error for prediction were: 0.98 and 8.6% ($p=0.029$); 0.94 and 12.5% ($p=0.042$); 0.97 and 9.5% ($p=0.037$), respectively.

Originality/value - The PLS-DA model predicted the adulteration for an external set of samples with high accuracy. The proposed method have the advantage of allowing rapid results, despite the storage time of the adulterated meat. It was shown that FTIR combined with chemometrics can be used to establish a methodology for identification of adulteration of game meat, not only for fresh meat but also for meat stored for different periods of time.

Keywords Food authentication, Adulteration of game meat, Meat adulteration, FTIR spectroscopy, PLS-DA

Paper type Research Article

1. Introduction

It is increasingly important to detect fraudulent food products, whether for economic, religious or public health reasons (Charlebois et al., 2016; Manning and Soon, 2014). Food fraud is a common term used to include the intentional adulteration of foodstuff by substitution or addition of ingredients or misleading statements about a product, for economic gain (Spink and Moyer, 2011).

Game meat consumption has been increasing because of various motivations, such as the particular flavor, its healthier composition and lower fat and cholesterol contents. It is prone to adulteration because of its high price. Selective methods are necessary to verify its authenticity (Druml et al., 2015). Several analytical methods have been applied for the determination of meat adulteration, such as protein-based methods (Al Ebrahim et al., 2013), the DNA-based methods (Lin et al., 2014) and the real-time PCR (Druml et al., 2015). These methods are laborious, expensive and destructive, and require sophisticated laboratory procedures.

Emerging technologies for authentication and traceability of fresh meat products include nuclear magnetic resonance imaging (Standal et al., 2010), fluorescence (Karoui and Blecker, 2011), near and mid infrared absorption spectroscopy (NIR and MIR) (Alamprese et al., 2013) and Raman spectroscopy (Zhao et al., 2015) coupled with Fourier transform and multispectral and hyperspectral imaging (Pu et al., 2015). These techniques are simple, nondestructive and allow real-time analysis.

Fourier transform infrared spectroscopy (FTIR) allows easy manipulation of the spectroscopic data (Bell, 2012). Transmission or attenuated total reflection (ATR) techniques are commonly used to obtain the absorption spectrum (Lohumi et al., 2015). There are several chemometrics methods applied in spectroscopy, namely principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), partial least squares regression and soft independent modelling of class analogy, among others (Gredilla et al., 2016; Saraiva et al., 2015). PLS-DA is a prevailing method for discriminant classification, particularly appropriate to deal with collinear data matrices, like in the case of spectroscopic data.

There are studies with FTIR for the detection of meat adulteration, for example, turkey meat in fresh, frozen-thawed and cooked minced beef (Alamprese et al., 2016), beef meatball adulteration with pork (Rohman et al., 2011), minced beef adulteration with turkey meat

(Alamprese et al., 2013) and rat meat in meatball formulation (Rahmania and Rohman, 2015). The consumer purchases meat with a range of different periods of storage. Although there are studies on the use of spectroscopy and chemometrics to discriminate adulterated meat, to our knowledge, few studies extend their experiments to stored meat. The aim of this work was to explore the potential of FTIR in tandem with PCA and PLS-DA in detecting the presence/absence of adulteration of wild fallow deer (*Dama dama*) meat with domestic goat (*Capra aegagrus hircus*) meat, not only for fresh samples but also for samples stored for different periods.

2. Material and Methods

2.1 Meat samples manufacture

Fallow deer (D) and goat (G) meat were excised from carcasses at 24 hours postmortem, cut in smaller pieces and minced. Four batches of fallow deer adulterated with goat were prepared and transformed in mini burgers (samples). Five formulations, with the values of 0, 25, 50, 75 and 100 %w/w of goat were considered.

The samples, weighing approximately 30 g, were packed in air and overwrapped with polyethylene film. Following packaging, samples were stored at 3ºC and examined after 12, 24, 36, 48, 72, 96, 120, 144, 168, 216, 264, 312, 360 and 432 h. At each sampling point (including t=0), the samples were analysed for three parameters, spectroscopic, pH and microbiological analysis. The microorganisms analysed were total mesophilic (TVC) and psychrotrophs. Four batches were evaluated, each one constituted by 75 samples (15 sampling points and 5 formulations), therefore, 300 samples were analysed.

2.2 Microbiological analysis

Aliquots (10 g) were homogenized with 90 ml tryptone salt (tryptone 0.1 per cent and NaCl 0.85 per cent) in a Stomacher for 90 s. Serial decimal dilutions were prepared in the same solution for microbiological determinations. TVC (ISO4833, 2003) and psychrotrophs (ISO6887, 2017) populations were obtained after incubation on plate count agar (Oxoid CM0325, England) at 30°C for 3 days and 7°C for 10 days, respectively. Results were expressed as logarithm of colony forming unit (cfu/g). In case the microorganism counts were below the detection limit, the result was considered to be zero for statistical purposes.

2.3 Physical-Chemical Measurements

pH

The pH was measured directly in the samples using a penetration electrode with a pH meter (Crison Instruments, Spain) and was evaluated in triplicate immediately after opening the packages (ISO2917, 2004).

FTIR Measurement

Infrared spectra were collected in a FTIR spectrometer (Mattson, Unicam Research Series, USA) equipped with a single reflection ATR module (Golden Gate, U.K.), a DLaTGS detector and a KBr beamspliter. The equipment is controlled by the WinFirst software - v1.1. The samples were placed on top of the ATR crystal which was kept at 30ºC, ensuring that the aerobic surface of the samples was in close contact with the crystal and then pressed. Supposing that samples are mainly composed of water, calculation using equation 2.7 of the study conducted by Stuart (2000) showed that the evanescent field was probing a depth of approximately 1.0 μm. All infrared spectra were recorded from 500 to 4000 cm-1 by co-adding 128 interferograms at a resolution of 4 cm-1. A new air background spectrum was taken after every scan. The ATR base was cleaned with ethanol and dried before measuring the next sample. The cleaning was verified by gathering a background spectrum and comparing to the previous one. For each sampling occasion, two replicates were analysed by FTIR, for each replicate two spectra were collected, and the average was calculated. These spectra were recorded as absorbance values.

2.4 Statistical Analysis

Spectral data set was initially submitted to smoothing based on the Savitzky–Golay algorithm (Savitzky and Golay, 1964) and, afterwards, were mean-centered and standardized (SNV).

Principal Component Analysis

For a preliminary exploration, the spectral data set was handled by PCA, which allowed determining its main features and to highlight relations among the original variables (absorbances at different wavenumbers). The PCA projects the large number of potentially correlated original variables in a representation space of smaller dimensions and calculates new

variables, called principal components (PC), that are linear combinations of the starting absorbances and, thus, reduces the size of the data set (Abdi and Williams, 2010).

Partial Least Squares Discriminant Analysis

For the classification of samples in three different levels of adulteration, A1 (pure meat, 0 per cent w/w G), A2 (adulterated, 25 and 50 per cent w/w G) and A3 (highly adulterated, 75 and 100 per cent w/w G), the PLS-DA algorithm was applied (Barker and Rayens, 2003). It is a supervised method applied for classification usually in multivariate regression (Wold et al., 2001). The latent variables (LVs) that maximize discrimination between groups of samples are calculated from the spectral data (X matrix) maximizing the covariance, with classes defined in the Y matrix. When the number of classes is more than two, the Y matrix is composed of several variables and the PLS2 algorithm must be applied (Abdi et al., 2016).

The data set was split into two subsets, for calibration (training set) and for prediction (external set). The number of LV of models was chosen by the criterion of lowest prediction error in leave-one-out cross-validation.

The quality of the models was scrutinized by the multiple coefficient of determination or regression coefficient (R^2, where R is the correlation factor) and the root mean square error of calibration (RMSEC) and of cross-validation (RMSECV) (Abdi et al., 2016).

The predictive ability of the model is evaluated from root mean square error of prediction (RMSEP), which is a measure of how well the model will make predictions, and is calculated exactly as RMSEC and RMSECV, except that the estimates of mean errors are now values obtained from the external set.

In addition, the evaluation of the PLS-DA models was performed using the goodness-off it parameter R^2Y, which represents the variation in class membership explained by the model, and the predictive ability parameter Q^2, known as the goodness of prediction (Sanz-Cortés et al., 2013). Fisher's probability was used as a quality probe of the classification (< 0.01 usually considered a good prediction) (Whitlock, 2005). Statistical calculations were performed using the Excel-based XLSTAT V2006.06 package (Addinsoft, Inc, NY, USA).

3. Results and Discussion

3.1 Microbial and pH Data

The evolution of TVC and psychrotrophs with storage time is shown in Table 1 for the five formulations. The TVC at the onset of storage (fresh samples) of fallow deer and of goat samples were (mean/standard deviation) 3.10±0.30 and 4.85±0.40 log cfu g^{-1}, respectively, and for samples stored for 432 h (completely spoiled) the values were 9.69±0.45 and 10.50±0.36 log cfu g^{-1}, respectively. Regarding psychrotrophs, for fresh samples of fallow deer and of goat the values were 2.50±0.25 and 4.14±0.36 log cfu g^{-1}, respectively, and for samples stored for 432 h (completely spoiled) were 8.43±0.39 and 9.35±0.38 log cfu g^{-1}, respectively. The TVC and psychrotrophs counts increase with storage time, as expected, following and exponential growth. The pure fallow deer has fewer initial TVC and psychrotrophs counts than goat and, therefore, the mixtures with increasing goat concentration have higher counts.

The pH value of meat is a parameter that indicates the quality of the meat (Watanabe et al., 1996). The evolution of the pH with the storage time for the five formulations and four sets of samples is shown in Table I. While for fresh samples, the pH was in a small range from 5.73 to 5.85, at the end of the storage period, the pH values spanned from 6.29 to 7.54 for the pure fallow deer and goat, respectively. These results confirm that the samples are highly deteriorated before the end of the storage time and are within the published values for game meat, for similar storage conditions (Spaziani et al., 2011). As can be seen in Table I, the time evolution of the pH for samples with 25, 50 and 75 per cent w/w of goat follows, within the experimental error, the corresponding values for pure fallow deer and pure goat. The high pH values observed in the goat samples at end of storage may be because of the production of basic compounds by some deteriorative microorganisms that degrade the nitrogen fraction of meat (Ammor et al., 2009).

Table 10. Evolution of TVC, psychrotrophs and pH with storage time for the five formulations of fallow deer adulterated with goat.

	TVCa (log cfu g^{-1})					psychrotrophs (log cfu g^{-1})					pH				
(% w/w	0	25	50	75	100	0	25	50	75	100	0	25	50	75	100
Time (h)															
0	3.1	3.3	3.6	4.0	4.8	2.5	2.8	3.2	3.9	4.1	5.72	5.84	5.82	5.85	5.78
12	3.2	3.4	4.1	4.3	4.9	2.6	3.0	3.3	4.1	4.4	5.79	5.91	5.86	5.88	5.83
24	3.2	3.6	4.3	4.7	4.9	2.8	3.0	3.4	4.0	4.4	5.74	5.82	5.86	5.90	6.01
36	3.2	3.9	4.4	4.8	5.1	2.7	2.9	3.6	4.0	4.3	5.92	5.93	5.97	6.03	6.09
48	3.6	4.0	4.7	5.3	5.5	2.8	2.8	3.5	4.0	4.4	5.94	5.99	6.02	6.09	6.16
72	3.7	4.4	4.9	5.3	6.1	2.8	2.9	3.5	4.3	4.4	5.90	5.97	6.00	6.10	6.12
96	3.8	4.8	5.3	5.7	6.5	2.8	3.1	3.6	4.5	4.9	5.92	5.98	6.03	6.08	6.18
120	3.9	5.1	5.9	6.5	7.2	3.2	3.3	3.8	5.4	6.1	5.95	6.01	6.06	6.12	6.16
144	4.0	5.2	6.0	6.8	7.6	3.3	3.7	4.8	6.1	7.1	5.92	5.99	5.99	6.07	6.11
168	4.0	5.8	6.4	7.1	7.8	3.1	4.0	5.5	6.6	7.7	6.02	6.09	6.09	6.17	6.21
216	4.7	6.0	7.1	7.8	8.8	3.3	4.7	6.5	7.4	7.9	6.04	6.07	6.15	6.22	6.33
264	6.2	7.7	8.4	9.2	9.7	4.2	5.8	7.1	8.2	8.8	6.02	6.03	6.18	6.45	6.51
312	8.4	8.8	9.3	9.7	10.3	5.0	6.6	7.8	8.9	8.8	6.10	6.24	6.50	6.60	6.67
360	9.1	9.2	9.5	10.1	10.4	6.3	8.0	8.6	9.1	9.2	6.15	6.32	6.74	7.24	7.39
432	9.6	9.8	9.9	10.2	10.5	8.4	8.5	8.7	9.2	9.3	6.29	6.62	6.96	7.28	7.54

Notes: Numerical values in parentheses are standard deviation of measurements. a) TVC-total viable counts. b) five formulations with %w/w of goat from 0 to 100. The TVC and psychrotrophs counts increase with storage time, following an exponential growth. The pure fallow deer has fewer initial TVC and psychrotrophs counts than goat and, therefore, the mixtures with increasing goat concentration have higher counts. The pH is a parameter that indicates the quality of the meat. While for fresh samples the

pH of both meats is similar, at the end of the storage period the pH values spanned a broader range. These vales confirm high deterioration, particularly evident in mixtures with 75 and 100% of goat meat, at the end of the storage time.

3.2 FTIR Measured Spectra

The infrared spectra of meat can provide information on biochemical changes occurring during storage (Ellis et al., 2004). The peptide group, the structural repeat unit of proteins, gives up to 9 bands called amide A, B, I, II, . . ., VII. Absorption bands because of Amide A, I and II are two major contributors of the protein infrared spectrum (Krimm and Bandekar, 1986). Absorption bands related with water, fat, carbohydrates and proteins appear in the region from, approximately, 1750 to 900 cm^{-1}, loosely called "fingerprint region" (Ammor et al., 2009). In the region from 4000 to 500 cm^{-1} there is a very broad band at around 3360 cm^{-1} related to, symmetric and asymmetric, N-H and O-H stretching of amide A and water, respectively. Around 1640 cm^{-1} there is a large absorption band related to the O-H bending (present in water) and to the C=O stretching of amide I (from protein). At 1550 cm^{-1} there is a band due to the N-H bending of amide II mixed with C-N stretching. Absorption bands at 1458 and 1401 cm^{-1} are due to C-H stretching of methylene (CH2) and methyl (CH3) groups (present in fat). Peaks at 1398, 1314 and 1238 cm^{-1} are related to C-N stretch, N-H bend, C-O stretching and O=C-N bending of amide III. The region from 1200 to 950 cm^{-1} is associated with C-O and C-C stretching vibrations (due to carbohydrates). Moreover, the peaks at 1460, 1240, and 1175 cm^{-1} can be attributed to C-O and at 1740 cm^{-1} to C=O (fat). Finally, the peaks arising from 1025 to 1140 cm^{-1} could be absorbance due to amines (C-N stretch) (Sinelli et al., 2010).

3.3 Preliminary Analysis of the Spectral Data Set

The FTIR spectra were exported from the equipment in native format and imported directly into the statistical software for all multivariate procedures. Data in the range 500 to 4000 cm-1, composed by 875 variables (wavenumbers), was used in further chemometric analysis.

First the spectra set expressing various combinations of the five formulations and storage periods were smoothed, mean-centered and standardized (SNV), and then PCA was used to determine the major sources of variance and to detect any unusual or outlying samples. The FTIR spectrum of the samples are very similar, meaning that gathering information requires the use of multivariate analysis.

The appropriateness to perform PCA was confirmed by Bartlett's sphericity test ($p < 0.0001$). The number of components retained in the final solution was based on the Kaiser-Meyer-Olkin criterion for the analysis of eigenvalues (> 1) and the proportion of variance retained (> 70%),

usually seen as the minimum needed to make the model suitable for explaining the original data. It was concluded that 88 PCs describe the variance of the data set represented by the original 875 variables. However, approximately 95% of the total variance was explained by the first nine PCs, among which PC 1, 2 and 3 accounted for 51.9, 21.3 and 5.4 % of the variability, respectively.

The variance that a given wavenumber is accounted for by all the PCs is given by the sum of the squared PCs loadings for all PCs for that given wavenumber, which is frequently called communality, and its initial value in PCA is 1. Therefore, it determines the variance in a given wavenumber explained by all the PCs (Abdi and Williams, 2010). Communality values smaller than 0.6 indicate that the wavenumber do not fit well in the PCA solution and should be release (Field, 2005; Stevens, 2002). Wavenumbers for which the communality value are higher than 0.6 are important to explain the variance and are potential wavenumbers to differentiate the five formulations (Nychas and Tassou, 1997).

From the PCA, the average communality of the wavenumbers from 2000 to 900 cm^{-1} (275 variables), was higher than 0.45, with most of the wavenumbers having communality higher than 0.55. This range of wavenumbers which were then selected for additional analyses

A new PCA was then calculated (using the aforementioned wavenumber range), and it was determined that the variance could be explained by 29 PCs, among which the first four explain ~95 % of the total variance. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy is 0.854. The communality value of the first four principal components was higher than 0.6 for wavenumbers from 1138 to 1180, 1304 to 1477, 1535 to 1556 and from 1728 to 1759 cm^{-1}.

Among these wavenumbers, those in the range 1304 to 1322 and from 1372 to 1403 cm^{-1} are related to biomolecules such as amides, amines and the biochemical changes during storage of the meat, as mentioned earlier by other authors (Ammor et al., 2009; Ellis et al., 2004; Ellis et al., 2002).The wavenumbers from 1728 to 1759 cm^{-1} are related to the lipid constitution of each type of meat and, in our opinion explain the separation achieved between the five different mixtures of fallow deer and goat. This is, probably, an effect of different feeding regimes (Kimet al., 2017; Paengkoum et al., 2013).

From the observation diagram PC2 and PC3, represented in Fig. 1, is evident the separation of the samples according to the formulation and, within each formulation, it is clearly seen the

effect of the storage time. From this diagram it may be conclude that it is possible to distinguish the level of adulteration, disregarding the storage time of the meat. It must be observed that, while the samples corresponding to the five formulations are distributed along the PC2 axis, the PC3 coordinate is closely correlated to the storage time for each formulation.

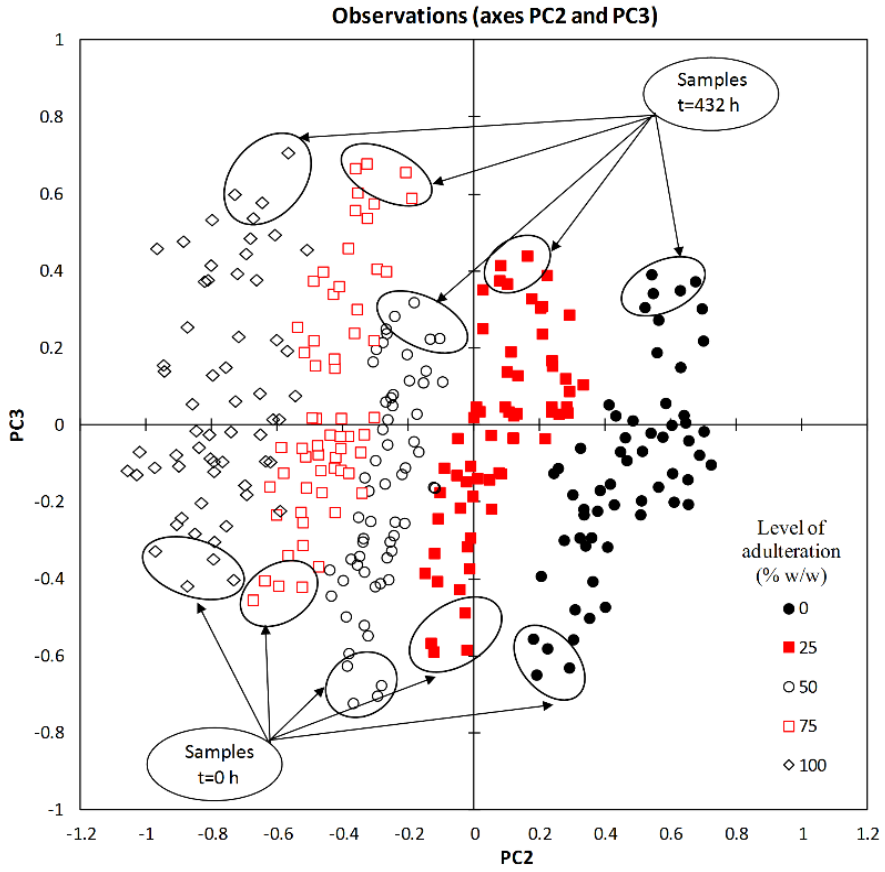

Figure 10. Observations diagram from the PCA of the spectral data for the five formulations of fallow deer adulterated with goat: 0, 25, 50, 75 and 100 %w/w of goat. Cluster of samples corresponding to the five formulations are easily distinguishable. Moreover, fresh (t=0) and spoiled (t=432h) samples are located in opposite sides of the diagram. This preliminary result indicates that infrared spectroscopy can identify different level of fallow deer adulteration, even in the case of samples stored for long periods. PC2 and PC3-principal components of the principal components analysis – PCA.

The loading plots of the PC2 and PC3 are represented in Fig. 2 whose interpretation is not straightforward, but a few correspondences to important functional groups can be pointed out.

Analysis of the PC2 loading plot reveals that the major features are mostly related to the lipid content of the samples. Namely, besides the bands at 1312 and 1400 cm^{-1}, related to amide II,

and from 1030 to 1180 cm^{-1}, related to amines, the bands at 1174, 1236 and 1476 cm^{-1} and, most important, the band at 1745 cm^{-1} are due to lipid content.

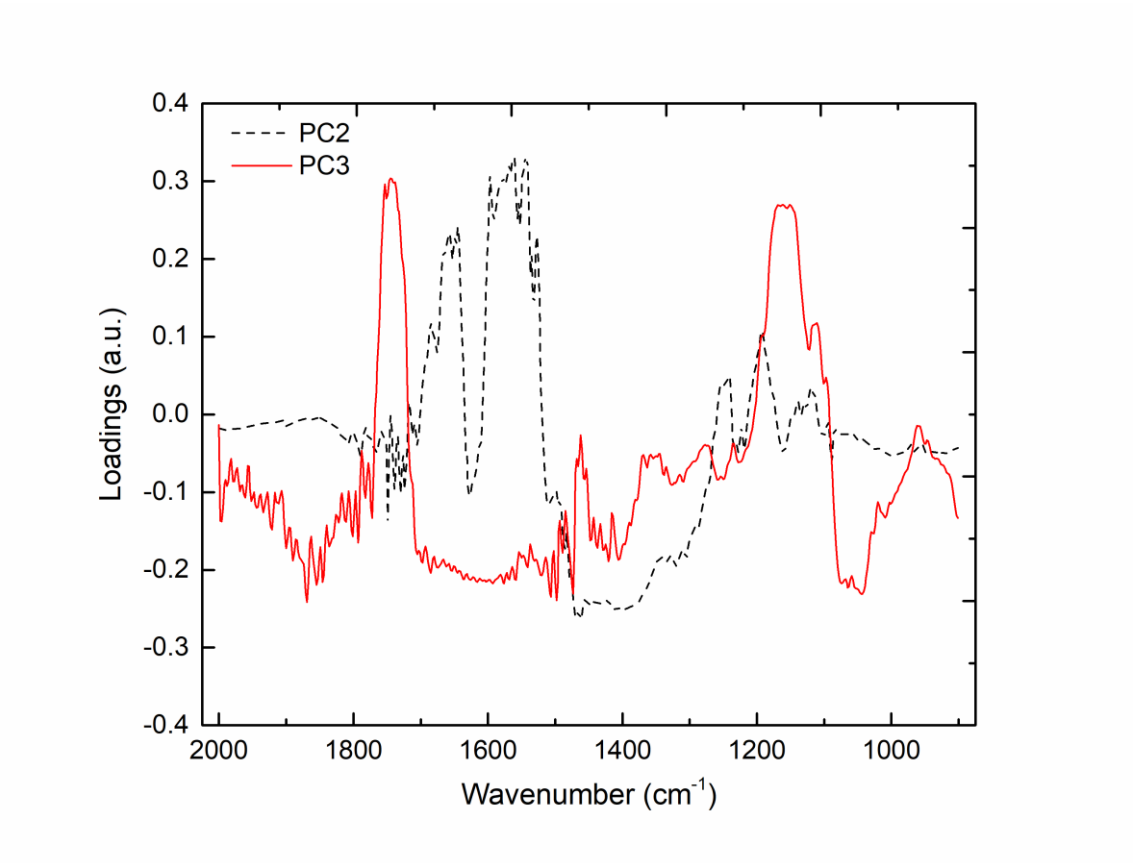

Figure 11. Loading plots calculated from the PCA of the spectral data for the five formulations of fallow deer adulterated with goat: 0, 25, 50, 75 and 100 %w/w of goat. Major features of PC2 are related to the lipid content of the samples and biogenic amines and PC3 is mostly related to the protein content, carbohydrates and lipids. The lipid content, accounts for the differences between the five formulations. It is reasonable to assume that fallow deer, feeding from the wild, has fat tissues that are a fingerprint. PC2 and PC3-principal components of the principal components analysis – PCA.

The loading plot for PC3 are mostly related to the content and structure of protein, but also to carbohydrates and lipids. It may be seen a band at 1460 cm^{-1} related to lipids, a band at 1194 cm^{-1} due to carbohydrates, but the most important bands are at 1640 cm^{-1}, due to amide I, and at 1546 cm^{-1}, related to amide II.

As indicated above, component PC2, mostly related to the lipid content, accounts for the separation obtained between the two types of meat. It is well known that animal feed plays a

role in the characteristics of the meat. For instance, dietary plant extract affects lipid oxidation levels of pork meat (Rossi et al., 2013). As stated above, it is reasonable to assume that the fallow deer, feeding from the wild, may possess fat tissues with characteristics that act as a fingerprint.

On the other hand, component PC3, correlated with the storage time, possesses loadings features related to biochemical changes in the meat owing to a combination of autolytic and microbiological proteolysis of meat muscle proteins. In fact, an increase in absorption at certain wavenumbers was reported, suggesting the production of free amino acids and peptides (Alexandrakis et al., 2012). Hydrolysis of proteins points to the production of metabolites related to spoilage such as ammonia and volatile amines (Ammor et al., 2009).

3.4 Partial least square discriminant analysis

After selecting the wavenumbers that provides more information for the discrimination, the next step was to build a classification model using PLS-DA in order to evaluate the possibility of predicting the level of adulteration. The wavenumbers between 2000 and 900 cm^{-1} were used as X variables, while the Y variables were associated with three different levels of adulteration A1, A2 and A3. Each Y variable corresponds to a different level of adulteration, with the value 1 or 0 depending on whether or not it belongs to a certain level of adulteration.

A subset of 225 samples, the training set, was used to calculate the RMSEC and the RMSECV parameters of the PLS-DA model, and are shown in Table 2. High correlation factors and low RMSE values were obtained: RMSEC between 5.1 and 9.2 and RMSCV between 4.2 and 10.3. Figure 3 represents the scores plot of the PLS-DA, where the three levels of adulteration A1, A2 and A3 are clearly discriminated. Note: Parameters of the partial least squares discriminant analysis (PLS-DA). a) Classes of adulteration: A1 (pure meat), A2 (adulterated) and A3(highly adulterated. b) regression coefficients, all with $p < 0.05$. Two set of samples were used: a “training set” and an “external set” (samples not used to build the predictive model). The expected error when measuring real adulterated samples using the spectroscopic method is below ~12%w/w of goat. Previous development of models (“Calibration”) and internal verification (“Validation”) use the training set. High regression coefficients (>0.9), with low p-value, and low error means high accuracy and predictive quality of the models developed.

Table 11. Quality of the predictive model. The effective (in real world) predictive ability ("Prediction") of the model was tested using the external set.

	R2 b			Error (%w/w of goat)		
Classesa	Calibration	Validation	Prediction	Calibration	Validation	Prediction
A1	0.99	0.99	0.98	5.1	7.2	8.6
A2	0.97	0.95	0.94	9.2	10.3	12.5
A3	0.99	0.98	0.97	7.4	9.4	9.5

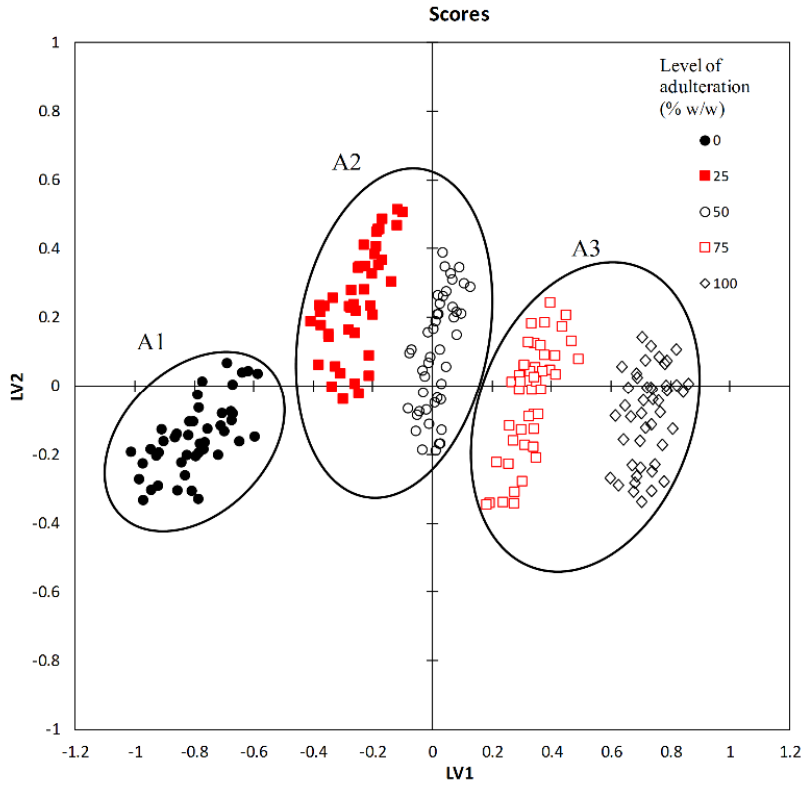

Figure 12. Scores plot of PLS-DA predictive model using the spectroscopic data. Groups of samples corresponding to three levels of adulteration, A1(pure meat), A2(adulterated) and A3(highly adulterated) are positively noticeable. Furthermore, pure fallow deer samples are detached from any adulterated sample, even in the case of samples stored for long periods. This plot indicates that infrared spectroscopy can identify pure fallow deer from adulterated meat. LV1 and LV2-latent variables of the partial least squares discriminant analysis, PLS-DA. Goodness-of-fit R2Y = 0.62; goodness-of-prediction Q2 = 0.51; p-value = 0.0027.

For a second subset of 75 samples, the external set, (of which 15 belong to A1, 30 belong to A2 and 30 belong to A3), reasonable values of R2 (between 0.94 and 0.98) and RMSEP between 8.6 and 12.5, were obtained. Results of the application of the model to the prediction of the level of adulteration for those samples is presented in Table 3. Predicted level of adulteration variable (Apred) near 1 means belonging to the corresponding class and, on the other hand, a value near 0 means not belonging to the that class. Therefore, very accurate classification was achieved because the predicted values were always very close to 1 for classification within the class, and values were near to 0 for classification outside the class. Therefore, it was confirmed the quality of the predictive ability of the PLS-DA classification model, presenting a goodness-of-fit value of R2Y = 0.62 and a goodness-of-prediction value of Q2 = 0.51 (Q2 > 0.5 is generally considered good, (Sanz-Cortés et al., 2013)), and p-value = 0.0027. We may be say that the present predictive model is capable of classifying real samples with 100% accuracy.

Table 12. Results of the application of the model. The present's predictive model is capable of classifying real samples with high accuracy.

Classesa	A1		A2		A3	
	Arefb	Apredc (std)d	Arefb	Apredc (std)d	Arefb	Apredc (std)d
A1	1	1.010 (0.009)	0	-0.077 (0.016)	0	0.007 (0.004)
A2	0	0.008 (0.001)	1	0.953 (0.102)	0	0.220 (0.060)
A3	0	-0.008 (0.001)	0	0.197 (0.049)	1	1.096 (0.125)

Note: Partial least squares discriminant analysis (PLS-DA). a) Classes of adulteration: A1(pure meat – 15 samples), A2(adulterated – 30 samples) and A3(highly adulterated – 30 samples). b) Aref-actual classification (0/1); c) Apred-average predicted classification for each class. d) std-standard deviation of predicted classification for each class. Goodness-of-fit R2Y = 0.62; goodness-of-prediction Q2 = 0.51; p-value = 0.0027. A very accurate predictive model classifies a sample blonging to a certain with a value Apred = 1 (means belong to the class) or Apred = 0 (means not belong to the class). Application of the predictive model to the external data set (samples not used in development of predictive models) led to average Aprev values near 1 for classification within the classe, and average values near 0 for classification outside the classe; in both cases the standard deviation of predicted classification is very small.

In principle, the difference of lipids and proteins in the two types of meat was the key to the discriminate fallow deer meat from goat meat by spectroscopy and chemometrics. In general, the greater the difference in meat composition, the more accurate can be achieved by infrared spectroscopy. When using infrared spectroscopy to detect different kinds of meat, such as cattle, llama and horse (Mamani-Linares et al., 2012), pork and beef (Kuswandi et al., 2015), pork and lamb (Kamruzzaman et al., 2013), the accuracy of meat classification was very high. However, for similar breeds of meat, such as white pigs and Iberian pigs (Guillén et al., 2010), adult steers and young cattle (Prieto et al., 2008), Duroc and Iberian pork (Del Moral et al., 2009), the accuracy declined and the meat samples were easily confused. It is conceivable that when the variety approaches, the chemical composition of the meat itself is more similar, reflecting the smaller difference in the spectrum.

The wild fallow deer is a ruminant mammal belonging to the family *Cervidae.* The domestic goat is a subspecies of goat domesticated from the wild goat of southwest Asia and Eastern Europe and is a member of the family *Bovidae.* They belong to different families, thus it is expected that the discrimination would be very complex.

The results presented in this paper showed that wild fallow deer meat can be reliably identified using the mid infrared spectroscopy combined with the PLS-DA method. However, the model is only applicable to the distinction of these two types of meat. To identify more types of meat, we need to increase the number of species and of samples. The model will be more adaptable and robust if the calibration set is extended with more samples, of different breeds and areas (Mamani-Linares et al., 2012).

4. Conclusions

A reliable and low-cost method based on the combination of mid infrared spectroscopy with the PLS-DA technique has demonstrated great potential in the discrimination and in the classification of fallow deer meat adulterated with goat. Using this methodology, the two types of meat can be easily distinguished with high accuracy.

The PCA analysis led to the conclusion that the most important absorption bands for discriminating the adulteration level of the fallow deer are contained in the wavelength range from 2000 to 900 cm^{-1}, encompassing specific bands normally associated to lipid and protein compounds.

The process of classification using the PLS-DA allowed discrimination of samples in three different levels of adulteration, A1 (pure meat), A2 (adulterated) and A3 (highly adulterated) was successfully carried out using fresh samples and stored for different periods of time, and at diverse stages of the deterioration process.

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PAPER V – SOUSA, NUNO; MOREIRA, MARIA J.; SARAIVA, CRISTINA; ALMEIDA, JOSÉ M.M.M. 2018. "APPLYING FOURIER TRANSFORM MID INFRARED SPECTROSCOPY TO DETECT THE ADULTERATION OF SALMO SALAR WITH ONCORHYNCHUS MYKISS", FOODS 2018, 7(4), 55; DOI:10.3390/FOODS7040055 (ANNEX V)

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Applying Fourier transform mid infrared spectroscopy to detect the adulteration of *Salmo salar* with *Oncorhynchus mykiss*

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Abstract:

The aim of this study was to evaluate the potential of Fourier transform infrared (FTIR) spectroscopy coupled with chemometric methods to detect fish adulteration. Muscles of Atlantic salmon (Salmo salar) (SS) and Salmon trout (Onconrhynchus mykiss) (OM) muscles were mixed in different percentages and transformed into miniburgers. These were stored at 3°C, then examined at 0, 72, 160 and 240 hours for deteriorative microorganisms. Mini-burgers was submitted to Soxhlet extraction, afterwards lipid extracts were analysed by FTIR. The principal component analysis (PCA) described the studied adulteration using four principal components with an explained variance of 95.60%. PCA showed that the absorbance in the spectral region from 721, 1097, 1370, 1464, 1655, 2805 to 2935, 3009 cm-1 may be attributed to biochemical fingerprints related to differences between SS and OM. The partial least squares regression (PLS-R) predicted the presence/absence of adulteration in fish samples of an external set with high accuracy. The proposed methods have the advantage of allowing quick measurements, despite the storage time of the adulterated fish. FTIR combined with chemometrics showed that it can be established a methodology to identify the adulteration of SS with OM, even when stored for different periods.

Keywords: Food fraud; Food authentication; Salmo salar adulteration; FTIR spectroscopy; Chemometrics methods.

1. Introduction

Atlantic salmon (SS) is economically important in the daily life of consumers, since it is a good source of polyunsaturated fatty acids, namely two important omega-3 fatty acids: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Castejón et al., 2016, Haq et al., 2017). They are composed of 5–10% red muscle and 90–95% white muscle (Cai et al., 2014). The red/orange colour is due to the presence of carotenoid pigment, named astaxanthin, which has antioxidant activity, leading to a high oxidative stability (Fidalgo et al.).

The salmon trout (*Onconrhynchus mykiss*) (OM) and the SS are visually similar, namely in muscle colour, rich in EPA and DHA and are the major species of European aquaculture because of the pressure on the wild fish population, consequently, access has become limited (Lundebye et al., 2017). The pigmentation of OM is caused by pketo-carotenoids astaxanthin and canthaxanthin (Choubert and Baccaunaud, 2006).

In the last decade, the issue of food safety has acquired increased importance, due to the rapid changes in the agro-food system. Fraud is a major concern for the food industry. It is defined as the intentional act of substituting, adding, adulterating, tampering or misrepresentation of ingredients, and/or packaging (Rady and Adedeji, 2018).

This not only decreases the quality of products, but also misleads consumers and may involve associated health risks (Nunes et al., 2016, Spink et al., 2016).

There are different types of food adulteration namely, unauthorized partial or total substitution of commercial valuable species with cheaper products (Boyacı et al., 2014), frozen-thawed product sold as fresh (Cheng et al., 2015, Ottavian et al., 2013), classification fraud of species or origin (Standal et al., 2010) and the presence of genetically modified organisms.

In the past, a variety of standard analytical methods were applied to detect the adulteration of proteins, such electrophoresis (polyacrylamide gel electrophoresis), immunological analysis (immuno-diffusion techniques, immuno-electrophoresis, and linked immune-adsorption assays), chromatographic and DNA-based procedures (polymer-chain reaction) (Al-Kahtani et al., 2017). However, these methods need skilled technicians and a relatively long time for sample preparation and analysis (Rady and Adedeji, 2018).

Presently, are being developed innovative and non-destructive spectroscopy techniques. Those techniques require small samples; no complex preparation is necessary, thus allowing simple, fast, and accurate measurements (Kamruzzaman et al., 2015, Alamprese et al., 2013)

Emerging non-destructive mapping technologies for authentication and traceability include visible/ near infrared, mid infrared, fluorescence spectroscopy (Lohumi et al., 2015), Raman spectroscopy (RS) sometimes coupled with Fourier transform infrared (FTIR) technique.

FTIR spectroscopy has substantial potential as a quantitative method in the food industry. When used together with an attenuated total reflectance (ATR) module and chemometric, FTIR offers methodologies capable of discriminating qualitatively and quantitatively foodstuff based on the spectral characteristics of the food matrix (Lohumi et al., 2015, Beasley et al., 2014).

Chemometric use mathematical and statistical techniques to select the best experimental procedure and treatment of chemical analysis data (Roggo et al., 2007). There are several chemometrics methods applied to spectroscopy, namely partial component analysis (PCA), discriminant analysis, principal least squares discriminant analysis, partial least squares regression (PLS-R), among others (Lohumi et al., 2015).

There are few studies to quantify fish adulteration using FTIR spectroscopy coupled with chemometric. This study explores the potential of FTIR as a rapid and accurate method to detect and predict the adulteration of SS with OM, regardless of their storage period.

2. Material and Methods

2.1. Sampling

SS and OM fish were eviscerated, skin removal was carried out and muscle was crushed separately in a mincer under sterilized conditions. Mini-burgers of SS adulterated with OM, from 0 to 100 %w/w in steps of 10% w/w, were produced. For each sampling point, 4 mini-burgers were produced, 2 for fat extraction and FTIR and 2 for microbiological analysis.

The mini-burgers weighing approximately 15g were prepared by mixing the fish and later packed in air overwrapped with polyethylene film. Following packaging, samples were stored at 3ºC and examined for microbiological parameters at intervals of 0, 72, 160 and 240 h.

The microorganisms analysed were total mesophilic (TVC) and psychrotrophic (TP). In addition, after each predefined storage period, the samples were submitted to Soxhlet extraction and the lipid extracted were analysed by FTIR.

The experiment was repeated 4 times, each batch having 176 samples, totalling 704 mini-burgers: 352 for FTIR measurements and 352 for microbiological determinations.

2.2. Microbial Analysis

Samples were homogenized with tryptone salt broth (tryptone 0.1 % and NaCl 0.85 %) in a stomacher for 90s. Serial decimal dilutions were prepared in the same solution for microbiological determinations. TVC (ISO4833, (2003)) and TP (2307:1987, (1987)) populations were obtained after incubation on plate count agar (PCA) (Oxoid CM0325, England) at 30 ºC for 3 days and 7 ºC for 10 days, according to ISO4833 of 2003 and NP2007 of 1987, respectively.

2.3 Determination of Moisture Content

The measurement of the moisture content consisted in drying the samples in an oven at 100°C. The weight of the samples was controlled at 60 minutes intervals using an analytical balance with a resolution of 0.001 g. The process stopped when the mass of the last two weighing, separated from 60 minutes, did not differ by more than 0.1% and then stored in a desiccator with silica.

2.4 Determination of Free Fat Content/ Soxhlet Extraction

Fat extraction was carried out by n-hexane in the dehydrated samples. The dried sample and traces of the sample on the petri dish were removed using cotton wool moistened with n-hexane and later placed in an extraction thimble. Afterwards, the extraction thimble was positioned in the extraction tubes together with n-hexane, and a flask was adapted to the extractor apparatus.

The extraction process lasted 8 hours, then the flask was placed in a water bath at 90°C to remove n-hexane, leaving only the fat. After this process, the flask was placed in the oven for 1h at 103°C to remove n-hexane residues. These procedures (drying and weighing) were repeated until the results of both successive weighing, separated by 1h, did not differ by more than 0.1% (Aloglu and Harrington, 2018).

2.5. Fourier Transform Infrared Measurement

The infrared absorption spectra were collected in a FTIR spectrometer (Shimadzu, Japão) equipped with an ATR module (Golden Gate, U.K.), a DLaTGS detector and a KBr beamspliter.

Samples of fish fat were placed on top of the ATR crystal whose temperature was set to ~35°C. The collection time for each sample spectrum was approximately 2 minutes. Spectrum was recorded in the region between 4000 and 500 cm-1 with a resolution of 4 cm -1 and 32 scans. In the ATR module the infrared radiation undergo total internal reflection when the incident angle at the interface between the sample and the crystal is higher than the critical angle, which is a function of the refractive indices of the two surfaces allowing the penetration of radiation into the sample (Beasley et al., 2014). The ATR base was carefully cleaned in situ by scrubbing with pure ethanol (Sigma Aldrich, Germany) before measuring the next sample. For each sample, two spectra were collected and the average was calculated.

2.6. Mathematical Treatment

2.6.1. Principal Component Analysis

Spectral data collected between 500 and 4000 cm-1 were divided into two ranges, from 650 to 1850 and from 2800 to 3050 cm -1. Spectral data set was initially submitted to smoothing based on the Savitzky-Golay algorithm and afterwards were mean-centered and standardized (SNV) (Savitzky and Golay, 1964).

For a preliminary exploration, the spectral data set was handled by PCA, which allowed determining its main features and to highlight relations among the original variables (absorbance at different wavenumbers). The PCA projects the large number of potentially correlated original variables in a representation space of smaller dimensions and calculates new variables, called principal components (PC), that are linear combinations of the starting absorbances and thus reduces the size of the data set (Abdi and Williams, 2010).

2.6.2. Partial Least Squares Regression

For quantitative analysis, the measured factors, contributing to the variance of the data set, were regressed using PLS-R onto the referred variables (Liang and Kvalheim, 1996, Wentzell and

Montoto, 2003). This multivariate calibration technique, sometimes called factor analysis, transformed the original variables (FTIR spectra absorbencies) into new ones (known as latent variables), which are linear combinations of the original variables (Miller and Miller, 2005). The method relied on two phases, the so-called calibration, and cross-validation steps. Calibration consists in building a mathematical model to establish a correlation between the matrix of FTIR spectra (predictor variables, X) and the concentration of analytes of interest (response variables, Y) which use a set of observations usually named calibration set. Cross-validation is performed using the calibration model used to calculate the concentration of samples not used to set up the model (De Luca et al., 2009).

The relative performance of the established model was accessed by the root mean square error of calibration (RMSEC), root mean square error of cross-validation (RMSECV), and multiple coefficient of determination or regression coefficient (R^2) (Divya and Mishra, 2007). The selected model was then used to determine the concentration of samples in an independent prediction set. The predictive ability of the model is evaluated from the root mean square of prediction (RMSEP). The lower the RMSEP value, the higher the degree of accuracy of the prediction result provided by the calibration model (Corgozinho et al., 2008).

PCA, DA, and PLS-R calculations were performed using the Excel-based XLSTAT V2006.06 package (Addinsoft, Inc, NY, USA) and statistical software Unscrambler V9.6 package (Camo, Oslo, Norway).

3. Results and Discussion

3.1. Microbial Analysis

Table 1 shows the evolution of TVC and of TP with storage time of pure SS and pure OM. The TVC and TP counts increase with storage time, as expected, following and exponential growth. Both species have very similar counts at time 0, but after 240 h pure OM showed a more pronounced development of both TVC and TP.

Table 13. Total mesophilic (TVC) and total psychrotrophic (TP) microorganism counts (mean and standard deviation) in Salmo salar (SS) and Onconrhynchus mykiss (OM) samples, according to storage period.

		Time (hours)			
		0	72	168	240
TVC	SS	3.44±0.46	4.67±0.10	6.82±0.23	7.75±0.22
	OM	3.89±0.61	6.26±1.12	7.98±0.25	8.81±0.21
TP	SS	3.19±0.52	4.61±0.03	6.16±0.06	7.47±0.25
	OM	3.89±0.61	5.39±0.31	8.00±0.25	8.86±0.21

3.2. Determination of Moisture Content

Prior to fat extraction, the samples were dehydrated. They were weighed hourly during drying to determine the evolution of water loss. The SS samples have a slightly higher relative humidity value compared to OM samples, 66.44 and 64.76 respectively, at time 0.

The SS samples retained more water, so its loss was more pronounced during first few minutes of drying. However, the longer storage time, in this case at 3°C, the greater the loss of moisture upon the first 60 minutes of drying. This is due to the interaction of lipid oxidation with proteins, which causes loss of water retention capacity (Tironi et al., 2010). Therefore, the loss of water in the first 60 minutes is higher in samples with longer storage time than in the most deteriorated ones, due to increased water availability.

3.3. Determination of Free Fat Content/ Soxhlet Extraction

To determine the fat content the Soxhlet method was used. Table 2 shows that OM samples had a higher percentage of fat than SS samples. Thus, OM is more susceptible to lipid oxidation, which may lead to a more pronounced deterioration than SS.

Table 14. Fat content of Salmo salar (SS) and Onconrhynchus mykiss (OM) samples in (% w/w).

Mixture (%w/w of OM/SS)	Fat content (%w/w)
0	11.75±0.78
10	11.62±0.71
20	11.71±1.11
30	12.6±1.34
40	12.75±0.81
50	12.62±0.67
60	13.31±0.71
70	13.15±1.19
80	13.78±0.66
90	14.21±0.57
100	13.65±1.35

Values of Mixture are %w/w of OM in SS.

3.4. Fourier Transform Infrared Measured Spectra

3.4.1. Preliminary Analysis of the Spectral Data Set

Figure 1 shows the absorption spectra of the fat extracted from the samples in the medium infrared region between 500 and 4000 cm-1 of the pure samples on time 0 and 240 h. Several peaks, which correspond to different functional groups, can be observed in Fig. 1 and Table 3 outlines the principal peaks and presents its origin (Rohman et al., 2011, Carton et al., 2008, Guillén et al., 2004).

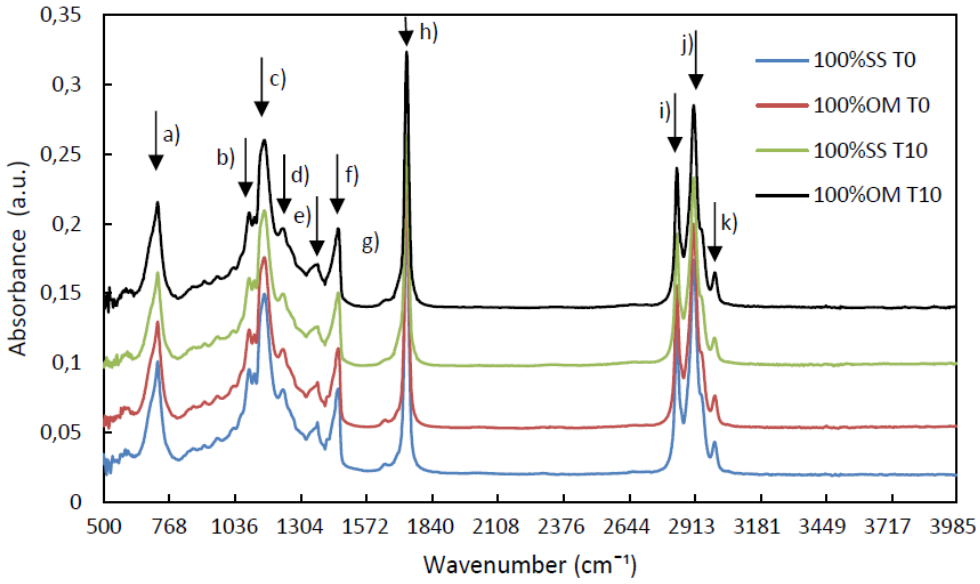

Figure 13. Fourier transform infrared spectroscopy (FTIR) spectra of fat extracted from fresh samples of Onconrhynchus mykiss (OM) and Salmo salar (SS) and stored at 3°C for 240 h.

Table 15. Assignment of functional groups present in Salmo salar and Onconrhynchus mykiss fat responsible for infrared absorption.

Assignment	Wavenumber (cm-1)	Functional group responsible for IR absorption
a)	721	cis-disubstituted olefins (- CH2- , - HC=CH- (cis))
b)	1097	ester of the –C-O group
c)		-C-O, CH2 group and are correlated with saturated acyl groups
d)		-C-O, CH2 group and and are correlated with saturated acyl groups
e)	1370	CH3 group
f)	1464	CH2 and CH3
g)	1655	Unsaturated acyl group (-C = C-)
h)		C = O group of triglycerides
i)	2850 to 2925	symmetrical and asymmetric methylene (CH2)
j)	2850 to 2925	symmetrical and asymmetric methylene (CH2)
k)	3009	cis olefinic CH double bonds (= C-H)

The appropriateness to perform PCA was confirmed by Bartlett's sphericity test ($p < 0.0001$). The number of components retained in the final solution was based on the Kaiser-Meyer-Olkin (KMO) criterion for the analysis of eigenvalues (>1) and the proportion of variance retained (> 70%), usually seen as the minimum needed to make the model suitable for explaining the original data. The FTIR spectroscopic data corresponding to the various adulteration levels of SS with OM were subjected to PCA. The KMO sample adequacy measurement was 0.859, which means that the suitability of the sample was good.

It was concluded that 36 PCs describe the variance of the data set represented by the original variables. It was observed that 95.90% of the variance was explained by only two main components, F1 and F2 principal components describing 42.34% and 53.56% of the variation, respectively. PCA was used to verify the possibility of using FTIR to distinguish SS samples with different adulteration levels of OM. Figure 2 shows the graph of observations for components F1 and F2. It can be observed that the samples with the same percentages of adulteration are grouped into clusters, regardless of storage time.

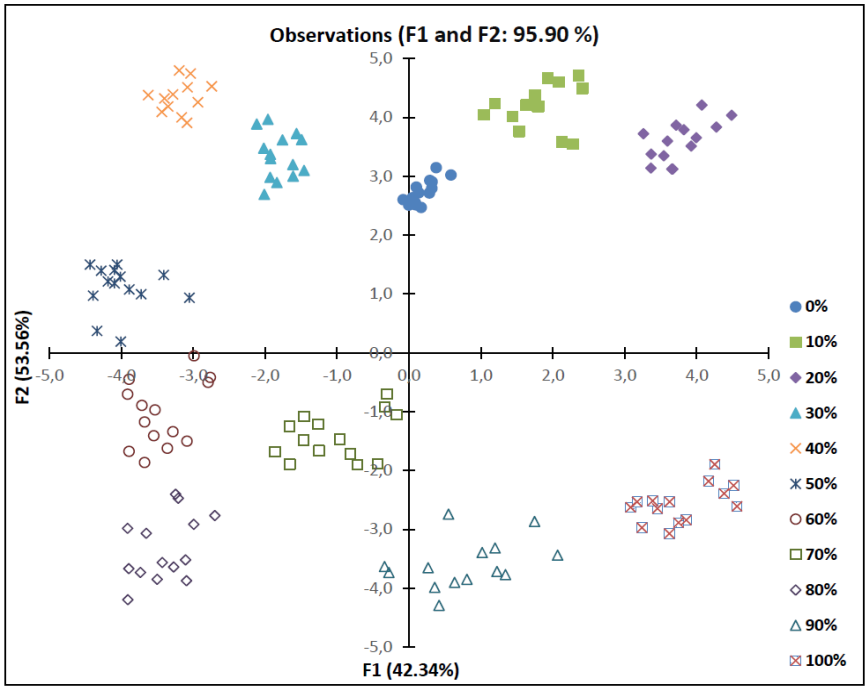

Figure 14. Observations diagram obtained by principal component analysis (PCA) using the Fourier transform infrared spectroscopy (FTIR) spectral data for the 11 formulations of Onconrhynchus mykiss (OM) and Salmo salar (SS).

3.4.2. Partial Least Squares Regression Models for Prediction Based on Spectral Data Set

PLS-R calibration were performed to determine the feasibility of establishing a relationship between the predictive variables (x, absorbances) and the percentage of adulteration (y, response variables).

PLS-R regression was performed using the frequency regions of two ranges, from 650 to 1850 cm-1 and from 2800 to 3050 cm-1. PLS regression was performed on the same frequency regions used for PCA. The quality of the fitting was scrutinized by RMSEC, multiple R2, and by RMSECV.

To validate the developed PLS-R models, leave-one-out cross-validation (LOOCV) was the method applied to a training set of 341 samples to evaluate the adequacy of the PLS-R technique. One sample at a time is randomly excluded. Then, the properties of the removed sample were predicted with a model constructed with the remaining samples (the training set). This procedure was repeated until each sample was excluded once (Picard and Cook, 1984). The ability of models to predict the properties of a set of 11 samples not used to construct the model (the external set) was inspected by evaluating the RMSEP.

Table 4 presents the quantitative performance of multivariate calibrations determined in this study, in terms of multiple R2, RMSEC, RMSECV and the RMSEP. The high value of R2 and the low values of RMSE indicate good accuracy and precision of PLS-R models (Huishan et al., 2005). The values of R2 and RMSE were 0.988 and 5.6 w/w, respectively, for calibration. When data were subjected to cross-validation the RMSE increased to 6.7 w/w. However, in the calculation of the adulteration level of the external set of 11 samples, the value of the RMSEP increased to 8.7 w/w. Figure 3 illustrates the accuracy and performance of the models that correlate the measured and estimated adulteration values from the FTIR data set.

Table 16. Quality parameters of the multivariate model for quantification of adulteration of mixtures of Salmo salar (SS) and Onconrhynchus mykiss (OM).

Number of factors	R2			RMSE (% w/w of OM/SS)		
	Calibration	Validation	Prediction	Calibration	Validation	Prediction
4	0.988	0.991	0.992	5.6	6.7	8.7

Figure 7

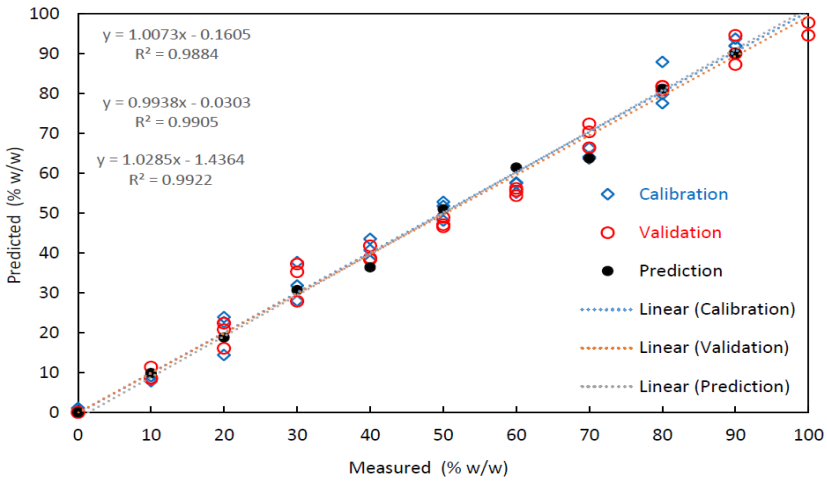

Figure 15. Illustration of the quality of prediction models obtained by Fourier transform infrared spectroscopy (FTIR) for the observed and estimated values for the different mixtures of Salmo salar (SS) and Onconrhynchus mykiss (OM).

4. Conclusions

The use of FTIR coupled with chemometric methods allowed to estimate accurately the percentage of adulteration of SS with OM. The process of classification using PLS-R allowed discrimination of samples in 10 levels of adulteration, in steps of 10%, was successful carried out using fresh samples and stored for different period of time, and at diverse stages of the deterioration process.

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Conflict of interest: The authors declare no conflict of interest.

Compliance with Ethics Requirements Nuno Filipe C. Sousa, Maria João P. Moreira, José Manuel M. M. De Almeida and Cristina Saraiva Declare That They Have No Conflict of Interest. the Present PAPER Does Not Contain Any Studies With Human or Animal Subjects.

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CHAPTER V. EXTENDED DISCUSSION

Food labelling is an important tool for communication between consumers and food industry regarding a food product or food processing (Charlebois et al., 2016). Consumers´ concers are associated to the constitution of foodstuffs they consume and recognize the importance of the information provided in the food label, including the list of ingredients, information about country of origin and the food processes applied in the transformation or manufacture of final product (Tonkin et al., 2016). The present work assesses respondent's opinion about the information provided in food label, its confidence about foods constitution and the perceptions about adulterations and food fraud.

It was observed that most respondents not read food labels for large variety of reasons. The lack of time (23.7%) has been referred as the most important reason. The brand name was less important for consumers (18.5%) in contrasts to the reported by Bandara et al., 2016 that was highly valued (85.56%). However, respondents pay more attention to other compulsory mentions of food label such as shelf-life (79%), product constitution (74.89%) and nutritional value (60.11%), as also referred by Bandara et al. (2016).

With regard of nutritional composition, respondents indicated they not spend much time to read the information displayed, probably associated to the lack of knowledge or the difficult to understand the technical information provided. This result is in opposition to the reported by Vemula et al. (2014) detected that nutrient information on labels was not often read because most consumers either lacked nutrition knowledge or found the information too technical to understand. A positive association was found between education level and checking various aspects of food labels. Sociodemographic characteristics may also influence the perception of information displayed in food label. Factors such as age, education, marital status, and practice of sports influenced the results obtained. Thus, respondents referred some difficult to read the information of food labels due to the small size of the text, and some authors also indicated the font size as a barrier (Bandara et al., 2016). Regarding gender, Van der Mere et al. (2014) indicated that women spend more time reading labels than men, but no significant differences between genders were observed in our study. In overall, respondents considered all the compulsory mentions displayed in food labels important. Our results indicated that price and label design showed the same importance. On contrary, in general they consider the usefulness of nutritional information is low, but according to socio-demographic consumers'

characteristics those follow a healthy life, practice sports or declared some health condition (e.g., food allergies) pay more attention to the nutritional information, food label design, allergens or intended use.

Also, food producer or country of origin were not relevant characteristics for respondents, which can be related to economic issues.

Verification and official control of food composition is necessary to prevent adulteration. Regarding food from animal origin, the latest reports of food fraud suggests the need of an effective identification of the species included (Andrée et al., 2010, Ballin, 2010, Standal et al., 2010b, Lin et al., 2014b).

Liu and Niyongira (2017) concluded that food label for consumer presented inappropriate font size, brand credibility influenced the choice, the respondents had lack of time for read food labelling or they observed deficient health claims (Liu and Niyongira, 2017). Respondents believe that label information ensures nutritional information, food quality and safety, and helps to choose healthy products. Education may improve the consumers understand of information displayed in food labels and the differences between food products. However, consumers with higher education are less likely to believe in nutritional labels. Also, respondents believe that information provided by food label does not prevent fraud, although ensures its traceability. These results are unclear because traceability is intended to ensure the quality along the food chain.

Kendall et al. (2019) indicated that food fraud causes an increased lack of consumer trust in food products and may represent a hazard for public health.

Consumer trust depends on the type of foods. Thus, our study showed that fish, milk and dairy products and frozen products displayed better confidence.

The consumer must have access to accurate information on the origin of products and protection of public health. Hence, the implementation of new food safety strategies, such as food defense plans throughout the food chain, will certainly be the next step developed by food authorities to improve public health (Davidson et al., 2017). The results of the present study highlight the need of campaigns to give information by public health authorities to show the importance and advantages of reading food labels. Public health authorities must ensure that food labels possess essential information and information which is quickly and clearly seen by consumers.

It is important to develop methodologies that allows the correct identification of the composition of food products. Actually, a diversity of analytical techniques has been developed for identification of adulteration or fraud, such as, polymerase chain reaction, random amplified polymorphic DNA, single nucleotide polymorphism analysis and enzyme-linked immunosorbent assay (ELISA). The detection ranges based on these techniques (from 0.01% to 10%) are constricted, and are far lower than the actual adulteration proportion. These techniques are all time-consuming, require specialized equipment and skilled personnel and preparation of samples (Velásquez et al., 2017). In this sense, the use of rapid, reliable and accurate methods to detect fraud or adulteration can be of great importance. Accordingly, two studies were carried out to detect the meat adulteration of wild fallow deer (*Dama dama*) with domestic goat (G) (*Capra aegagrus hircus*) and the fish adulteration of Atlantic salmon (*Salmo salar*) (SS) and Salmon trout (*Onconrhynchus mykiss*) (OM), for different periods of time, using FTIR spectroscopy coupled with chemometric. In this study, PCA showed a high communality value of the first four principal components for wavenumbers from 1138 to 1180, 1304 to 1477, 1535 to 1556 and from 1728 to 1759 cm^{-1}. Among these wavenumbers, those in the range 1304 to 1322 and from 1372 to 1403 cm^{-1} are related to biomolecules such as amides, amines and the biochemical changes during storage of the meat, as mentioned earlier by other authors (Ammor et al., 2009, Ellis et al., 2002, Ellis et al., 2004). The wavenumbers from 1728 to 1759 cm^{-1} are related to the lipid constitution of each type of meat and, in our opinion, explain the separation achieved between the different mixtures of fallow deer and goat. This is, probably, an effect of different feeding regimes, as referred by Kim et al. (2017) and Paengkoum et al. (2013).

The results presented in this paper showed that wild fallow deer meat can be reliably identified using the mid infrared spectroscopy combined with the PLS-DA method. However, the model is only applicable to the distinction of these two types of meat. To identify more types of meat, we need to increase the number of species and of samples. The model will be more adaptable and robust if the calibration set is extended with more samples, of different breeds and areas (Mamani-Linares et al., 2012a).

Therefore, many researchers are attracted by spectroscopic methods to study on meat adulteration, and some works (Velioglu et al., 2018, Nunes et al., 2019) have been carried out to determine the content of the non-native meat.

Similar to our study, it was achieved the quantification of dog meat (DM) in beef meatball with FTIR spectroscopy combined with chemometrics methods. Lipid extracts obtained from the samples were scanned using FTIR spectrophotometer at 4000–650 cm^{-1}. The results showed that combined frequency regions of 1782–1623 cm^{-1} and 1485-659 cm^{-1} gave optimum prediction. Coefficient of determination (R^2) for correlation between the value of DM and FTIR predicted value was 0.993 in calibration model and 0.995 in validation model. The region from 1,238 to 1031 cm^{-1} is associated with C-O and C-C stretching vibrations (because of carbohydrates) and at 1,746 cm^{-1} to C=O (fat) (Rahayu et al., 2018).

Deniz *et al.* (2018), using Fourier transform infrared (FTIR) spectroscopy coupled chemometrics methods, showed that PCA yielded more applicable results for classification of adulterated beef in raw meat mixtures than hierarchical cluster analysis (HCA) for identification of adulterated, incorporating chicken or turkey meat in different proportions. As observed in our work, similar peaks at 1,196 cm -1 can be attributed to C-O. Peaks at 1,305 cm^{-1} are related to Amide III, N–H bending, C–H stretching: proteins. Absorption bands at 1,456–1,455 cm^{-1} are because C–O–H, bending modes of methyl groups: proteins, lipid. Around 1650 cm^{-1}, there is a large absorption band related Amide I; 80% C=O stretching, 10% N–H bending, 10% C–N stretching proteins (Deniz et al., 2018).

Yang *et al.* (2018) in a study using infrared spectroscopy coupled with chemometric methods determined adulteration pork meat in beef or mutton in the market samples. Similar results were achieved with our, the peaks at 2925 cm^{-1} and 2855 cm^{-1} were due to lipids (CH_2 stretching). The peak at 1745 cm^{-1} was due to lipids (C=O stretching). The peak at 1645 cm^{-1} was due to water (O-H stretch) and amide I (C=O stretching). The peaks at 1401 and 1464 cm^{-1} were due to lipids (C-H stretching) and amide III. Other absorption peaks between 900 and 1200 cm^{-1} were associated with carbohydrates (C-O or C-C stretching).

In the Mahalanobis distance method, the coefficient of test sets was increased from 0.93 to 0.99; the RMSEC and RMSECV were decreased from 0.17 to 0.09 and 0.21 to 0.11 accordingly. The coefficient of determination in-between the calibration and testing sets in PLS-DA reached 0.99 and 0.99, RMSEC was 0.06, and both the RMSECV and RMSEP were 0.08. In contrast, in SVM, methods were 0.97 and 0.96. The RMSEC, RMSECV, and RMSEP were 0.15, 0.17, and 0.24, respectively (Yang et al., 2018).

In other study, Ropodi *et al.*, 2018 determined frozen-then-thawed minced beef labeled as fresh using multispectral imaging (MSI) and Fourier transform infrared spectroscopy (FTIR). Results showed 100% overall correct classification for test and external validation MSI data, while FTIR data yielded 93.3 and 96.7% overall correct classification for FTIR test set and external validation set respectively. The area 1650–1620 cm^{-1} is associated with water/moisture and therefore relevant to the thawing process and 1560–1530 cm^{-1} assigned with the amide II band, comparable to our study (Ropodi et al., 2018).

In other similar studies researches distinguished adulterated Norwegian salmon with Heilongjiang salmon using a Fourier transform infrared spectroscopy (FTIR) coupled with PLS-DA model, several pre-processing methods, including standard normal variate (SNV). The two salmon were easily distinguished and the accuracy of the result was 100%. When Norwegian salmon meat was mixed at 20, 40, 60, or 80% with Heilongjiang salmon meat and tested using the spectra within the waveband range covering 450 to 4000 cm^{-1}, 90% accuracy could be reached. The peaks at 1164, 1770, 2940, and 2855 cm^{-1} gave significant contributions to the weighted regression coefficients. These peaks are also the absorption peaks of CH_2, C=O, C–O–C stretching vibration from lipids or protein as reported (Wu et al., 2018).

The FTIR spectroscopy coupled with chemometrics is a powerful analytical technique for authentication of meat and fish products. In addition the methodology provides a rapid screening of adulteration of meat and of fish. However, some confirmation methods like PCR should be used to confirm the presence of meat adulterant (Rohman, 2019). Though, the spectral techniques are promising alternative to the costly food identification techniques (Kumar and Chandrakant Karne, 2017).

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CHAPTER VI. CONCLUSIONS

Food labelling is one way for communication from food business operators to consumers, and it may influence the consumer's buying decision. The enforcing of the food labelling policy by the implementation of new policy was aimed to improve guarantee the food safety and public health with new mandatory information and nutritional values concerning each product. However, the effort undertaken by food and health authorities can be compromised, if consumers do not read the food label as observed in the present study. Thus, lack of time and excessive information were referred as the main factors for the absence of food labelling reading. Furthermore, it was observed that food labelling is more useful for specific consumers groups, such as athletes, consumers with health conditions or consumers concerned with a healthy lifestyle.

Regarding consumers´ confidence about the information displayed on food labelling, over half of respondents stated that information provided is reliable. However, respondents showed a lack of confidence on food composition that still decrease in processed foodstuffs such as meat products. Food fraud is recognized slightly over half of respondents with a higher perception of those practices that imply a risk to public health than those related to economic motivation. Age and education revealed the most important socio-demographic factors regarding food label perception, confidence on its information and also knowledge about food fraud.

The results of the present study highlight the need of information campaigns by public health authorities to show the importance and advantages of reading food labels, as well as ensuring food labels with essential information which are not only quickly and clearly seen, but also understood by consumers. To improve consumer trust in food labelling, the information provided should be clear and easy to interpret. Educational programmes about food labelling information should be implemented to increase knowledge about health and safety and the consumer trust in food products.

To prevent and detect fraud or adulteration, the use of rapid and reliable methods could be an important tool used by food industry and laboratories. Then, the combination of mid infrared spectroscopy with the PLS-DA technique has demonstrated great potential in the discrimination and in the classification of fallow deer meat adulterated with goat and *Salmo salar* (SS)

adulterated with *Onconrhynchus mykiss* (OM). Using this methodology, the two types of meat and of fish were easily distinguished with high accuracy.

The PCA analysis led to the conclusion that the most important absorption bands for discriminating the adulteration level of fallow deer are within the wavelength range from 2,000 to 900 cm^{-1}, encompassing specific bands normally associated to lipid and protein compounds. The process of classification using the PLS-DA or PLS-R allowed to the discrimination of samples in different levels of adulteration during different periods of storage time and at diverse stages of the deterioration process.

Within this research area of the food industry, the future perspective is the application of multispectral imaging to other types of foodstuff to determine its authenticity. Also, it seems possible the use of FTIR to determine the quantity of certain contaminants. The application of other spectroscopy techniques, such as Raman, may led to an improvement in the determination of food adulteration.

In future, along the entire logistic chain could be developed and implemented portable systems and that meat of poor quality or fraudulent could be rapidly identified by distributors and consumers. The development of equipment's that enable in situ, robust, and product-to-product on-line inspection and control it is one of the major advantages for industry and for consumers.

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APPENDICES

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